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# Extractability of *Thevetia Peruviana* Glycoside using Various Organic Solvents

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**Abstract**
This work was designed to investigate the suitable organic solvent(s) for the effective removal of *Thevetia peruviana* glycoside in the seed. The anti-nutritional status of the seed was also assessed. Of all anti-nutrients screened (Tannins, Phlobatannins, Saponin, Flavonoids, Cardiac glycoside and Lectins) in the seed meal, only Cardiac glycoside (at 7.98 w/w % of the seed meal) was found. Attempt to remove the cardiac glycosides content using various organic solvents (Methanol, Ethanol, Ethyl acetate, n-hexane, n-butanal, Chloroform and Acetone) shows that 80% methanol/ethanol solvent mixture (8:2) reduces the toxic cardiac glycoside from the seed meal optimally.
**Keywords**: Thevetia peruviana seed, cardiac glycoside, aglycones, detoxification

## 1. Introduction

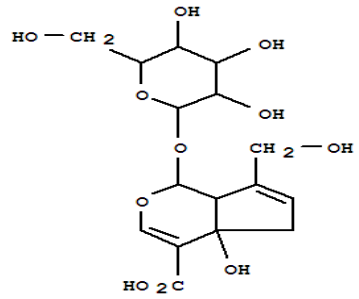
The sources of plant proteins are not only limited but also very expensive because of the competing needs of these sources by man and other livestock. ( Taiwo *et al*., 2004). Oil seeds are major sources of vegetable proteins and oils for human and animal nutrition which also constitute an indispensible part of our industrial raw materials. (Usman *et al*., 2009). But, a number of these seeds which could have been alternative sources of protein for animal feed contain toxins and anti-nutrients which affect their nutritive values and utilization. (Oluwaniyi *et al*., 2007). Toxicological evaluation in plants is therefore very important before any plant that can find utilization and application as protein source in animals feed formulation or human food. This is to determine the safety of the plant.

*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 is thevetin between 3.6 and 4.0% in the seed (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).

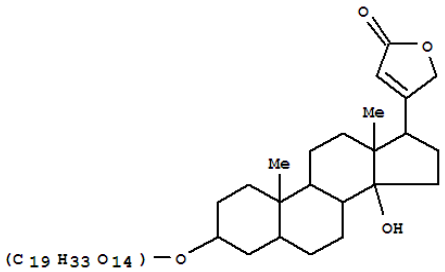
There are two varieties of the *Thevetia peruviana* plant, one with yellow flowers, yellow oleander, and the other with purple flowers, nerium oleander. Both varieties flower and fruit all the year round proving a steady supply of seed. (Usman *et al.*, 2009). As reported by Taiwo *et al*., (2004) the proteins content of various feeds: 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 the seed by acid and 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 completely as there were deaths, low feed consumption and performance of the experimental birds. (Atteh *et al.,*1995, Odetokun *et al.,* Taiwo *et al.,* 2004). Oluwaniyi *et al*., 2007 attempted to detoxify the seed using alcohol extraction (ethanol/methanol mixture). The result showed that this method of using organic solvent was promising, even though it is cost effective. This work therefore was designed to look into other potential organic solvents that could be use to effectively extract out the toxins from the seed.

