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Evaluation of CyanogenicGlucoside Contents in Some Edible Nuts and Seeds in Girei, Adamawa State, Nigeria

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Abstract: *Cashew nut (Anacardium L.), Red beans (Vignaaguiculata L.), Baobab seed (Adanosiadigitata L.), Soya bean (Glycine max L.), Tiger nut (Cyperusesculentus L.), White bambara nut (Vignasubsterranea L.), Kampala groundnut (Arachishypogaea L.), white bean (Vignaaguiculata L.), Sorghum (Sorghum bicolor L.) and Maize (Zee mays L.) were analyzed for the presence of hydrogen cyanide in the form of cyanogenicglucoside. Qualitative analysis showed that all the samples studied contain hydrogen cyanide in form of cyanogenicglucosides. FTIR analysis also shows that all the samples have nitrile CN functional group within the range of 2280-2240 cm^{-1} absorption band, confirming the presence of the cyanide in the samples. Quantitative analysis was carried out on both distill water and methanol cyanide extracts using UV-Vis spectrophotometer. The least and highest concentration obtained for the distill water extract were in baobab seed (130±1 mg/kg) and cashew nut (300±1.58 mg/kg), while methanol extract were in tiger nut (210±1 mg/kg) and cashew nut (370±1 mg/kg) respectively.The melting points of the hydrazones in all the samples indicate thatbenzaldehyde was present asaglycone. Aprecautionary detoxification is therefore imperative.*

Key words: *Benzaldehyde, cyanide,edible nuts,hydrazones,toxicity.*

I. Introduction

Food is any substance consumed to provide nutritional support for the body. It contains essential nutrients such as carbohydrates,fats, proteins, vitamins. Today food is required to feed the ever increasing population of the world. In Nigeria, these food crops include groundnuts (*Arachishypogeae L.)*,soya bean (*Glycine max L.)*, maize (*Zeamays L.*) etc. Some of these foodstuffs contain Cyanogenicglucosides. Cyanogenicglucoside is an organic compound containing sugar moiety, and is capable of yielding cyanide on hydrolysis [1, 2].The cyanogenic compound is present mainly as glucoside in more than 2650 plant species [3]. Apricot kernel, peach kernel, cassava, almond, bamboo shoot, sorghum, Japanese apricot, flaxseed among others have been consumed by man worldwide either as food or as herbal medicine [4]. About ten cyanogenicglucosides including amygdalin, prunasin, dhurrin, linamarin, and taxiphyllin have been reported in edible plants [5].Hydrocyanic or prussic acid (HCN) is toxic and rapidly acts as a common poison [1, 2].The primary role of cyanogenicglucosides is in the organization of chemical defense system in plants and in plant-insect interactions [6]. Cyanogenicglucosides may serve an important functionin primary metabolism astransporters of nitrogen andglucose. This was first demonstrated in studies with *H.brasiliensis* [7, 8].

However report has it that consumption of food substance containing HCN may cause death within few minutes to three hours, depending on the concentration consumed in the food [9]. In Turkey, apricot seeds are the most common food causing acute cyanide poisoning in children [10]. The toxic action of HCN is due to the cyanide ion whose toxic properties are shared by all the soluble inorganic cyanide salts present in the samples [2, 11].Cyanide is highly poisonous to humans, it inactivates the cytochrome oxidase in the mitochondria, and thus inhibits cellular respiration. Beyond acute poisoning, several diseases result from long-term ingestion of cyanide [12].

Many of the cyanides in soil and water come from industrial processes. The major sources of cyanides in water are discharges from some metal mining processes, organic chemical industries, iron and steel plants or manufacturers, publicly owned wastewater treatment facilities, and use of cyanide-containing pesticides. Plants absorb these cyanides while some occur naturally in the fruits, seeds, roots, and leaves of numerous plants, and are released to the environment from natural biogenic processes from higher plants, bacteria, and fungi [13].

