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A Study of the Extractability of Thevetia Glycosides with Alcohol Mixture

Omolara O. Oluwaniyi and Samuel A. Ibiyemi
Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

Abstract: The aim of this research was to investigate the most effective extraction conditions for the production of thevetia seed protein concentrate of reduced cardiac glycoside content. Alcoholic extraction of the glycosides was studied as a function of time, solvent to meal ratio and solvent composition. Thevetia seed meal was extracted with 10:1, 15:1 and 20:1 solvent to meal ratios, for 45min, 24, 48 and 72 h. Varying concentrations-50 to 100% (v/v) aqueous alcohol were also used. A concentration of 70 or 80% aqueous alcohol resulted in the lowest glycoside content, while a solvent to meal ratio of 15: 1, extracted over a period of 72 h appears to give the best compromise between glycoside extraction and cost of extraction solvent. All treatments resulted in an increase in the protein content of the samples.

Key words: Detoxification, alcoholic extraction, cardiac glycosides thevetia seed meal

INTRODUCTION

Thevetia peruviana otherwise known as the milk bush, be-still tree, lucky nut or yellow oleander is an evergreen plant often grown as an ornamental plant in spite of the high oil content of the seed (60-65% oil) and equally high protein content of the defatted meal (30-37% protein) (Ibiyemi *et al*., 2002). This is as a result of the toxic nature of the plant. Thevetia seed contains toxic compounds which are mostly cardiac glycosides and their free aglycones such as thevetin, theveridoside, theveside, cerberin, peruvoside, perusitin, digitoxigenin etc (Sun and Libizor, 1965; Perez-Amador *et al*., 1994; Lang and Sun, 1965; Sticher, 1970; Huang *et al*., 1966; Arora *et al*., 1967; Arnold *et al*., 1935; El Tanbouly *et al*., 2000).

Ingestion of thevetia seed by either man or animal has been reported to result in severe cardiac toxicity which produced marked poisoning symptoms that culminated in death (Oji and Okafor, 2000; De Silva *et al*., 2003; Fonseka *et al*., 2002). Symptoms include nausea, vomiting and giddiness within hours. Other clinical features are severe diarrhoea, abdominal pain, dilated pupils and occasionally convulsions (Fernando and Widyaratna, 1989; Samal *et al*., 1992).

A detoxification of this seed will be required before the seed can find usability as a possible protein source in animal feed formulation. Several attempts have been made in the past to detoxify the seed cake with little success. Some of the methods that have previously been employed include dry-heating, autoclaving, fermentation etc (Odetokun *et al*., 1999; Taiwo *et al*., 2004).

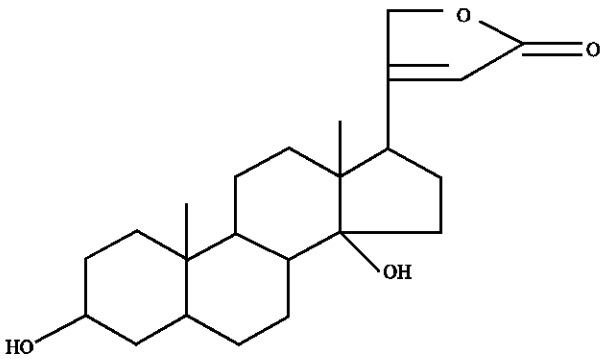
Finnigan and Lewis (1985) have shown that oilseed protein quality may be improved by alcoholic extraction of undesirable components to leave a protein concentrate. Van Megan (1983) had also reported ethanolic extraction of defatted rapeseed to be successful in detoxification. We also recently reported the reduction in the glycoside content of thevetia following alcoholic extraction of the defatted meal (Oluwaniyi *et al*., 2007). This current study therefore aims at investigating the most favourable condition for the alcoholic extraction of the glycosides of thevetia seed meal with respect to the time taken for extraction, quantity and composition of extractant used, reduction in the content of the glycosides as well as the quantity of protein in the residual meal.

