

Proximate and Cardiac Glycoside Composition of *Thevetia* (*Thevetia neriifolia*. JUSS) Seed as Affected by Soaking in Water, Brine and Ethanol

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Abstract

Effects of soaking using water, ethanol and brine on proximate and cardiac glycoside composition of *Thevetia neriifolia* seed (TS) were undertaken. Each 100g portion of TS was soaked in either water or ethanol for 24 hours or in 2.5, 5.0 and 7.5% brine solution for 2, 3 or 4 hours. The samples were then either sun-dried for 3 days or toasted after soaking and thereafter analyzed for chemical and residual glycoside composition. There were significant ($P<0.05$) variations in the composition of ash which ranged from 13.0% in the raw meal (T_R) to 11.04% in T_4 (TS soaked in fine-grade ethanol and sun-dried). Obtained values for nitrogen free extracts was between 12.56% in T_1 (TS soaked in water and toasted) and 28.31% in T_4 . These values varied significantly ($P<0.05$) among the groups except in T_2 (TS soaked in water and sun-dried) (19.47%) and T_3 (TS soaked in fine-grade ethanol and toasted) (20.09%) where the values were similar ($P>0.05$). Toasting significantly ($P<0.05$) reduced the crude protein (CP) in water and ethanol treated samples from 19.35% in raw sample to 18.14 and 18.43%, respectively. The CP increased significantly in sun-dried water (22.1%) and brine treated (23.1%) samples. Soaking in 7.5% brine for 3 hours significantly ($P<0.05$) reduced glycoside content from 4.7% to 0.07% (98.51% reduction of glycoside).

Keywords: residual cardiac glycoside content, thevetia seed proximate composition, potential oilseed cake, feedstuff detoxification, thevetia seed meal.

1. Introduction

Animal nutritionist have not relented in their quest to explore and establish the potential of other numerous and lesser known plant and animal products as feed ingredients in livestock industry. Nutritional properties of plants and animal products such as pigeon pea, lima beans, cottonseed, sunflower, locust bean, cassava peel, feather meal, and blood meal have been elucidated through meaningful research to reduce or totally remove the toxins and anti-metabolites in them thereby making the feedstuff more important particularly as ingredients in livestock feed formulation. (Akande, 2009; Akande et al., 2010; Akintunde et al., 2010).

The plant *Thevetia neriifolia* (Juss) grows wild in the humid zone of West Africa including Nigeria. Also known as yellow oleander, milk bush, trumpets flower or best still tree that belongs to the family apocynaceae. It is a perennial shrub, 6m in height, native of central and tropical South America and named after the French monk F. Andre Thevet (1502-1592) (Burrkilol, 1985). It is an ornamental plant propagated for its flowers and used mainly as hedge plant. Cake contains 30-53% protein (Steintz, 1962; Ibiyemi et al., 2002; Oluwaniyi et al., 2007). The seed contains 60-65% oil while the defatted cake contains 30-53% protein (Steintz, 1962; Ibiyemi et al., 2002; Oluwaniyi et al., 2007)

Documentation of the nutritive potentials of TS has been very scanty. Atteh et al. (1990) investigated the replacement value of *Thevetia* oil for palm oil in broiler chicks' diets and recommended further processing of the oil prior to its being used effectively as a feed ingredient. Oderinde and Oladimeji (1990) analyzed the composition of the oil and indicated a total unsaturated fatty acids of 75.4% of which oleic acid was 31.46% and linoleic acid 43.90%. It has the characteristic of good edible oil, if the bitterness could be removed by alumina and alkali treatment. Various ways of eliminating toxic components and improving the nutritive value of feedstuff like fermentation, ensiling, sun drying, oven drying, boiling, soaking in water or other solvents, autoclaving and so on

depending on the nature of the substrate have been documented (Verma, 2006; Olomu, 2011; Vasudevan et al., 2011).

