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Evaluation of Antinutrient and Toxic Constituents in Shell of *Vitellaria paradoxa* Fruit Obtained from Different Geopolitical Zones of Niger State.

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ABSTRACT
The environmental factors in the areas where plants grow have an impact on their chemical makeup. This study evaluated the concentrations of saponin, tannin, oxalate, phytate, cyanide, and nitrate in the shell of *Vitellaria paradoxa* fruits collected across three geopolitical zones in Niger State, Nigeria. Fresh fruits were obtained from Zone A (Mokwa, Bida, and Lapai), Zone B (Suleja, Kuta, and Paiko), and Zone C (Wishishi, Kagara, and Kontogora). Antinutrients: Saponin, tannin, and nitrate concentrations were determined via spectrophotometric methods, while phytate and oxalate were analyzed using titrimetric methods. Cyanide levels were estimated through the alkaline picrate method, with data subjected to ANOVA and Duncan's Multiple Range Test. The results of this present research, revealed that the fruit shells from Zone A had considerably greater amounts ($p < 0.05$) of tannin (3.05 ± 0.51 mg/g), oxalate (13.55 ± 6.80 mg/g), phytate (1.58 ± 0.06 g/100g), cyanide (287.98 ± 140.91 mg/kg), and nitrate (743.92 ± 101.81 mg/kg) than the other zones. Zone B has the highest concentration of saponins (33.72 ± 7.44 mg/g). Oxalate and cyanide levels were higher than allowed thresholds, however saponin, tannin, phytate, and nitrate quantities were all below allowable safety standards. According to the findings, the bioaccumulation of these compounds by *V. paradoxa* shells varies according to their geopolitical origin. As a result, their direct ingestion or usage in feed formulations may cause toxicities, hence they must be properly processed before being used.

Keywords: V*itellaria paradoxa*, Antinutrients, Toxic substances, Fruit shell, Geopolitical zones, Saponin.

INTRODUCTION
Vitellaria paradoxa recently has gained importance as an economic crop because of the increasing demand for its butter, both domestically and internationally. Shea fruits serve as an important source of food for many organisms and other animals including birds, bats, elephants, sheep and pigs (Choungo *et al.*, 2021). The fruits also contribute to food security in forest areas of Nigeria, mainly for the rural poor, especially since their ripening happen together with the lean season of food production (Fobil, 2010). It plays a crucial role in its highly nutritional capacity, food security and availability during the period of low food availability. The production of shea nut and processing of the

butter serves as one of the main sources of employment for the rural women and children who are engaged in gathering it. Shea butter is important edible oil for the people of Southwestern and Northern Nigeria and most of Western Africa, being the most essential source of fatty acids and glycerol in their diet (Choungo *et al*., 2021). It is also useful in the pharmaceutical and cosmetic industries as an important raw material and a precursor for the manufacture of soaps, candles, and cosmetics (Ojo *et al*., 2021). Shea nuts are also more and more being exported for the utilization in the cosmetics industry as a constituent in lotions, makeup, baby ointments, hair care products and soaps (Moldovan *et al.,* 2021; Ojo *et al*., 2021).

Regardless of being increasingly substituted by commercially produced lotions in many communities, shea butter is traditionally used as a skin and hair moisturizer and for protection against the sun (Lin *et al*., 2017). The healing properties and effects of shea butter are thought to be somewhat attributable to the existence of allantoin, a substance known to trigger the growth of healthy tissue in ulcerous wounds (Ojo *et al*., 2021). It is also traditionally smeared on pregnant women during childbirth, on new born babies and adolescents because of its relieving effects (Blanks *et al*., 2023; Ojo *et al*., 2021). Though the nuts being an essential export commodity, its fruit pulp nevertheless is widely consumed. It also plays a major role in the local economy and diet as well as occupies an important period of time in the annual local dietary cycle (Choungo *et al*., 2021; EJESM, 2026). The edible part of the shea fruit is extremely nutritious and has important nutrients for the human body. The fruit provides important source of food for communities and rural poor particularly at the period of food shortages, hunger and other catastrophes. In addition to providing health benefits, the green fruit has a fleshy edible pulp, which contains protein, dietary fibre, carbohydrate, mineral elements (such as Fe, Ca, Mg, Na, and K). The pulp is also a rich some vitamins such as ascorbic acid, vitamins complex (Fobil, 2010).