II. Materials

Beakers, volumetric flasks, measuring cylinders, conical flasks, weighing balance,test tubes, wash bottle, spatula, funnel, water bath, filter paper, melting point model, UV- Visible Spectrophotometer and FTIR Spectrophotometer.

Reagents: Chloroform, Picric acid, Sodium Carbonate, Potassium Cyanide, Methanol, Distilled water, Benzaldehyde,Acetic acid, Sodium acetate and 1,2-dinitrophenyl hydrazine.

Sample collection

All samples were collected from different markets in Girei Local Government Area of Adamawa State. The samples studied includes Kampala groundnut (*Arachishypogaea L.*), Cashew nut (*Anacardium L.*), red bean (*Vignaaguiculata L.*), baobab (*Adasoniadigitata L.*), soya bean (*Glycine max L.*), tiger nut (*Cyperusesculentus L.*), white bambara nut (*Vignasubsterranea L.*), maize (*Zee mays L.*), sorghum (*Sorghum bicolor L.*), and white bean (*Vignaaguiculata L.*).

Sample preparation

All samples were air dried at room temperature (25°C) in the laboratory. After drying, they were grinded into powder.All reagents are of analytical grade.

Qualitative analysis of cyanide

The method by Nkafamiya and Manji[2] was used to test for the presence of cyanide in all the samples. Ten grams of the powdered samples was moistened and placed in a test tube. Moistened sodium picrate paper was inserted in the test tube, taking care that it does not come in contact with the samples. A few drops of chloroform were added and the tube was stooped tightly. The sodium picrate paper gradually turned to reddish brown colour indicating the presence of HCN in the form of cyanogeneticglucoside.

FTIR Analysis

IR analysis was conducted to measure the absorption spectra of cyanide (nitrile) as a functional group in all the samples according to the method described by Enaamet al., [14].

Extraction and quantitative determination of cyanide

Cyanide content of the samples was determined using the method described by Bradbury et al. [15] and Adeniran et al.[16] with a modification in the solvent used for the extraction. Five gramsofeach sample was dissolved in 50ml distilled water in a corked conical flask to extractcyanide. The cyanide extraction was allowed to stay overnight. The extract was filteredthrough a filter paper. Alkaline picrate solution was prepared by dissolving 1g of picric acidand 5g of sodium carbonate in warm water in a volumetric flask and making up the volumeto 200ml with distilled water. To 1 ml of the sample filtrate was added 4ml alkaline picrateand this was incubated in water bath for 5min for color development. After the developmentof the reddish brown colour, the absorbance of the solution was read at 490nm on a Cole-Parmer UV-7504 spectrophotometer. Standard cyanide solution was prepared fromdifferent concentrations of potassium cyanide solution. The concentration of the Cyanide was expressed in mg/kg.

Melting point determination of hydrazones

The melting point of the hydrazones of the various samples was determined to identify the type of glucoside present according to the method described by Vogel [17] and Nkafamiya and Manji[2]. This was done using Stuart SMP3 melting point model. Zero point one grams of the sample was added to 3 cm^3 of 1,2-dinitrophenyl hydrazine, 0.1 g of sodium acetate was also added with 10 drops of acetic acid. The mixture was shaken for about five minutes. A crystalline precipitate appeared; the precipitate was oily at first but became crystalline upon standing. The precipitate was filtered and dried. Then the melting point was determined which detect the type of glucosides present in the samples.

Quantitative determination of benzaldehyde

The concentration of the benzaldehyde was determined according to the method described byNkafamiya et al. [18], using a UV spectrophotometer. The standard pure benzaldehyde was preparedfromdifferent concentrations and the absorbances were read at 310nm. Five grams of each sample was dissolved in 50ml methanol in a corked conical flask to extractcyanide. The cyanide extraction was allowed to stay overnight. The extract was filteredthrough a filter paper, and the absorbance of the 5ml filtrate was also read at 310nm. The concentration of the Benzaldehyde was expressed in mg/kg.

