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Full Length Research Paper

EFFECT OF PROCESSING ON *THEVETIA PERUVIANA* GLYCOSIDE

[1]Markus, V., [2*]Abdulsalami, M.S., [3]Mustapha, M and [4]Abdulgamiyu, A.
[1]Dept. of Biochemistry, Faculty of Science, Kaduna State University, Kaduna.
[2]Dept. of Biological Sciences, Nigerian Defence Academy, Kaduna.
[3]Dept. of Science Lab. Technology, School of Technology, Matan fada road, Kawo.
[4]Dept. of Animal Production, Federal University of Technology, Minna, Nigeria.
*Corresponding author's E-mail: abdulsalamimosan@yahoo.com

ABSTRACT
The nutritional content of the meal of *Thevetia peruviana* seed is comparable to that of soybean and peanut meals but is rarely consumed due to its toxicity, resulting from high content of cardiac glycoside. This work therefore was designed to investigate suitable method(s) for the effective removal of the cardiac glycoside from seed kernel meal. The various methods of processing employed were: fermentation, boiling in water, soaking in water, autoclaving, acid and alcohol treatments. The results showed that fermentation gave the least reduction in the cardiac glycoside (59.87%); boiling gave better (76.42%) reduction than all methods except alcohol treatment which gave the best reduction (84.97%). Further investigation using combined method (boiling and alcohol treatment) with temperature and time variation showed that boiling with 80% methanol/ethanol solvent mixture (8:2) for 30 minutes at 45^0C is sufficient to attain the optimum (91.0%) reduction of the toxic cardiac glycoside from the seed meal.
Key words: *Thevetia peruviana* seed, processing, cardiac glycoside, aglycones

INTRODUCTION
Thevetia peruviana, juss, more commonly known as yellow oleander, be-still tree, milk bush etc, belong to the order apocynales and the family apocynaceae. It is a native of tropical America, but has naturalized in the tropical and subtropical regions of the world including Nigeria where it is mainly grown as an ornamental plant because of the toxic nature of the plant, mostly cardiac glycoside and their free aglycones (Oluwaniyi *et al*., 2007, Olatunji *et al.*, 2011). The major glycoside reported being thevetin and that the seed kernel contains between 3.6 and 4.0% thevetin (Sun and Libizor, 1964). Other glycosides that have been reported from thevetia plant include theveside, neriifolin, cerberin, peruvoside, theveridoside, digitoxigenin among others (Lang and Sun, 1965; Huang *et al*., 1966; Arora *et al*., 1967; Sticher, 1970; Perez-Amador *et al*., 1994; El Tanbouly *et al*., 2000; Oluwaniyi *et al*., 2007;Oluwaniyi and Ibiyemi, 2007).

There are two varieties of the *Thevetia peruviana* plant, one with yellow flowers, yellow oleander, and the other with purple flowers, nerium oleander. Both varieties flower and fruit all the year round, proving a steady supply of seed (Usman *et al.*, 2009). As reported by Taiwo *et al*., (2004) the protein content of various feeds ranged from 39% in cane molasses, 3.9% in cottonseed meal to 40% in soybean meal and peanut meal. These results show that the meal of *Thevetia peruviana* seed can be comparable to the quality of soybean and peanut meal. Currently, there is virtually no reported

human dietary or commercial demands for the seed, this makes it very cheap compared to other conventional protein concentrates like peanut and soybean.

Several attempts have been made to detoxify thevetia seed by single method: acid treatment, heat treatment, autoclaving, fermentation, and utilizing the treated seed in the formulation of birds feed. The results showed that the methods used did not remove the toxins as desired as there were deaths, low feed consumption and performance of the experimental birds (Atteh *et al.*, 1995, Odetokun *et al.*,Taiwo *et al.,* 2004). This shows that employing one method may not give the desired removal of the toxin and a combination of two or more methods may be far better. (Akande and Fabiyi, 2010). Oluwaniyi *et al.,* (2007) attempted to detoxify the seed using combined method of acid treatment followed by alcohol extraction (80% ethanol/methanol mixture). The result showed that combining the two methods brought marked reduction in the *Thevetia peruviana* glycoside. This work therefore was designed to look into other potential methods that could be combined to effectively remove the toxins from the seed.