MATERIALS AND METHODS

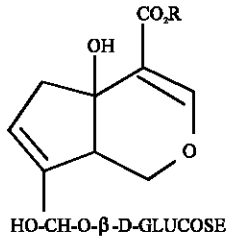
Matured fruits of thevetia plant were collected from a location in Ilorin, Kwara State, Nigeria, by direct plucking of matured (black) fruits from plants and by picking those that have fallen off plants. The fruits were cracked to remove the hard pericarp and mesocarp and the soft seeds crushed into a paste. The paste was defatted first by mechanical pressing, followed by solvent extraction using pre-distilled n-hexane.

General extraction methods: Alcoholic extraction of thevetia seed meal was studied as a function of time, solvent to meal ratio and solvent composition as described by Finnigan and Lewis (1988). All extractions were performed at ambient temperature. A sample of Thevetia Seed Meal (TSM) was placed in a flask and

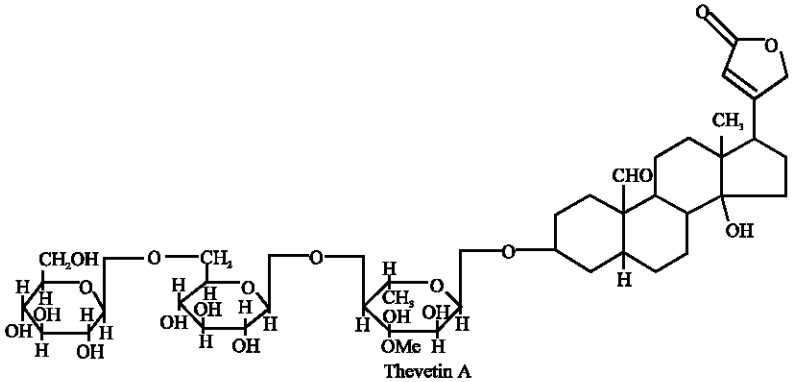
Corresponding Author: Omolara O. Oluwaniyi, Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria



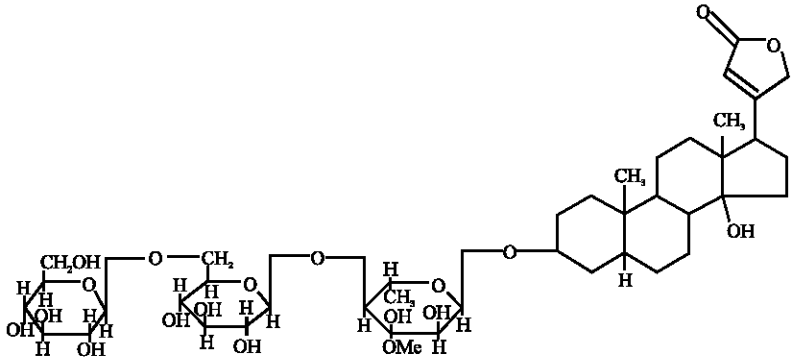
Digitoxigenin (Thevetigenin)



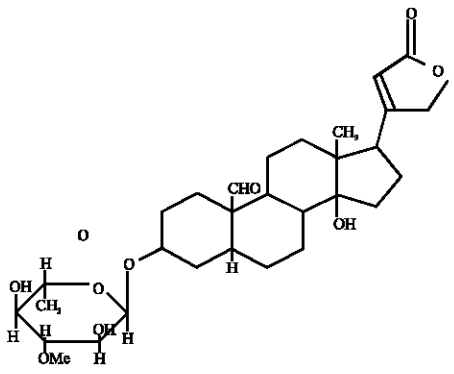
(Theveside: R = H; Theveridoside: R = Me)