Recent efforts directed towards detoxification of the seed for inclusion into the diets of one or two classes of livestock (Ibiyemi & Oluwaniyi, 2003; Oluwaniyi et al., 2007) met with some degree of success. The practicality of the methods of detoxification employed (in terms of cost and expertise) among the targeted clientele (local poultry farmers) left much to be desired. In a bid to detoxify *Thevetia* seeds, a method of detoxification that would significantly increase the cost of production would be undesirable, as was the case with the alcoholic extraction (Oluwaniyi et al., 2007). In addition, the process must be practicable in terms of technicality and handling by local farmers that would be the main user of the method.

The present endeavor was aimed at investigating the effects of soaking using water, ethanol and brine on the proximate and cardiac glycoside composition of *Thevetia neriifolia* seed.

2. Materials and Methods

Thevetia neriifolia fruits that have fallen off plants and turned black due to decomposition of the pericarp were handpicked and manually cracked to remove the soft seeds. The picking and cracking of fruits were done concurrently within the duration of a week in Ado-odo / Ota Local Government area of Ogun State, Nigeria. The choice of fruits that have fallen off plant was to be sure that only matured fruits were selected.

Four portions of TS weighing 100g each were either soaked in water or fine grade ethanol for three days (72 hours) as described by Oluwaniyi and Ibiyemi (2007). The extracts were then drained and the seeds were either sun-dried or toasted for subsequent analysis residual glycoside content.

Other portions weighing 100 g each were soaked in brine solution of three different graded concentrations of 2.5, 5.0 and 7.5% for 2, 3 and 4 hours concurrently at room temperature with intermittent stirring. The extract were thereafter drained and the residues sun-dried until friable and then stored for analyses. A portion of raw seeds weighing 100g was also milled and stored for analyses.

2.1 Chemical Analyses

Proximate analysis of all samples was determined according to AOAC (2000). The cardiac glycosides in raw and treated sampled of the extract was evaluated using a modification of El-Olemy et al. (1994). The gross energy was calculated based in the procedure of Ekanayake et al. (1999) and the metabolizable energy value of the samples was estimated according to the procedure of Pauzenga (1985).

2.2 Statistical Analysis

Data were analyzed using SAS (1999) to estimate the variances and significant variations compared using Duncan multiple range test of the same software.

3. Results

The proximate composition of raw, water and ethanol-detoxified TS is summarized in Table 1. Dry matter content significantly ($P < 0.05$) increased in the toasted samples (T_1 and T_3) from 88.60% in raw sample (T_R) to 90.40% in T_1 and 91.02% in T_3 . The CP content decreased significantly ($P < 0.05$) from 19.35% in raw sample to 18.14% in T_1 (TS soaked in water and toasted) and 18.43% in T_3 , but T_2 (TS soaked in water and sun-dried) had significantly ($P < 0.05$) higher value of 22.11%.

A similar trend was observed for crude fibre (CF), with a drop from 6.56% in T_R to 5.62% in T_1 . However, variations observed for CF in T_2 , T_3 and T_4 were not significant ($P > 0.05$). The lowest ($P < 0.05$) value of 35.20% for ether extract was observed for T_3 (TS soaked in fine-grade ethanol and toasted) but this value was not significantly ($P > 0.05$) different from the value observed for T_4 (TS soaked in fine-grade ethanol and sun-dried). However, the variations observed for ether extract for T_1 , T_2 , and T_3 were not significant ($P > 0.05$). There were significant ($P < 0.05$) variations in the composition of ash. Mean values obtained ranged from 13.0% in the raw meal (T_R) to 11.04% in T_4 (TS soaked in fine-grade ethanol and sun-dried). Treatments 1 and 2 (T_1 and T_2) however, were statistically similar. Values obtained for nitrogen free extracts ranged from 12.56% in T_1 to 28.31% in T_4 . These values varied significantly ($P < 0.05$) among the groups except in T_2 (19.47%) and T_3 (20.09%) where variations were not significant ($P > 0.05$). There was a general decrease in gross energy from 5.94 kcal/g DM in the raw sample T_R to 5.02 kcal/g DM in T_4 but the apparent differences observed between T_R and T_2 were not significant ($P > 0.05$).