Statistical analysis: This is the statistical method used in my research work i.e mean and standard deviation.

Readings,
Mean**N=** Number of readings [19].

III. Results And Discussion

Table 1 presents the qualitative analysis of cyanogenicglucosides. The results indicate that all the samples contain cyanide in form of cyanogenicglucosides. This may be due to the use of pesticides containing cyanide, combustion of polyurethane around the environment, catalytic converters that generate cyanide, some industrial activities, industrial waste water discharged ornaturally occurring Sodium or Potassium cyanide in the area. Similarly report has been presented byNkafamiya and Manji [2] and Nkafamiyaet al.[18], showing the presence of cyanide in Some edible nuts.Nwaichi et al. [20] and Orjiekweet al.[21] also reported the presence of cyanogenicglucosides in cassava (*Manihotexculenta L.).*

Table 1: Qualitative determination of Cyanogenicglucosides in different samples

Samples	Observation
Soya beans (*Glycine max L.*)	+
White beans (*Vignaaguiculata L.*)	+
Red beans (*Vignaaguiculata L.*)	+
White bambaranut (*Vignasubsterranea L.*)	+
Kampala groundnut (*Arachishypogaea L.*)	+
Tiger nut (*Cyperusesculentus L.*)	+
Cashew nut (*AnacardiumL.*)	+
Maize (*Zee mays L.*)	+
Sorghum (*Sorghum bicolor L.*)	+
Baobab seed (*Adanosiadigitata L.*)	+

The positive sign (+) shows the presence of cyanogenicglucosides in all the samples.

The FTIR analysis carried out for all the samples are presented in Figures 1a - j. From the Figures, the absorption band from 2280-2240 cm^{-1} indicates the presence of nitrile CN, which confirms the presence of cyanide in all the samples. The spectra also indicates the presence of functional groups such as; amide between 1680-1630cm^{-1}, aliphatic nitro compounds between 1560-1540cm^{-1}, aliphatic carbonate between 1760-1740cm^{-1}, aldehyde between 1740-1725cm^{-1}, CO between 1725-1705 cm^{-1}, O-H group around 3570-3200cm^{-1}, 993cm^{-1}due to C-H stretching etc. Result is similar to the reports byNkafamiya et al. [18] and Enaam et al.[14].