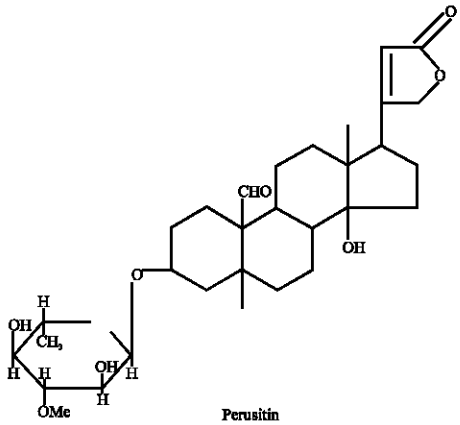
Thevetin A



Thevetin B (Cerberoside)



Peruvoside



Perusitin

solvent was added to give the appropriate solvent to meal ratio. Extraction at 45 min was achieved using a magnetic stirrer while the others were covered and left.

Alcoholic extraction was performed using aqueous 8: 2 ethanol/methanol mixture. Solvent to meal ratios of 10: 1; 15: 1 and 20: 1 were investigated.

Effect of time and solvent to meal ratio: Ten g each of TSM was extracted with 80% (v/v) aqueous alcoholic mixture at solvent to meal ratios of 10: 1; 15: 1 and 20: 1. The extractability of each solvent to meal ratio was also investigated as a function of time. Each was extracted for 40 min, 24, 48 and 72 h. At the end of the experimental time, the samples in the flasks were filtered, the solvent squeezed out and the residue air-dried.

Effect of solvent composition: TSM was extracted with 50, 60, 70, 80, 90 and 100% (v/v) aqueous alcohol mixture at a solvent to meal ratio of 15: 1 for 72h after which each was filtered, squeezed and dried.

Moisture and protein content determination: The moisture content of each sample (treated and untreated) was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105°C (AOAC, 1984). The nitrogen content was determined by the Kjeldahl digestion method (Kjeldahl, 1883), using 2.0 g samples. The crude protein was calculated by multiplying the nitrogen content with a factor of 6.25.

Glycoside content determination: The quantity of cardiac glycosides in the raw and treated samples was evaluated using Baljet's reagent (95 mL aqueous picric acid + 5 mL 10% aqueous NaOH) as described by El-Olemy *et al.* (1994). One gram of each sample was extracted by soaking for 2 h with 10 mL of 70% alcohol and filtered. The extracts were then purified using lead acetate and Na_2HPO_4 solutions before the addition of freshly prepared Baljet's reagent. The intensity (absorbance) of the colour produced was then measured using a spectrophotometer at 495 nm. A blank was carried out at the same time using distilled water and Baljet's reagent. The intensity of the colour produced is proportional to the concentration of the glycoside.

RESULTS AND DISCUSSION

Analysis of thevetia seed meal showed that the untreated meal contains 11.83% moisture, 54.4 g kg^{-1} (or 5.44 g %) total cardiac glycosides and 542.5g kg^{-1} (54.25%) of crude protein.

Effect of time and solvent to meal ratio: The result of the effect of time and solvent to meal ratio on the extractability of the toxins of thevetia meal (which are mainly cardiac glycosides) is illustrated in Fig. 1. The glycoside content was reduced by 91.5% with 10: 1 alcohol to TSM, 94.1% with 15: 1 and by 95.6% with 20: 1 alcohol to meal after 72 h. The protein content as presented in Table 1 also shows a gradual increase in the protein content of the residual protein meal after treatment with the different quantities of solvent. Extracting with 15:1 solvent to meal ratio appears to give the best result as far as conserving the protein is concerned. The protein contents ranged between 68.70-69.09%, whereas treating with either a higher or lower proportion of solvent resulted in a decrease in the protein content. The length of time used for treating the meal does not seem to have an effect on the protein content of the product.

