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Enhancing the Chemical Composition of *Balanites aegyptiaca* Seeds through Ethanol Extraction for Use as a Protein Source in Feed Formulation

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Abstract

Over dependence on conventional feedstuff has contributed to a continuous rise in the prices of feeds. *Balanites aegyptiaca* is a perennial tree and its seeds, if properly processed could be a cheaper alternative source of protein for livestock feed formulation. In this study, *Balanites aegyptiaca* seeds were subjected to ethanol extraction, to examine the effect on the nutrient, phytochemical, organoleptic as well as textural properties of the seed kernel. The result showed a significant (P<0.05) decrease in lipid content from 37.11% to 9.98% and a significant (P<0.05) increase in protein content from 31.73% to 37.68%. There was a reduction in the level of tannin from 0.0690 to 0.0043 mg/100g, phytic acid 108.65 to 36.65 mg/100g and oxalate 30.01 to 15.03 mg/100g. The results show that ethanol extraction is an effective processing technique for enhancing the suitability of *Balanites aegyptiaca* seed kernel as an alternative protein source in animal feeding.

Keywords: *Balanites aegyptiaca*, extraction, chemical composition

1. Introduction

The continuous rise in the cost of animal products has partly been blamed on the over dependence on conventional feedstuff for feed manufacture (Oboh, 2006; Ojewola, & Udom, 2005). This is because soybeans and groundnuts which are the conventional sources of protein locally used in animal feed formulation (Ghadge et al., 2009) are also used as food by humans (Singh & Singh, 1991). A combination of factors such as population growth and urbanization has resulted in a constant increase in the price of such food items as soybean and groundnut, thus contributing to an increased cost of feed production and the market price of animal protein (Oboh, 2006).

One of the ways to increase the protein supply for feed formulation is to make more plant proteins available for human consumption and develop the production of proteins from unconventional sources for animal feeding (Ayssiwede et al., 2011; Safaloah, 2006). There are a number of lesser known legumes and oil seeds in Nigeria who's nutritional and economic values could be determined and exploited for use as commercial feedstuff so as to reduce the dependence on conventional feed sources (Ayssiwede et al., 2011; Samuel et al., 1997).

One of these is the seed kernel of *Balanites aegyptiaca*, known as Desert date in English (Chothani & Vaghasiya, 2011). It is a desert plant that is widely distributed in the arid zone of Nigeria. It is also found in other African countries of Senegal and Sudan, as well as in India (Chothani & Vaghasiya, 2011, Ndoye et al., 2004, Pandey, 2005). The tree is remarkable because it is available during the dry season, when foliage is difficult to obtain and prices of conventional feedstuff are more on the rise, and is found in many kinds of habitat, as it grows in a variety of soil types (Chothani & Vaghasiya, 2011).

Available reports on the nutritional and antinutritional profile of *Balanites aegyptiaca* seed powder shows that the seed powder contains a relatively high amount of protein and lipid (Samuel et al., 1997). However, in addition to the nutrients, the seed contains high level of antinutritional factors; tannins, oxalate and phytic acid (Chothani & Vaghasiya, 2011 Samuel et al., 1997). Tannins are secondary plant metabolites that are rich in phenolic hydroxyl groups and have been implicated in the inhibition of non-heme iron absorption, by complex formation with iron in the gastro intestinal lumen (Brune et al., 1989). Tannins are also known to inhibit oxidation of alkaloids and morphine and form colored complexes with iron, thus reducing the bio-availability of

this important mineral (Brune et al., 1989, Tuntawiroon et al., 1991).

Oxalate or oxalic acid is a strong organic acid. This acid has the ability to form a strong bond with various minerals such as sodium, potassium, magnesium and calcium (Curhan, 1993). When this occurs, the compounds formed are usually referred to as oxalate salts. Some of these salts are practically insoluble in water; an example is calcium oxalate (Williams et al., 1990). If consumed by animals or humans in significant quantity, calcium oxalate has the propensity to precipitate (or solidify) in the kidneys or in the urinary tract to form calcium oxalate crystals (Curhan, 1993) leading to diseases such as kidney stone. Binding of oxalates to calcium renders such calcium metabolically unusable (Barten, 1993).

Phytate is a major storage form of phosphorus (Lorri, 1995). Phytate is normally found in form of complexes with polyvalent cations like iron, zinc, magnesium calcium and proteins. Phytate inhibits non heme absorption (Hurrell et al., 2003; Kappas & Galbraith, 1993), thus affecting cation bio-availability. Since there is limited information on the potential effects of various processing methods and techniques on the level of nutritional and antinutritional components present in *Balanites aegyptiaca*, it becomes necessary to examine how ethanol extraction as a processing technique, reduces these antinutritional components.