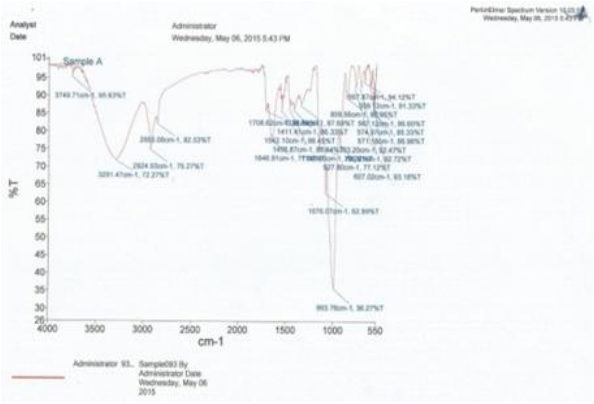

Figure 1a: Absorption spectra for maize extract

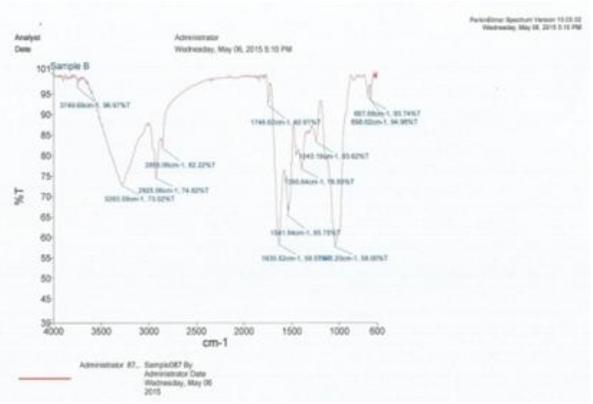

Figure 1b: Absorption spectra for soya bean extract

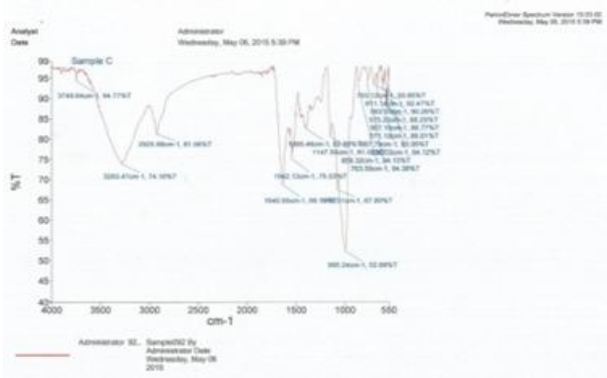

Figure 1c: Absorption spectra for red beans extract

Figure 1d: Absorption spectra for bambara nut extract

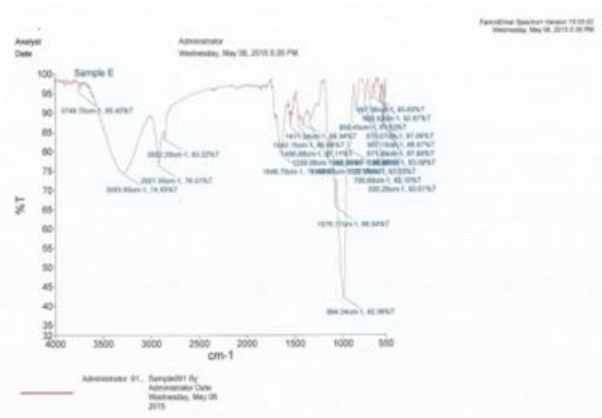

Figure 1e: Absorption spectra for sorghum extract

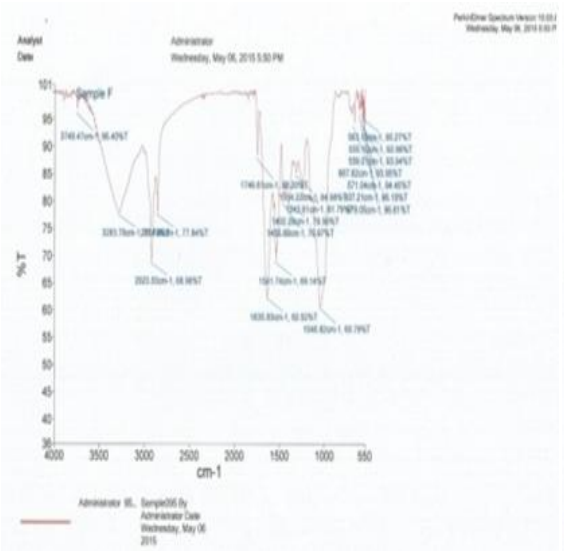

Figure 1f: Absorption spectra for kampala groundnut

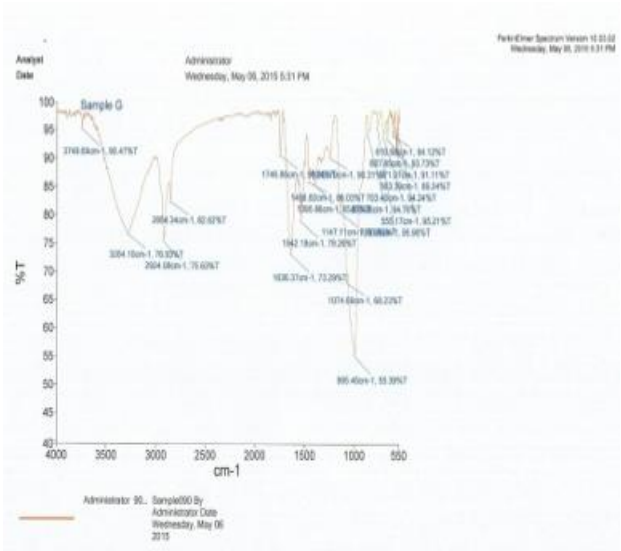

Figure 1g: Absorption spectra for bambara nut extract

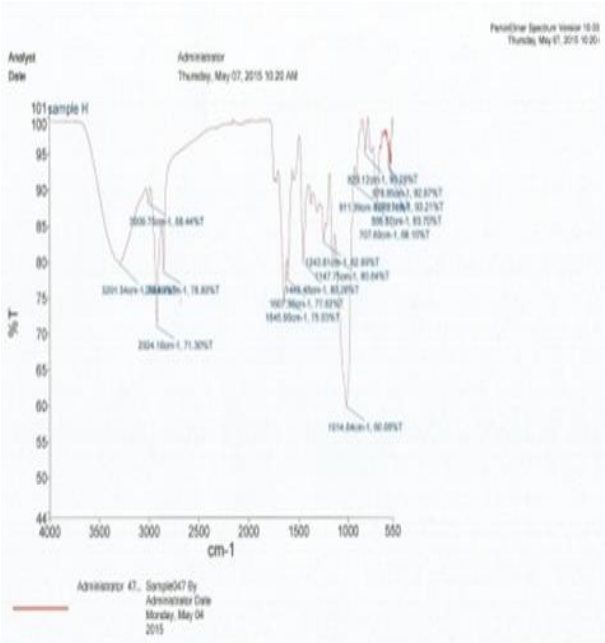

Figure 1h: Absorption spectra for cashew nut extract

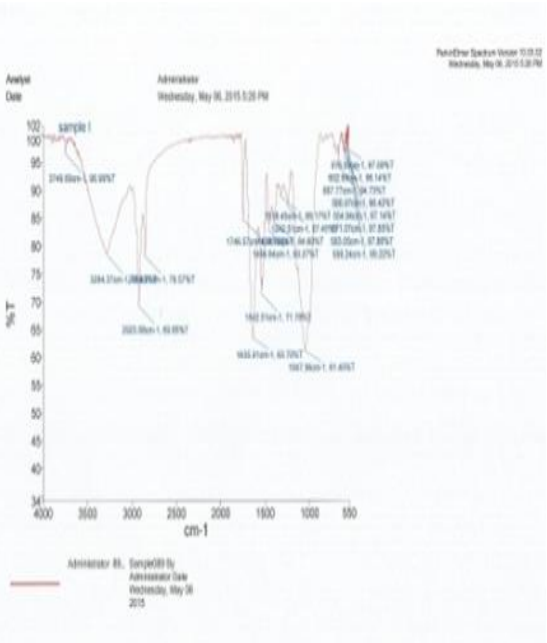

Figure 1i: Absorption spectra for baobab seed extract

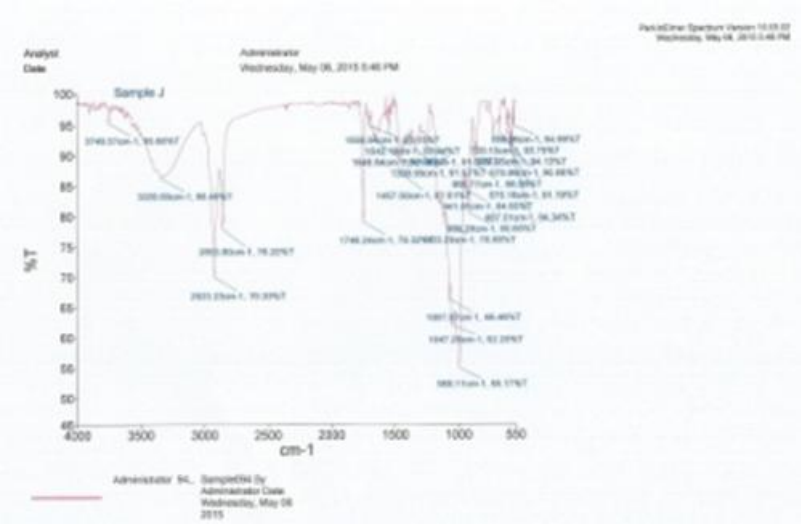

Figure 1j: Absorption spectra for tiger nut extract

Table 2 presents the concentration ofcyanide using distilled water for the extraction.Cashew nut (*AnacardiumL.)* had the highest concentration of 300±1.58 mg/kg, this may be due to its inherent nature, its exposure to more pesticides than the rest of the samples or due to the difference in the composition of the soil type it was grown on, that is to say the soil contains more Sodium or Potassium cyanidethan the rest of the soil on which the other samples were grown. Baobab seed (*Adonasiadigitata L.