Despite the importance of shea butter fruit, the *V. paradoxa* fruits like other fruits may contain some antinutrients and toxic substances that may cause health and nutritional problems. The chemical contents and compositions in plants are generally influenced by environmental factors where they are grown. This study is therefore designed to investigate the influence of geographical locations on the antinutrients and toxic substances content in the shell of shea fruit obtained from the three different geopolitical zones of Niger State, Nigeria.

MATERIALS AND METHODS

Study Area

Three Local Government Areas were selected from each of the three Zones in Niger State. Namely, Zone A (Lapai, Bida and Mokwa), Zone B (Suleja, Kuta and Paiko) and Zone C (Wishishi, Kagara and Kontogora). The fresh fruits of the *V. paradoxa* used for the analysis were obtained from three different locations in each of the Local Government Area and were identified as *V. Paradoxa* by a Botanist from the Botany Department, IBB University, Lapai, Niger State.

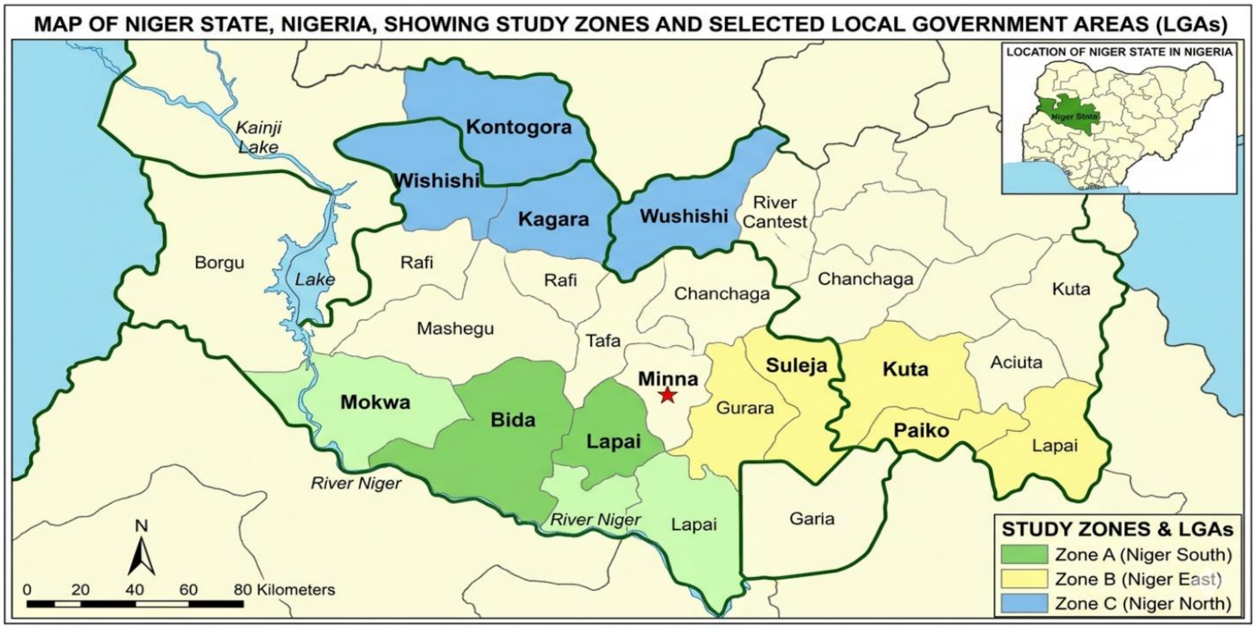

Fig 1: Map of the study Areas in Niger State, Nigeria.

Source of Samples

Vitellaria paradoxa fruits were obtained from three Local Government Areas from each Zone in Niger State in Middle belt, Nigeria. Shea fruit collected from Lapai, Bida and Mokwa represent zone A, Those from Suleja, Kuta and Paiko represent zone B while those collected from Wishishi, Kagara and Kontogora represent zone C. The fruits were kept in sterile polythene bags and taken to the laboratory of Department of Biochemistry, Ibrahim Badamasi University, Lapai. Niger State. The shells of the fruits were used for evaluation of the antinutrient constituents.