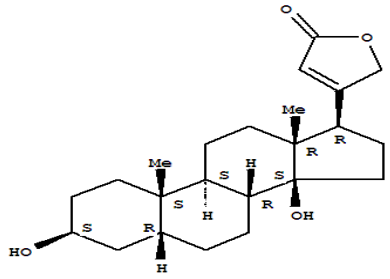
 



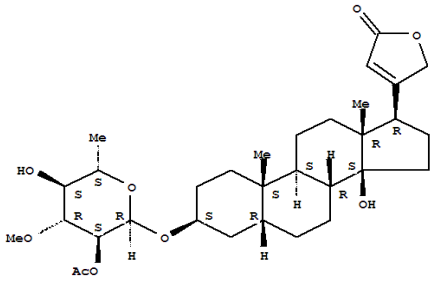
Theveside



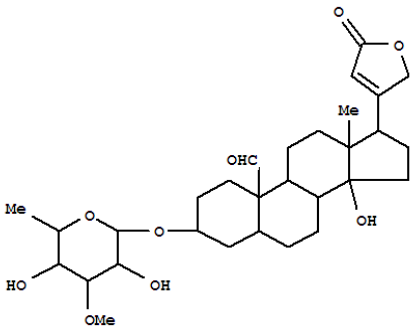
Thevetin



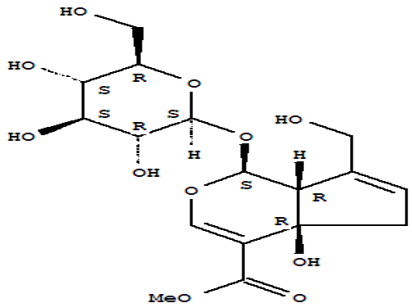
Digitoxigenin



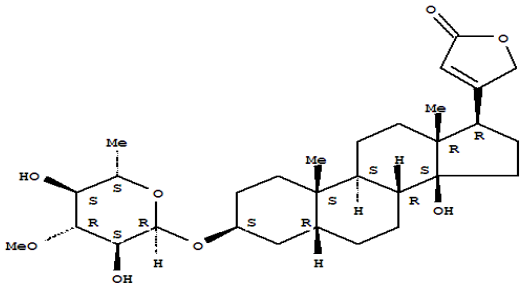
Cerberin



Peruvoside



Theveridoside



Neriifolin

Figure.1 Structures of free aglycones in *Thevetia peruviana* seed

## 2. Materials and Methods

### *2.1 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 were 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 two portions: one subjected to qualitative analysis, and the other, defatted and divided into parts for the solvent extraction using the various organic solvent.

### *2.2 General Solvent extraction method*

The solvent extraction method with different organic solvents was done using a modified method of Finnigan

and Lewis (1988) as reported by Oluwaniyi and Ibiyemi, (2007). 10ml of 80% aq. 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

*2.3 Qualitative Analysis of the Thevetia peruvian seed*

The qualitative analysis was carried out on the aqueous extract and powdered specimens using standard procedures described by Edeoga *et al.,* (2005) and Majaw and Moirangthem (2009) for the determination of the anti-nutritional factors, except lectins.

*2.3.1 Test for Tannins*

0.5g of the dried powered sample was boiled in 20ml of water in a test tube and the filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

*2.3.2 Test for Phlobatannins*

Deposition of a red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

*2.3.3 Test for Saponin*

2g of the powered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth (foam). The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

*2.3.4 Test for Flavonoids*

5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of the plant seed extract followed by addition of concentrated $H_2SO_4$. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

*2.3.5 Test for Cardiac glycoside (Keller-killani test)*

5ml of the seed extract was treated with 2ml of acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated $H_2SO_4$ . A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

*2.3.5 Test for Lectins*

An aqueous extract of the sample was prepared (solvent: meal) 10:1. The extract was mixed with blood of mice after the animal was sacrificed at different volume of the aqueous extract (5ul, 10ul, 15ul, 20ul) in a microtitre plate. Agglutination of the red blood cell was viewed using the microscope for the presence of lectins.

*2.4 Quantitative Analysis of Thevetia Peruvian seed*

2g of the sample were 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). 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 made 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.

## 3. Results and Discussion

Table 1: Results for qualitative and quantitative analysis of anti-nutritional factors in the seed kernel of *Thevetia peruviana*

| Anti-nutritional factor | Inference | Amount (w/w%) |
|---|---|---|
| Flavonoids | _ | 0.00 |
| Tannins | _ | 0.00 |
| Phlobatannins | _ | 0.00 |
| Saponin | _ | 0.00 |
| Lectins | _ | 0.00 |
| Cardiac glycoside | + | 7.98 |

+ indicates presence of constituents; □ indicates absence of constituent

Analysis of the *Thevetia peruviana* seed meal showed that, of all the anti-nutritional factors screened only Cardiac glycoside was found as shown in Table1. This result comes in agreement with the many works already published establishing the presence of cardiac glycoside in the seed of *Thevetia peruviana* (Perez-Amador *et al*., 1994; El Tanbouly *et al*., 2000; Oluwaniyi and Ibiyemi, 2007; Oluwaniyi *et al.,* 2007; Usman et al., 2009). On the amount of Cardiac glycoside in the seed, it was found to be at 7.982g w/w% of the seed meal.

Sun and Libizor, (1964) reported that the seed contain between 3.6 and 4.0% thevetin (the major glycoside in the seed). Also, Oluwaniyi and Ibiyemi, (2007) reported that the seed contain 5.44g% cardiac glycoside. The variation seen here from the results could be as a result of the variety of the plant used or the location where the plant was grown.