Results obtained for the level of glycoside in treated and untreated TS were shown in Table 2. The level of glycoside significantly reduced from 47.2 g/kg in the raw sample (T_R) to 0.08 g/kg in T_3 (TS soaked in fine-grade ethanol and toasted) which was about 98.3% reduction in glycoside composition. The reduction in glycoside

composition in water treated samples T₁ (TS soaked in water and toasted) and T₂ (TS soaked in water and sun-dried) was 41.28% and 45.74% respectively. Differences observed in values for water treated and ethanol treated samples were significant (P<0.05). The values observed for ethanol treated samples T₃ (TS soaked in fine-grade ethanol and toasted) and T₄ (TS soaked in fine-grade ethanol and sun-dried) were 0.08 and 0.09 about 98.30% and 98.09% respectively.

Table 1. Proximate composition (g/100g) of raw and detoxified *Thevetia* seed

Nutrients	Treatments					SEM
	T _R	T ₁	T ₂	T ₃	T ₄	
Crude protein (%)	19.35 ^b	18.14 ^d	22.11 ^a	18.43 ^d	18.86 ^c	0.08
Crude fibre (%)	6.56 ^a	5.62 ^c	6.16 ^b	6.04 ^b	6.12 ^b	0.06
Ether extract (%)	44.01 ^a	42.02 ^a	40.12 ^a	35.20 ^b	35.67 ^b	1.21
Ash (%)	13.00 ^a	12.06 ^b	12.14 ^b	11.26 ^c	11.04 ^c	0.08
Nitrogen free extract (%)	17.08 ^c	12.56 ^d	19.47 ^b	20.09 ^b	28.31 ^a	0.17
Dry matter (%)	88.60 ^b	90.40 ^a	87.11 ^c	91.02 ^a	86.22 ^d	0.23
Gross energy (kcal/g DM)	5.94 ^a	5.20 ^b	5.89 ^a	5.11 ^b	5.02 ^b	0.10

a,b,c,d- Means along the same row with any identical superscripts are not significantly different (P>0.05); T_R = untreated TS; T₁ = *Thevetia* seed (TS) soaked in water and toasted; T₂ = TS soaked in water and sun-dried; T₃ = TS soaked in fine-grade ethanol and toasted; T₄ = TS soaked in fine-grade ethanol and sun-dried. SEM = Standard Error of Means; DM = Dry matter.

Table 2. Glycoside content of raw and treated *Thevetia* seed

Sample	Total glycoside (%)	Reduction in glycoside level (%)
T _R	4.70 ^a	0.00 ^c
T ₁	2.76 ^b	41.28 ^b
T ₂	2.55 ^b	45.74 ^b
T ₃	0.08 ^c	98.30 ^a
T ₄	0.09 ^c	98.09 ^a
SEM	0.56	3.45

Values were means of three determinations. a, b, c means along the same row with any identical superscript are not significantly different (p>0.05). SEM = Standard error of means.

The proximate composition of brine-treated *Thevetia* seed is shown in Table 3. There were significant (p<0.05) differences in the mean values observed for crude protein. Crude protein, significantly (p<0.05) increased in all brine-treated samples. The observed mean values for crude protein ranged from 23.20% in B₄ (TS soaked in 5.0% brine for 4 hours) to 19.36% in the raw sample T_R. The apparent variations observed for brine-treated samples A₃ (TS soaked in 2.5% brine for 3 hours), A₄ (TS soaked in 2.5% brine for 4 hours), B₂ (TS soaked in 5.0% brine for 2 hours), B₃ (TS soaked in 5.0% brine for 3 hours), B₄ (TS soaked in 5.0% brine for 4 hours), C₂ (TS soaked in 7.5% brine for 2 hours), C₃ (TS soaked in 7.5% brine for 3 hours) and C₄ (TS soaked in 7.5% brine for 4 hours) were not significant (p>0.05).

