Thevetia peruviana is one out of 250,000 species of plant endowed with seed oil, proteins and biologically active principles which can be harnessed for food, bio-fuel and medicinal purposes in Kenya. However its utilization has been hampered by the coexistence of the oil, proteins and the biologically active principles in the seed kernels of the plant.

Phytochemical screening for biologically active principles was done by solvent extraction, thin layer chromatography (TLC) for detection of the biologically active principles and gas chromatography (GC) for fatty acid determination. Extracts of the biologically active principles were obtained from fermented and non fermented samples from mature T. peruviana plants, young germinated seedlings and tissue cultured material. Authentic sample for TLC of peruvoside was bought from SIGMA-ALDRICH Inc. of United States of America. The plant species has high potential for production of seed oil, pharmacologically active principles and proteins especially in the dry land areas along the Lake Victoria basin region in Kenya.

The seeds contain about 62% pale yellow non drying oil, 30% proteins and about 8% of biologically active cardiac glycosides. The oil hydrolyses to give 64% oleic, 6.3% linoleic, 17% palmitic, 11.8% stearic and 0.4% arachidonic acids. The oil has a density of 0.9108, refractive index of 01.0682 at 210°C, acid value of 0.6, saponification value of 191, esterification value of 190 and an iodine value of 73 [1,2]. The n-hexane oil extract is free from the cardio-active glycosides and has been recommended as a suitable replacement for peanut and almond oils [2]. Hydrogenation studies using cobalt chloride-molybdenum oxide catalyst supported on activated charcoal has also been reported, Kareem and Kadiri, [3]. The crude oil is also suitable for industrial applications in bio-diesel manufacture and soaps [4].

Eighteen amino acids including essential and non essential amino acids have been isolated from the leaves from Indian cultivars. These include glutamic acid, leucine, glucine, isoleucine which are predominant over arginine, valine, alanine, proline, phenylalanine, aspartic acid, cysteine, lysine, serine, tyrosine, histidine, threonine, methionine and tryptophan [5]. Attempt by Atteh et al. [6] to detoxify the T. peruviana cake using hydrolysis and solvent extraction failed as reflected by the mortality recorded in the broilers placed on the detoxified seed cake-based diet.

Usman (2009) [7] modified the method of Atteh et al. [6] by using 0.1–0.5 M HCl, NaOH and Ca(OH)2 solutions.

The detoxification was monitored by the level of bitterness of the cake. HCl and NaOH treated cake were devoid of bitter taste irrespective of concentrations of de-toxicaents. However, only the cakes treated with 0.4 and 0.5 M Ca(OH)2 were freed of bitter principles. The effect of these treatments on the crude protein, albumin and globulin content were monitored. The nutrient estimated in detoxified cake compared favorably well with Nigerian grown Groundnut cake. The leucine to lysine ratio in the raw, ethanol treated and acid treated cake are 1.23, 0.99 and 1.23 respectively. This compares favorably well with soybean with a value of 1.23. The leucine/lysine ratio that is lower than 4.6 will enhance the utilization of iso-leucine and lysine in the diet. This shows that raw and treated seed cake has better leucine/lysine ratio than most
Nigerian grown cereals and Usman et al., The crude protein content of the defatted and de-toxified seed cake is 66% third to soya 70% and sesame 68%.

The following steroidal (cardiac) glycosides have been isolated from the seed kernels, thevetin A, thevetin B, peruvoside and nerifolin. Of all the glycosides, peruvoside is the most important as it is already used in Germany as a cardio-tonic drug despite its natural occurrence in the plant in trace amounts. Flavanone and flavonol glycosides from the leaves of Thevetia peruviana and their HIV-1 reverse transcriptase and HIV-1 integrase inhibitory activities have been reported [8].

Materials and Methods
Sample collection and preparation
Calli tissues were obtained from the in vitro regenerates and leaves harvested from young germinated seedlings. Seeds were harvested from mature plants growing in Kenya. 100 g of the seeds were de-shelled, pestled and extracted by soxhlet method using n-hexane. The defatted seed kernels were fermented at 37°C for two days then extracted with ethanol and chloroform. Calli from tissue cultured materials and leaves from young seedlings were also extracted with ethanol.