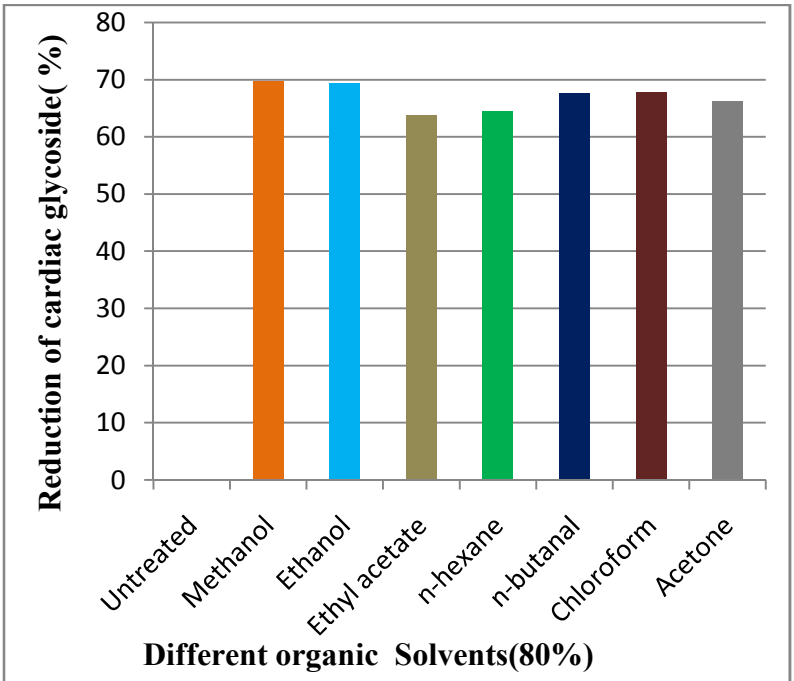



Figure 2. Extraction of Cardiac glycoside from *Thevetia peruviana* seed meal using different organic solvent.

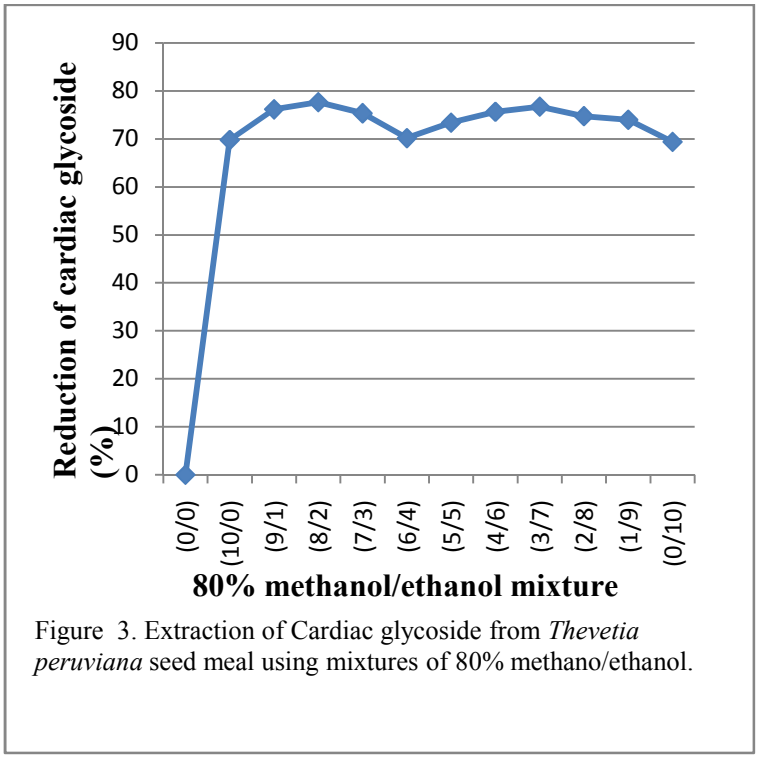



Figure 3. Extraction of Cardiac glycoside from *Thevetia peruviana* seed meal using mixtures of 80% methano/ethanol.

The result of the extractability of *Thevetia peruviana* glycosides using various organic solvents (figure 2) showed a reduction in the toxic cardiac glycoside content irrespective of the organic solvent employed.

However, methanol and ethanol gave better reduction of the cardiac glycoside in the seed. Further investigation using various methanol/ethanol solvent mixtures showed that 8:2 and 3:7 ratios extracted the cardiac glycoside better than other ratios, however with 8:2 ratio slightly better than 3:7 ratio (Figure 3). This result corresponds to the work reported by Oluwaniyi *et al*., (2007) when same methanol/ethanol solvent mixture (8:2) was employed in detoxifying the *Thevetia peruviana* seed in a 10:1 solvent to meal ratio.

## 4. Conclusion

At the end of this research, only Cardiac glycoside (at 7.982g% of the seed kernel) of all the anti-nutrients screened in the thevetia seed was found. It also shows that extractability with methanol/ethanol solvent mixture (8:2) gave a better reduction of the toxic cardiac glycoside from the seed meal. Further research is recommended to be carried out on *Thevetia peruviana* seed employing specific enzymes that act only on the glycoside to enable it excellent detoxification.

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