MATERIALS AND METHODS

Materials

Matured fruits of *Thevetia peruviana* plant were collected from Marafa, Kaduna North local government area of Kaduna State. The sample was identified by a herbarium curator, Department of Biological Science, Ahmadu Bello University, Zaria. The voucher number of the plant is 165. The mesocarp of the fruits was removed and the hard endocarp cracked. The soft seeds were air dried, crushed in a wooden mortar and milled to powder. The powder was then divided into portions: one subjected to qualitative analysis, and the other, divided into parts for the solvent extraction using the various organic solvents.

Methods of processing

The methods of processing employed were: Boiling, Fermentation and Autoclaving (modifications of the ones described by Ugwu and Oranye (2006)); Alcohol treatment and Acid treatment (adopted from Oluwaniyi *et al.,* (2007)); Soaking (a modification of the method described by Akande and Fabiyi, (2010)).

Boiling: Five gram (5g) of sample were weighed and boiled for 90 minutes in water at 100^0C. The seeds were then oven-dried at 60^0C. The dried seeds were milled and pass through 0.250mm aperture mesh size sieve.

Fermentation: Three gram (3g) of the sample were weighed and left to ferment for 48h. 0.1g yeast was introduced to start the fermentation. The presence of bubbles was observed during the process. After the 48hrs have elapsed, the fermented sample was oven-dried.

Autoclaving: Four gram (4g) of the sample was weighed and autoclaved at a pressure of 15 Ib (120^0C) for 30 minutes. The autoclaved seeds were oven dried at 60^0C and milled into flour.

Alcohol treatment: The solvent treatment was done using a modification of the method of Finnigan and Lewis (1988) . 10ml of 80% aqueous solution of the reagent was used to soak the defatted meal twice. A solvent to meal ratio of 10:1 was used the first time and the mixture was stirred well and left overnight. The solvent was then decanted and fresh solvent added (ratio 5:1) and this was also left overnight. The final product was then pressed free of solvent and the caked air-dried

Acid treatment: 0.1M HCl was used for the treatment and mixed with defatted cake in the ratio 4:1 (solvent: meal). The treated cake was then air dried.

Soaking: Water was used to soak the sample and the mixture was stirred well and

left overnight. The water was then decanted and fresh water added, this was also left overnight. The final product was then pressed free of water and the sample air-dried

Quantitative Analysis of *Thevetia Peruvian* seed

Two gram (2g) of the sample was defatted with 100ml of diethyl ether using a soxhlet apparatus for 2hrs.The quantitative analysis was carried out using the standard procedure described by El-Olemy *et al.*, (1994). The quantity of glycoside in the raw and treated samples was evaluated using Baljet's reagent (95ml 1% aqueous picric acid + 5ml 10% aqueous NaOH). One gram (1g) of each sample was soaked overnight with 10ml of 70% alcohol and filtered. The extracts were then purified using 12.5% lead acetate and 4.77% Na_2HPO_4 solution before the addition of freshly prepared Baljet's reagent. Digitalis cardiac glycosides develop an orange red color with Baljet's reagent. The intensity of the color produced is proportional to the concentrate of the glycoside. This color formation is used for the quantitative estimation of cardiac glycoside present. The intensity (absorbance) of the color produce was measured using spectrophotometer at 495nm. A blank was carried out at the same time using distilled water and Baljet's reagent.