Observed values for crude fibre ranged from 6.56% in raw sample T_R to 5.25% in B₄ (TS soaked in 5.0% brine for 4 hours). These variations were however not significant (p>0.05). Composition of ether extractives varied significantly (p<0.05) from 44.01% in raw sample to 32.75% in A₄ (TS soaked in 2.5% brine for 4 hours). The variations observed in A₄ (TS soaked in 2.5% brine for 4 hours), B₄ and C₄ were not significant (p>0.05). Similarly, the variations observed for A₂, A₃, B₂, B₃, C₂ and C₃ were not significant (p>0.05). Observed values for ash ranged from 15.20% in C₄ (TS soaked in 7.5% brine for 4 hours) to 13.00% in raw sample T_R. Apparent variations observed in values for A₃, A₄, B₂, B₃ and B₄ were not significant (P>0.05). Similarly, the apparent variations observed in values for C₂, C₃ and C₄ were not significant (p>0.05), but significantly (P<0.05) higher than values

observed for A₂ (TS soaked in 2.5% brine for 2 hours) and TR (raw sample) which are 13.89% and 13.00% respectively.

Observed values for nitrogen free extractives ranged from 24.35% in B₄ (TS soaked in 5.0% brine for 4 hours) to 15.56% in C₂ (TS soaked in 7.5% brine for 2 hours). Values observed for A₄, B₄ and C₄ (24.04%, 24.35% and 23.13% respectively) were not significantly ($P>0.05$) different among these groups but significantly ($P<0.05$) higher than those observed for other treatments.

Table 3. Proximate composition of brine-treated Thevetia seeds

Nutrients (%)	A ₂	A ₃	A ₄	B ₂	B ₃	B ₄	C ₂	C ₃	C ₄	Raw	SEM
Crude protein	20.1 ^b	23.1 ^a	23.2 ^a	23.1 ^a	23.1 ^a	23.2 ^a	23.0 ^a	23.1 ^a	23.2 ^a	19.4 ^b	2.5
Crude fibre	6.1	5.7	5.8	5.8	5.6	5.3	5.9	5.7	5.7	6.6	2.1
Ether extract	42.9 ^b	38.4 ^b	32.8 ^c	40.7 ^b	38.4 ^b	32.8 ^c	40.6 ^b	38.4 ^b	32.8 ^c	44.0 ^a	1.1
Ash	13.9 ^c	14.3 ^b	14.3 ^b	14.4 ^b	14.4 ^b	14.4 ^b	15.0 ^a	15.2 ^a	15.2 ^a	13.0 ^c	2.1
NFE	17.0 ^b	18.7 ^b	24.0 ^a	16.1 ^c	18.5 ^b	24.4 ^a	15.6 ^c	17.6 ^b	23.1 ^a	17.1 ^b	2.1
Dry matter	88.8	88.8	88.9	88.8	88.9	88.9	88.9	88.9	88.9	88.6	1.1
Moisture	11.3	11.2	11.1	11.2	11.1	11.1	11.2	11.1	11.0	11.4	0.9

a, b, c, d = means along the same row with identical superscripts were not significantly different ($p>0.05$); Raw = Untreated Thevetia Seed, A₂ = Solution A + 100g Thevetia Seed soaked for 2 hours; A₃ = Solution A + 100g Thevetia Seed soaked for 3 hours, A₄ = Solution A + 100g Thevetia Seed soaked for 4 hours; B₂ = Solution B + 100g Thevetia Seed soaked for 2 hours, B₃ = Solution B + 100g Thevetia Seed soaked for 3 hours; B₄ = Solution B + 100g Thevetia Seed soaked for 4 hours, C₂ = Solution C + 100g Thevetia Seed soaked for 2 hours; C₃ = Solution C + 100g Thevetia Seed soaked for 3 hours, C₄ = Solution C + 100g Thevetia Seed soaked for 4 hours; A = 2.5% Sodium Chloride Solution, B = 5.0% Sodium Chloride Solution, C = 7.5% Sodium Chloride Solution. Figures 2, 3 and 4 stood for time in hours. NFE= Nitrogen Free Extract.