Thin layer chromatography
The crude ethanol and chloroform extracts from the fermented kernels, and leaf extracts were spotted onto a TLC plate at about 1 cm apart. Peruvoside from SIGMA Aldrich inc. was the standard glycoside. The solvent system used in elution was (ethyl acetate-methanol-water in the ratio of 81:11:8) and detection was by spraying with concentrated sulphuric acid. The TLC plate was 20 cm×20 cm, Polygram silica gel/ in the ratio of 81:11:8 and detection was by spraying with concentrated sulphuric acid. The naturally occurring glycosides were extracted with 95% ethanol from defatted non-fermented seed kernels, young leaf extracts from the wildtype and tissue cultured samples and separated by TLC. Peruvoside was separated by TLC from the defatted-fermented seed kernels extracted by ethanol and chloroform. The natural (three sugar glycosides) from leaf extracts had low RF values ranging from 0.1-0.2 while the peruvoside (monoglycoside) had high RF values ranging from 0.6-0.7. Peruvoside was detected in fermented-defatted seed kernels but not in non fermented defatted seed kernel extracts and crude leaf extracts. The non fermented kernel extracts and the leaves showed presence of the natural cardiac glycosides (tri-sugar glycosides). The oil and calli showed no glycosides presence. The chloroform extract had larger spots indicating that the glycoside were more soluble in chloroform than ethanol (Figure 1 and 2).

TLC Results summary
TLC Results summary mentioned in table 1

Results
Phytochemical screening of the seed oil and glycosides
The cardio-active glycosides stains dark blue to brownish on T.L.C plate after spraying with concentrated sulphuric acid. The naturally occurring glycosides were extracted with 95% ethanol from defatted non-fermented seed kernels, young leaf extracts from the wildtype and tissue cultured samples and separated by TLC. Peruvoside was separated by TLC from the defatted-fermented seed kernels extracted by ethanol and chloroform. The natural (three sugar glycosides) from leaf extracts had low RF values ranging from 0.1-0.2 while the peruvoside (mono-glycoside) had high RF values ranging from 0.6-0.7. Peruvoside was detected in fermented-defatted seed kernels but not in non fermented defatted seed kernel extracts and crude leaf extracts. The non fermented kernel extracts and the leaves showed presence of the natural cardiac glycosides (tri-sugar glycosides). The oil and calli showed no glycosides presence. The chloroform extract had larger spots indicating that the glycoside were more soluble in chloroform than ethanol (Figure 1 and 2).
Fatty acid composition by GLC analysis

The oil was found to contain oleic, linoleic, stearic and palmitic acids, which constituted over 90% of the total fatty acids. Myristic acid was only identified from mechanically extracted oil samples (Table 2). The oil was found to be rich in unsaturated fatty acids, which comprised over 60% of the total fatty acids present. These mainly included oleic 48–52% and linoleic 16–18% respectively. The short chain fatty acid present is myristic acid as shown in figure 3.

Discussion

The results indicate that peruvoside was only present in the defatted and fermented seed kernels. This indicates that during fermentation three sugar thevetia glycosides such as thevetin are converted to one sugar glycoside (peruvoside). Peruvoside is more potent as a cardio-active therapeutic drug than thevetin which has therapeutic dose near lethal dose. The glycosides are more soluble in chloroform than in ethanol. The absence of glycosides on the calli indicates that in vitro culture conditions does not induce glycoside synthesis, however field growing conditions may elicit glycoside biosynthesis in Thevetia peruviana. This helps to explain why Sen and Datta, reported loss of thevetin in dedifferentiating callus in 1981.

The results of T. peruviana seed oil compares favorably to the work reported in the literature review and to commonly used vegetable oils such as palm oil, coconut, Soya beans and sunflower in terms of fatty acid composition, physical and other chemical properties. Palm oil constitutes over 90% of the total vegetable oil import into the country from Malaysia and sunflower oils.

The high oleic acid (18:1, mono unsaturated fatty acid) content places the oil as a suitable vegetable oil for human diet, since oleic acid is recommended for alleviation of cardiovascular fat related ailments. The presence of linoleic acid (an essential fatty acid) makes the oil more suitable for dietary purposes. The low acid, value meets the recommended International codex standards for edible oils [9].

Conclusion

Thevetia peruviana growing in Kenya has high potential for peruvoside production. Fermentation is valuable for production of therapeutic cardio-active glycoside (peruvoside) from defatted T. peruviana.

Recommendations

• Tissue culture may be used as a tool to develop new varieties of T. peruviana free from the toxic glycosides.

• Molecular techniques may be used to identify and suppress the glycoside synthesising gene.

References