RESULTS AND DISCUSSION

Various methods of processing were employed to remove the cardiac glycoside from the seed (Figure 1). The result shows a marked reduction in the cardiac glycoside content irrespective of the detoxification method employed. It also shows that alcohol treatment and boiling gave better reduction of the cardiac glycoside in the seed, however, with alcohol treatment best among the processing methods. Fermentation gave the least reduction in the glycoside. Yeast cells were employed in the fermentation process. Although fermentation has been used in many detoxification processes of plant products like cassava, it could be seen here that it was the least effective method in reducing the cardiac glycoside. This suggests that the glycoside may not have O-linked glycosidic bonds since only these bonds are prone or susceptible to the enzyme actions produce by the yeast organism.

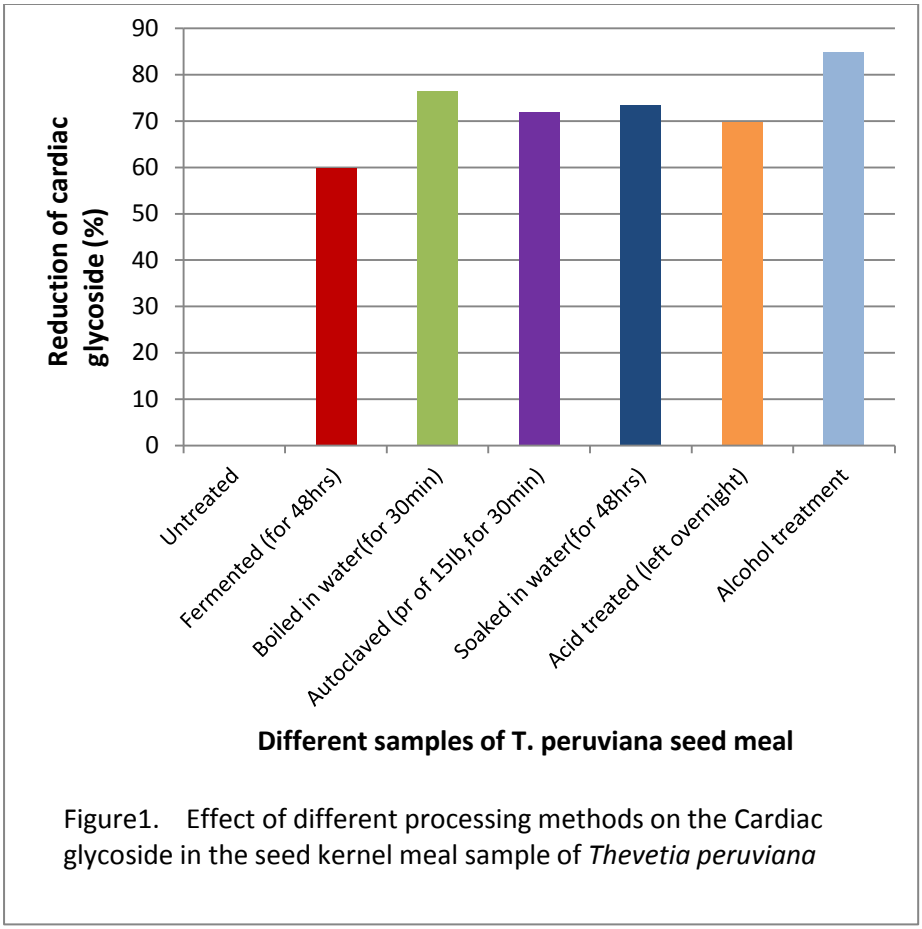

Figure1. Effect of different processing methods on the Cardiac glycoside in the seed kernel meal sample of *Thevetia peruviana*

Akande and Fabiyi, (2010) reported that in many instances, usage of only one method may not give the desired removal of toxic substances in a seed and a combination of two or more methods may be required to achieve the desired result. It was in this light that two methods were employed to see if the cardiac glycoside in the seed could be better removed.

Figure 2 and Figure 3 showed the usage of these two combined methods of processing: Methanol/ethanol mixture (8:2) and Boiling, with variation to boiling time and temperature. From the results obtained, it shows that boiling with methanol/ethanol solvent mixture (8:2) for 30 minutes at 45^0C is sufficient to attain 91.0 % reduction of the toxic cardiac glycoside from the seed meal. Further research is recommended to be carried out employing specific enzymes that act only on the glycoside.