Analytical Procedure

Determination of Nitrate and Cyanide Contents

The nitrate content in the shell of *Vitellaria paradoxa* fruits was determined by the colorimetric method of Sjoberg and Alanko (1994). Finely ground samples were extracted with hot distilled water and the filtrate was passed through a cadmium reduction column to reduce nitrate to nitrite. The reduced extract was treated with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to develop a magenta-colored azo dye whose absorbance was measured spectrophotometrically at 540nm using Jenway 6305 UV/Visible spectrophotometer (110V).

The cyanide concentration was determined by the alkaline picrate method of Ikediobi et al. (1980). The samples were hydrolyzed to set free hydrogen cyanide (HCN) and the latter was absorbed in a 20% sodium carbonate solution. An aliquot of this extract was combined with an alkaline picrate reagent and incubated in a water bath at 95°C for 5 min. The intensity of the developed reddish-brown color was measured using Jenway 6305 UV/Visible spectrophotometer (110V).at 520 nm after cooling at room temperature.

Evaluation of Oxalate and Phytate Contents

The oxalate content was determined by the titrimetric method of AOAC (1984). The samples were digested with 1.5M H_2SO_4 and a controlled precipitation of oxalate as calcium oxalate was obtained by the addition of ammonium hydroxide and calcium chloride. The precipitate obtained was washed and dissolved in hot H_2SO_4 and titrated against a standard solution of 0.05M $KMnO_4$ until a faint persistent pink endpoint was observed.

Phytate content was determined according to the method of Maga (1983). Samples for phytic acid were taken with 0.2N HCl. The extract was precipitated as ferric phytate on treatment with standard iron (III) chloride solution. After centrifugation, the iron remaining in the supernatant was determined by titration against a standard potassium thiocyanate (KSCN) solution. The phytate concentration was calculated from the iron to phytate phosphorus ratio.

Determination of Saponin and Tannin Contents

The determination of concentrations of saponin and tannin was carried out using colorimetric techniques as per AOAC (1984). For the saponin determination, a 20% ethanol extraction technique followed by purification with diethyl ether was used. Saponin extraction was treated with vanillin sulfuric acid reagent and absorbance readings taken at 544nm via Jenway 6305 UV/Visible spectrophotometer (110V).
In order to estimate the tannin content, Folin-Denis spectrophotometric method was used. Methanol extract of the plant was reacted with Folin-Denis reagent and sodium carbonate. This reaction mixture was allowed to equilibrate for 30 minutes in order to develop the colors completely and later the absorbance was noted at 760nm using Jenway 6305 UV/Visible spectrophotometer (110V).

Data Analysis

Data obtained was subjected to the Analysis of Variance (ANOVA) and mean values were separated using the Duncan's Multiple Range Test (DMRT). Significance will be tested at $P < 0.05$.

RESULTS

Concentration of antinutrients and toxic substances in the shell of *Vitellaria paradoxa* fruits

The effect of different geopolitical zones of Niger State on the bioaccumulation of antinutrients and toxic substances in the shell of *V. Paradoxa* fruits is presented in Table 1. The results showed that the concentration of saponin in the shell of *V. Paradoxa* fruits from zone A and C did not differ significantly from each other; however, its concentration in the shell of the fruits from zone A was significantly higher compared to those of the two other zones. The mean values of 9.76 ± 4.68, 33.72 ± 7.44 and 9.16 ± 3.72 mg/g of saponin in the shell of *V. paradoxa* were recorded for zone A, B and C, respectively. Likewise, the tannin content is the shell of the fruits obtained from zone A, B and C were 3.05 ± 0.51, 0.46 ± 0.08 and1.11 ± 0.81 mg/kg, respectively. The results showed that the concentration of tannin in the shell of the fruit of *V. Paradoxa* was significantly highest in zone A, closely followed by C and least from those obtained from zone B. The results also showed that the concentrations oxalate and phytate in the fruit shell of *V. Paradoxa* from zone B and C did not differ significantly from each other, however, the concentrations of these parameters were significantly in the shell of the fruits obtained from zone A. The mean concentrations of oxalate in the shell of fruits from zone A, B and C were 13.55 ± 6.80, 7.93 ± 0.89 and 10.90 ± 3.12 mg/g, respectively while the corresponding values for phytate were 1.58 ± 0.06, 0.56 ± 0.01 and 0.77 ± 0.35 g/100 g, respectively. The significant increasing order of cyanide and nitrate concentrations in shell of *V. Paradoxa* fruits from the three geopolitical zones were zone A > zone B > zone C. The mean values of 287.98 ± 140.91, 53.90 ± 28.67 and 6.91 ± 2.79 mg/kg were obtained for cyanide in the shell of the fruits from zone A, B and C, respectively while the corresponding mean values of nitrate were 743.92 ± 101.81, 381.08 ± 213.04 and 137.33 ± 71.14 mg/kg, respectively.