Because of the high protein content in *Balanites aegyptiaca* kernel (Samuel et al., 1997), it could be utilized as an animal feed source if its toxic substances are eliminated and its protein is extracted and concentrated. Identifying a suitable processing method will enhance the opportunities for a versatile utilization of *Balanites aegyptiaca* seed kernel meal as an alternative/additional and economical source of protein in livestock feeding. This research work seeks to determine the effect of ethanol extraction as a processing technique, on the chemical composition (proximate composition, antinutritional profile and phytonutrients) of *Balanites aegyptiaca* seed kernel meal. The choice of ethanol extraction for processing is because ethanol extraction has been reported as an effective method for processing oil seeds like Neem seed (James et al., 2007).

2. Materials and Methods

Balanites aegyptiaca seeds were obtained from the market and processed. The seed kernels were obtained by hard cracking and then sundried for 10 hours, modifying the drying technique adopted by Eshetu (2000) in drying a similar oil seed, *Nicotiana tabacum*. An oven was not used to dry at 70^0C because the oil seed kernel would begin to melt and boil. The sun dried seeds were ground to fine powder using a Fritsch laboratory grinding machine. The sample was divided into three portions for proximate analysis, phytochemical analysis and ethanol extraction. The extraction was repeated four times and all the analysis were performed in triplicates.

The powdered *Balanites aegyptiaca* kernel was extracted using the method of Roberts and Briggs (1963). Briefly 300 g of the seed powder was weighed into a 6 liter Pyrex round bottom flask. About 3000 ml of 60% ethanol was added to the seed powder to give the required meal: solvent ratio. After 3 hours, the slurry was distributed into 250 ml centrifuge bottles. The bottles were then placed in a 60^0C water bath and held for 30 minutes with periodic mixing. The mixtures were then centrifuged for 15 minutes at 2000 x g. The supernatant was decanted and replaced with fresh 60% ethanol at 60^0C. The extraction was repeated twice each time adjusting the pH to 7 using 20% NaOH. The extracted powder was heated at 108^0C for 30 minutes, followed by final drying in a convection oven at 50^0C for 12 hours.

2.1 Chemical Composition

The Proximate composition for both the raw and ethanol extracted *Balanites aegyptiaca* was determined according to the Standard Methods of the Association of Official Analytical Chemists (AOAC, 1990). The available carbohydrate content was calculated by difference.

Phytochemical screening for both the raw and ethanol extracted *Balanites aegyptiaca* was carried out according to the methods of Odebiyi and Sofowora (1978), Trease and Evans (1983) and Trease and Evans (1989).

2.2 Statistical Analysis

The results obtained are presented as Mean ± SEM and difference between treatments was analyzed by unpaired t- test using Graphpad version 4.03. P values < 0.05 were considered significant.

3. Results and Discussion

3.1 Results

The results for proximate composition of both the raw and ethanol extracted *Balanites aegyptiaca* seed kernel is shown in Table 1. The result shows that there was a significant (P<0.05) increase in the crude protein content of the extracted powder when compared to the raw powder. On the other hand, there was a significant (P<0.05) reduction in the lipid content of the extracted powder when compared to the raw powder. There was a significant

(P<0.05) increase in the percentage crude fibre, ash and phosphorus content of the extracted powder compared to the raw powder. There was no significant (P>0.05) difference in the Calcium content of the extracted and raw powders.

Table 1. (Percentage) Proximate composition of the raw and ethanol extracted seed powder of *Balanites aegyptiaca*

	Crude Protein	Crude Fibre	Lipids	Ash	Nitrogen Free Extract	Calcium	Phosphorus
Raw Powder	31.73	17.19	37.11	3.98	9.99	0.19	0.16
Ethanol Extracted Powder	37.68**	21.63**	9.98**	3.81**	26.90**	0.19	0.20**

** = P<0.001, SEM = 0.006.

Results of the antinutritional tests are shown on table 2. The result shows that the antinutritional factors; tannins, oxalate and phytic acid were significantly (P<0.05) reduced in the extracted powder when compared to the raw powder.