SEM = Standard error of means.

TSM = Thevetia seed meal.

3.1 Glycoside Level of Brine-Treated Thevetia Seeds

The level of cardiac glycoside (measured as digitoxin) in the samples is summarised in Table 4. Glycoside content of brine-treated samples significantly ($P<0.05$) reduced from 4.70% in the raw meal to 0.07% in C₃ (TS soaked in 7.5% brine for 3 hours). There was also a marked reduction in glycoside level in samples A₃ (TS soaked in 2.5% brine for 3 hours) and B₂ (TS soaked in 5.0% brine for 2 hours), (0.21% and 0.41% respectively). The level of glycoside reduction observed for samples A₃, B₂ and C₃ were not significant but the value observed for C₃ was the lowest, even below what was observed for the ethanol treated samples T₃ (TS soaked in fine-grade ethanol and toasted) and T₄ (TS soaked in fine-grade ethanol and sun-dried). Percentage reduction in glycoside composition ranged from 63.40% in C₄ (TS soaked in 7.5% brine for 4 hours) to 98.51% in C₃ (TS soaked in 7.5% brine for 3 hours). The highest value of 98.51% observed for C₃ was closely followed by 95.53% and 91.28% for A₃ and B₂ respectively. The observed value for percentage glycoside significantly ($P<0.05$) dropped from 75.53% in C₂ (TS soaked in 7.5% brine for 2 hours) to 63.40% in C₄, however values observed for B₄ (TS soaked in 5.0% brine for 4 hours) and C₂ were not significantly different ($P>0.05$).

Table 4. Glycoside Level of brine-detoxified *Thevetia* seed

Sample	Glycoside level (%)	Glycoside Reduction (%)
Raw	4.70 ^a	0.00
A ₂	0.48 ^d	89.79
A ₃	0.21 ^f	95.53
A ₄	0.66 ^d	85.96
B ₂	0.41 ^f	91.28
B ₃	0.68 ^d	85.53
B ₄	1.09 ^c	76.81
C ₂	1.15 ^c	75.53
C ₃	0.07 ^f	98.51
C ₄	1.72 ^b	63.40

a, b, c, d = means along the same row with any identical superscript are not significantly different ($p > 0.05$); Raw = Untreated *Thevetia* seed, A₂ = Solution A + 100g *Thevetia* Seed soaked for 2 hours, A₃ = Solution A + 100g *Thevetia* Seed soaked for 3 hours, A₄ = Solution A + 100g *Thevetia* Seed soaked for 4 hours, B₂ = Solution B + 100g *Thevetia* Seed soaked for 2 hours; B₃ = Solution B + 100g *Thevetia* Seed soaked for 3 hours; B₄ = Solution B + 100g *Thevetia* Seed soaked for 4 hours; C₂ = Solution C + 100g *Thevetia* seed for 2 hours; C₃ = Solution C + 100g *Thevetia* Seed soaked for 3 hours; C₄ = Solution C + 100g *Thevetia* seed soaked for 4 hours. The alphabet stands for the concentration of brine and the number stands for time in hours. A = 2.5% Sodium Chloride solution; B = 5.0% Sodium Chloride solution; C = 7.5% Sodium Chloride solution.