Effect of solvent composition: Figure 2 shows the effect of varying the concentration of aqueous alcohol used in the extraction of the cardiac glycosides. The results show that extracting with 70-90% aqueous alcohol gave the meal with the lowest glycoside content whereas using 100% alcohol resulted in the poorest extraction of the glycosides. Preliminary works done in our laboratory also

Table 1: Moisture and protein content of treated thevetia seed meal as a function of time and solvent to meal ratios

	10: 1 Solvent to meal ratio		15: 1 solvent to meal ratio		20: 1 solvent to meal ratio	
Time (h)	Moisture	Protein	Moisture	Protein	Moisture	Protein
00.75	17.27	64.92	12.10	69.09	15.80	65.97
24.00	16.95	65.76	11.13	68.70	15.63	66.05
48.00	16.09	63.45	12.80	68.91	15.80	66.34
72.00	18.00	65.77	12.89	68.88	15.46	65.91

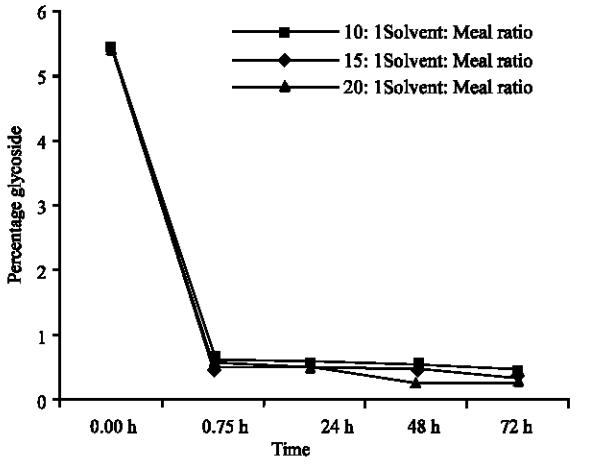


Fig 1: Extraction of cardiac glycosides from thevetia seed meal by 80% aqueous ethanol/methanol mixture

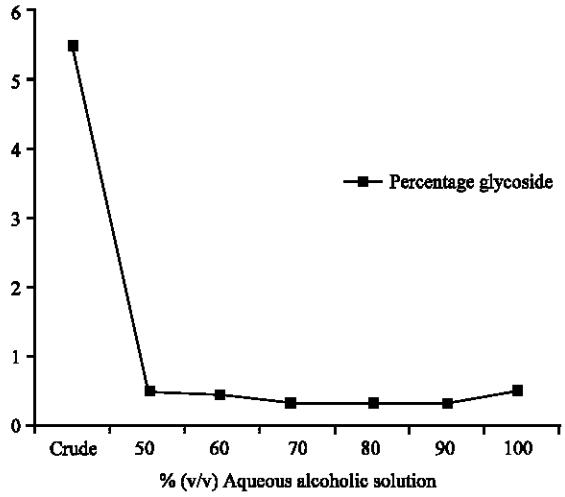


Fig. 2: Extraction of thevetia glycosides using varying concentrations of aqueous alcohol at 15: 1 solvent to meal ratio for 72 h

Table 2: Effect of varying concentrations of aqueous alcohol (15: 1 solvent to meal ratio) on the moisture and protein content of treated thevetia seed meal

Concentration of aqueous alcoholic mixture (%)	15: 1 Solvent to meal ratio soaked for 72 h	
	Moisture	Protein
50	25.89	62.13
60	22.82	65.35
70	15.35	67.98
80	13.39	68.95
90	14.44	65.50
100	15.87	62.35

The effect of varying the solvent composition on the protein content (Table 2) also follows a similar trend with the effect on glycoside content. Extraction with 80 and 70% aq. alcohol resulted in the highest protein content in the residual meal while the least protein content was obtained from the meal extracted with 50 and 100% aq. alcohol.

It was also observed that the protein concentrate remaining after treatment with 50% alcohol was very susceptible to spoilage, which is attributed to the high percentage of water in the solvent, thus confirming further that a solvent composition that is less than 70% or greater than 90% alcohol results in a poor extraction of the glycoside as well as a loss of the crude protein.

The final products, irrespective of the concentration and ratio of solvent or duration of treatment, still contain a residual amount of cardiac glycoside although quite minute. It is however possible that double extraction of the meal may result in a meal with undetectable cardiac glycoside content.

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