Table 2. Results of antinutritional factors analysis of the raw and ethanol extracted seed powder of *Balanites aegyptiaca*

	Tannin (mg/100g)	Phytic acid (mg/100g)	Oxalate (mg/100g)
Raw Powder	0.0690	108.65	30.00
Ethanol Extracted Powder	0.0043**	36.65**	15.00**

** = P<0.001, SEM = 0.006.

Results of the phytochemical tests are shown on Table 3. The phytochemical screening revealed the presence of saponins, cardiac glycosides and steroids in both the raw and extracted powders, though the positive reaction was stronger in the raw powder for the three parameters. Flavonoids and anthraquinones were absent in both the raw and ethanol extracted powders, while alkaloid was present in only the raw powder of *Balanites aegyptiaca* seed kernel.

Table 3. Results of phytochemical analysis of the raw and ethanol extracted seed powder of *Balanites aegyptiaca*

	Saponin	Cardiac glycoside	Steroid	Flavonoid	Anthraquinone	Alkaloid
Raw Powder	+++	++	++	-	-	+
Ethanol Extracted Powder	+	+	+	-	-	-

3.2 Discussion

The observed increase in the protein content of *Balanites aegyptiaca* seed powder after ethanol extraction is in agreement with James et al. 2007 who reported a significant increase in the protein content of Neem seed cake on ethanol extraction compared to water extraction. When compared to water extraction of *Balanites aegyptiaca*, ethanol extraction better enhanced the protein content as can be seen from the 37.68% obtained on ethanol extraction compared to 35.26 obtained by Samuel et al. 1997. The increase in protein content of the seed powder on extraction, compared to the raw powder can be attributed to hydrolytic reactions as well as removal of saponin and other compounds which bind to proteins (Manal et al., 2000).

The observed reduction in the saponin content of *Balanites aegyptiaca* powder on ethanol extraction is in agreement with Patil et al. 2010 who used various solvents (water, ethyl acetate, petroleum ether and methanol) for extraction of *Balanites aegyptiaca*, and reported methanol as a suitable solvent for *Balanites aegyptiaca* seed extraction compared to water as a solvent for extraction of oil seeds. The removal or a significant reduction of

saponin quantity in oil seeds by ethanol extraction has also been reported by other Researchers (Saetae & Suntornsuk, 2011; Abou-arab & Abou-salem, 2010). Saponin reduction on ethanol extraction enhances the nutritional value of *Balanites aegyptiaca* seed as a feed component. This is because saponins form complexes with proteins (Manal et al., 2000) thus reducing the nutritional quality of foods in which they are present.

There was also an observed reduction in the levels of tannins, phytic acid and oxalate after ethanol extraction. This is in agreement with the work of Saetae and Suntornsuk (2011) who reported a decrease in the level of anti-nutritional factors after carrying out ethanol extraction on *Jatropha curcas*, also an oil seed. Phytic acid inhibits amylase, pepsin and trypsin which are digestive enzymes (Dvorakova, 1998) necessary for metabolism. Phytic acid and tannins are known to complex proteins while oxalate complexes with minerals such as calcium thus reducing their bioavailability (Manal et al., 2000). Reduction in the levels of tannins, phytic acid and oxalates improves the nutritional quality of *Balanites aegyptiaca* seed powder.

Ethanol treatment was also noticed to have improved the flowability of the processed seed powder when compared to the unprocessed highly cohesive *Balanites aegyptiaca* seed powder, thereby enhancing the taste, texture and homogeneity of the powder with other ingredients during feed compounding. This may be attributed to the reduction in some of the phytochemicals and lipids (Juliano & Barbosa-Canovas, 2010).The presence of relatively higher deposits of lipid particles composition in the raw seed powder affects the texture and impedes the flowability of food powder (Juliano & Barbosa-Canovas, 2010).

4. Conclusion

Anti-nutritional factors in *Balanites aegyptiaca* can be efficiently reduced by extraction using ethanol as solvent to give a detoxified *Balanites aegyptiaca* seed powder which could be used as a protein source in feed formulation. The protein content in *Balanites aegyptiaca* seed powder after ethanol extraction was high (37.68%). It is recommended that *in vivo* studies using experimental animals fed with *Balanites aegyptiaca* as a source of protein and *in-vitro* digestibility studies should be carried out to ascertain the digestive and metabolic quality of the protein obtained from *Balanites aegyptiaca* seeds. Further studies should be carried out to determine the effect of feeding ethanol extracted *Balanites aegyptiaca* seed powder on biochemical blood parameters of the test animals fed with *Balanites aegyptiaca* seed powder.

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