*) had the lowest concentration of 130±1.00 mg/kg, this may be due to its naturally low content in the sample. Similarly, report has been presented by Bradbury et al. [15] and Adeniranet al. [16], presenting the concentration of cyanide in lime beans (5.20 mg/kg) found in Ibadan,Oyo state.Nwaichiet al. [20] also reported the concentration of cyanide to be 0.5mg/kg in maize (Zee mays L.), 0.05 mg/kg in soya bean (Glycine max L.) and 0.10 mg/kg in cassava (*Manihotexculenta L.*) found in Port –Harcourt, Rivers state. Orjiekweet al. [21] also reported the concentration of cyanide in cassava (*Manihotexculenta L*) flour, fufu and garri found in Okada town, Edo stateto be 30 mg/kg, 50 mg/kg and 25 mg/kg respectively. The concentrations obtained from this work were higher than these literatures. This may be due to a number of factors which include genetic and environmental factors, location, season, and soil types as reported by [22].The results indicate presence of the HCN in the form of cyanogeneticglucoside and it is below the threshold level of 600 mg/kg per day in adult [23, 18].

Table 2: Concentration of the Cyanide when Extracted with Distilled Water

Samples	**Concentration (mg/kg)**
Maize (Zee mays L.)	250±1.00
Soya bean (Glycine max L.)	210±0.70
Red bean (*Vignaaguiculata L.*)	225±1.00