3.2 Proximate Composition of Defatted TSM

Table 5 shows the summary of the proximate composition of TSM after extracting oil from it. The two methods of oil extraction employed gave significant variations ($P > 0.05$) in the values recorded for all the nutrient components except for CF, ash and dry matter. Crude protein significantly ($P < 0.05$) increased from 27.20%, with hydraulic extraction to 44.8%, with expeller extraction. Ether extract significantly reduced from 28.5% with hydraulic extraction to 5.1% with expeller. The observed variations in the mean value for CF, were not significant ($P > 0.05$). CF increased from 13.8% with hydraulic extraction to 14.2% with expeller. The composition of ash dropped ($P > 0.05$) from 8.0% with hydraulic extraction to 7.7% with expeller. Percentage NFE significantly ($P < 0.05$) increased from 18.5% with hydraulic extraction to 25.3% with expeller. The method of oil extraction had no significant ($P > 0.05$) effect on the percentage DM.

Table 5. Proximate Composition (g/100g DM) of Defatted *Thevetia* Seed Meal

Nutrient (%)	Hydraulic	Expeller	SEM
Crude protein	27.2 ^b	44.8 ^a	2.16
Ether extract	28.5 ^a	5.1 ^b	2.05
Crude fibre	13.8	14.2	0.34
Ash	8.0	7.7	0.41
NFE	18.5 ^b	25.3 ^a	1.94
Dry matter	96.0	97.2	3.85

NFE= Nitrogen Free Extract; SEM = Standard error of means; a, b, c, d = means along the same row with any identical superscript are not significantly different ($p > 0.05$).

4. Discussion

The processing methods used in this study resulted in a decreased crude protein content of the treated samples except for T₂ and the brine-treated samples where CP significantly increased from 19.35% in the raw sample to 22.11% in T₂ (soaked in water and sun-dried) and 23.2% in C₃ (7.5% brine). The changes observed especially in

sun-cured samples must have been due to activities of microorganisms during soaking (fermentation) and sun-curing which perhaps resulted in biochemical changes and significant modification of food quality as earlier reported (Campbell & Laherrere, 1998). This also revealed that fermentation could improve the nutritive qualities of food in both plant and animal tissues. The decrease in percentage CP for the toasted samples could be attributed to the negative impact of heat on proteins as reported elsewhere. (Vasudevan et al., 2011). The percentage composition of nutrients especially CP recorded in this study was below the values reported by Oluwaniyi et al. (2007). This could be due to differences in the location of the source of *Thevetia* seeds and methods of detoxification employed. The reduction in the level of cardiac glycoside measured as percentage digitoxin in all treatments agreed with other reports [Chen & Chen, 1933; Bai & Koshy, 1999] that glycosides of *Thevetia* were water and alcohol soluble as well those glycosides were more soluble in ethanol than in water. In the present study, water-extracted TS recorded 45.74% reduction in the level of glycoside compared to the raw sample, while ethanol extraction was 98.30%. The undesirable cost associated with the use of ethanol in the first trial gave rise to the use of brine as extracting solvent. Brine-treatment recorded a higher percentage (98.51%) reduction of glycoside in C₃ (7.5% brine soaked for three hours) compared to ethanol-treatment. This conformed to the report of Desai (2000) that cardiac glycosides have affinity for Na⁺ and/or K⁺ contained in brine. The reversal of the reaction after three hours could be attributed to lack of Na⁺ ions in the solution to combine with thevetin in the presence of ATPase resulting in a relapse of the reaction. Oil extraction increased the percentage CP of detoxified TSM. The CP of full-fat TSM increased from 23.12% in C₃ to 27.2 and 44.8% in the hydraulic and expeller-extracted samples respectively. This agreed with other earlier reports [Finnigan and Lewis, 1988; Lutz and Pryztulski, 2008] in which CP of oilseeds tends to increase when the oil content was reduced considerably. The differences observed in the nutrient composition of the cake from the two methods employed could be as a result of the fact that the proteins got more concentrated as more oil was extracted by the expeller method. This therefore suggested the superiority of electrical power over hydraulic press.

5. Conclusion

Thevetia seed when processed has favourable nutritional profile utilizable in the diets of farm animals. Soaking in brine reduced cardiac glycoside content more effectively when compared with either water or alcohol. Thus, the use of brine was relatively much easier and less expensive compared to ethanol.

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