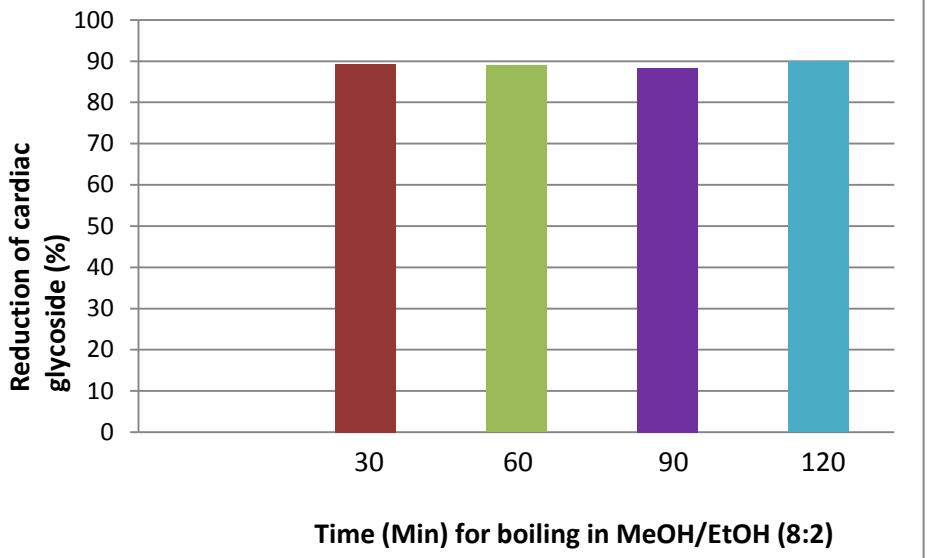

Figure 2. Extraction of Cardiac glycoside from *Thevetia peruviana* seed meal using methanol/ethanol solvent mixture (8:2) and boiling at 65^{0}C, with variation to time

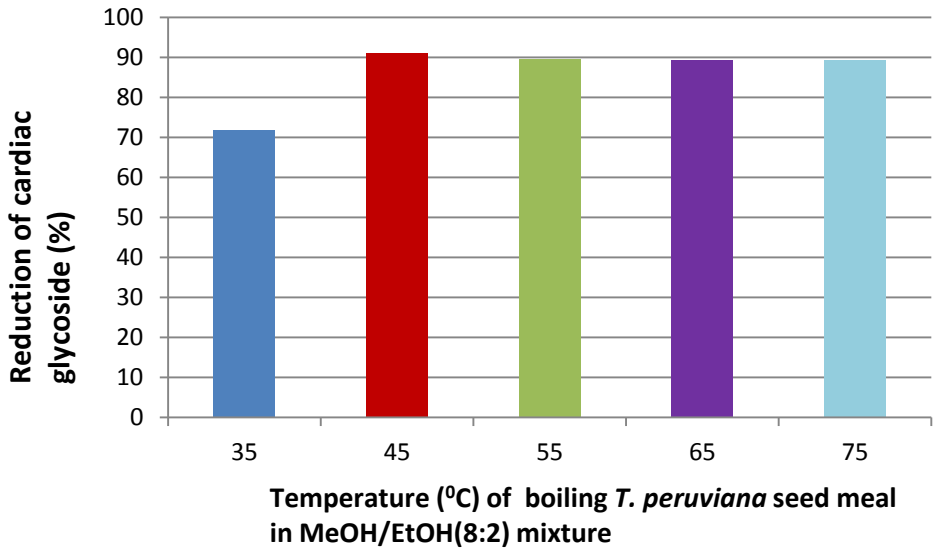

Figure 3. Extraction of Cardiac glycoside from *Thevetia peruviana* seed meal using methanol/ethanol solvent mixture (8:2) and boiling for 30 minutes, with variation to temperature.

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