White bean (*Vignaaguiculata L.*)	260± 0.70
Sorghum (*Sorghum bicolor L.*)	265±1.58
Kampala groundnut (*Arachishypogaea L.*)	240±1.00
White bambara nut (*Vignasubsterranea L.*)	175±0.70
Cashew nut (*Anacardium L.*)	300±1.58
Baobab seed (*Adonasiadigitata L.*)	130±1.00
Tiger nut (*Cyperusesculentus L.*)	180±0.70

Table 3 presents the concentration of Cyanide extracted with methanol. Cashew nut (*Anacardium L.*)had the highest concentration of 370±1 mg/kg, this may be due to its less polarity and hence high solubility in the solvent than the rest of the samples. Tiger nut (*Cyperusesculentus L.*) had the lowest concentration of 210±1 mg/kg, this may be because of its high polarity and hence low solubility in the solvent than the rest of the sample. The concentrations of the cyanide obtained when extracted with methanol were higher than the ones extracted with distilled water. This is due to difference in polarity and hence the difference in solubility of plant samples [24]. Water is more polar than methanol but most organic compounds with less polarity have high solubility in a less polar solvent, this is because likes dissolve likes. Hassan et al. [25] and Idris et al.[26] also presented a similar report. The results indicate presence of the HCN in the form of cyanogenetic glycoside is very much below the threshold level of 600 mg/kg per day in adult [23, 18].

Table 3: Concentration of the Cyanide when Extracted with Methanol

Samples	**Concentration (mg/kg)**
Maize (*Zee mays L.*)	340±0.70
Soya bean (*Glycine max L.*)	280±1.58
Red bean (*Vignaaguiculata L.*)	360±1.00
White bean (*Vignaaguiculata L.*)	315±0.70
Sorghum (*Sorghum bicolor L.*)	350±1.00
Kampala Groundnut (*Arachishypogaea L.*)	265±1.58
Bambara nut (*Vignasubsterranea L.*)	240±0.70
Cashew nut (*Anacardium L.*)	370±1.00
Baobab seed (*Adonasiadigitata L.*)	260±1.58
Tiger nut (*Cyperusesculentus L.*)	210±1.00

Table 4 presents the melting point of the hydrazones of the various samples. The melting points of all the samples indicate that benzaldehyde was present, which is one of the hydrolysed products of Amygdalin with melting point of 237^{o}C. Amydalin on hydrolysis yields benzaldehyde, glucose and hydrogen cyanide. Nkafamiyaet al. [18]reported the presence of benzaldehyde as one of the hydrolysed product of amygdalin.

Table 4: Melting Point of the Hydrazones

Samples	Melting points
Maize (*Zee mays L.*)	233^{o}C
Soya bean (*Glycine max L.*)	232^{o}C
Red bean (*Vignaaguiculata L.*)	233^{o}C
White bean (*Vignaaguiculata L.*)	234^{o}C
Sorghum (*Sorghum bicolor L.*)	236^{o}C
Kampala groundnut (*Arachishypogaea L.*)	233^{o}C
White bambara nut (*Vignasubsterranea L.*)	238^{o}C
Cashew nut (*Anacardium L.*)	232^{o}C
Baobab seed (*Adonasiadigitata L.*)	238^{o}C
Tiger nut (*Cyperusesculentus L.*)	234^{o}C

Table 5presents the concentration of the benzaldehyde. Bambara nut (*Vignasubsterranea L.*) has the highest concentration of 0.3390±0.7 mg/kg, this may be due to its naturally high content in the sample. Cashew nut (*Anacardium L.*) has the lowest concentration of 0.169±1 mg/kg, this may be due to its naturally low content, since it has the highest content of cyanide. Similarly a report has been presented by Nkafamiyaet al. [18].

Table 5: Concentration of Benzaldehyde

Samples	Concentration(mg/kg)
Maize (*Zee mays L.*)	0.216±1.00
Soya bean (*Glycine max L.*)	0.265±0.70
Red bean (*Vignaaguiculata L.*)	0.234 ±1.00
White bean (*Vignaaguiculata L.*)	0.216±0.70
Sorghum (*Sorghum bicolor L.*)	0.230±1.00
Kampala groundnut (*Arachishypogaea L.*)	0.228±1.58
White bambara nut (*Vignasubsterranea L.*)	0.339±0.7
Cashew nut (*Anacardium L.*)	0.169 ±1.00
Baobab seed (*Adonasiadigitata L.*)	0.212±0.70
Tiger nut (*Cyperusesculentus L.*)	0.223 ±1.00

IV. Conclusion

The qualitative and quantitative analysis of cyanogenicglucosides content in common edible nuts and seeds considered in this study has quantitatively indicated the presence of the compound. The work also reveals the efficiency of the laboratory method used in the qualitative analysis of this substance as confirmed in the FTIR spectra. The efficiency of cyanide extraction was investigated using water and methanol. Methanol however gives a better extract of cyanide from the nuts and seeds.Amaygdalin was also identified as the form of glucoside present in the nuts and seed from the quantitative presence of benzaldehyde in them. In general,the cyanide levels of theedible nuts and seedsevaluated in this study are below the maximum acceptable level

600mg/kg. However, accumulation of small doses of cyanide over a long period of time can be dangerous. Therefore, precautionary detoxification may be recommended before the consumption of the seeds and nuts in which cyanide has been presented to be relatively high in this study.

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