Table 1: Antinutrients and toxic substances content in the shell of *Vitellaria paradoxa* fruits obtained from geopolitical zones of Niger State.

Antinutrients	Zones		
	A	B	C
Saponin (mg/g)	9.76 ± 4.68^{a}	33.72 ± 7.44^{b}	9.16 ± 3.72^{a}
Tannin (mg/g)	3.05 ± 0.51^{c}	0.46 ± 0.08^{a}	1.11 ± 0.81^{b}
Oxalate (mg/g)	13.55 ± 6.80^{b}	7.93 ± 0.89^{a}	10.90 ± 3.12^{a}
Phytate (mg/100g)	1.58 ± 0.06^{b}	0.56 ± 0.01^{a}	0.77 ± 0.35^{a}
Cyanide (mg/kg)	287.98 ± 140.91^{c}	53.90 ± 28.67^{b}	6.91 ± 2.79^{a}
Nitrate (mg/kg)	743.92 ± 101.81^{c}	381.08 ± 213.04^{b}	137.33 ± 71.14^{a}

Means ± standard error on the same row with different letter superscripts are significantly different ($p < 0.05$) from each other. Key: Zone A (Mokwa, Bida and Lapai), Zone B (Suleja, Kuta and Paiko), Zone C (Kotongora, Wushishi and New Bussa).

DISCUSSION

The result of this present research revealed that the average cyanide levels (287.98 ± 140.91 mg/kg) in Zone A, exceeded the safety threshold of 200mg/ kg by a substantial margin, and were significantly higher when compared to both Zones B and C as explained in table 1. These levels can be classified as possibly lethal forms of respiratory poisons this is in agreement with Mohammad *et al.*, 2022 and could prevent mitochondrial use of oxygen, a situation which was earlier reported by Nyirenda, (2021). Although this was not a complete mixture, the vertical autumn involved heavy metals providing direct toxicity or indirectly causing toxic effects through bioaccumulation. This is in stark contrast to the observations made for Zones B and C, where cyanide levels were well below tolerable limits.

Also, there was a similar pattern for nitrate content, which was highest in Zone A (743.92 ± 101.81 mg/kg). Although these results fall within the acceptable level in terms of daily consumption at normal amounts, the high concentration in Zone A implies that there is more nitrogen in the soil or in the runoff from agriculture along the Mokwa-Bida axis. This is consistent with the claims recently made by Oloya *et al.,* (2024) on the relationship between geographical location and potential cyanogenic and nitrate content of crops.

One key finding in this study is that the oxalate concentrations in all shell samples from the various zones (7.93-13.55 mg/g) were significantly higher than the average concentration in common fresh weight vegetable foods such as spinach. This finding is supported by Van't Klooster *et al.* (2022) when they emphasized that some of the tropical fruit parts have the capacity to accumulate high amounts of oxalates depending on the availability of soil calcium. The high oxalate concentrations in particular Zone A pose health risks since they have the potential to cause kidney stone formation and hinder the absorption of calcium.

The Results of this research showed that the amounts of saponin, tannin, and phytate in the various zones were within permissible safety levels. The amount of saponin was notably high in Zone B (33.72 ± 7.44 mg/g) than Zones A and C. This result aligns with those of Choungo *et al.*, (2021), who reported that the amount of saponin in shea parts tends to vary depending on the environmental stress level in different regions.

The amount of tannin was also remarkably high in Zone A (3.05 ± 0.51 mg/g). Although these results are relatively low, tannin is a recognized defense strategy against microbial pathogens and plant-eating animals. The amounts of phytate were below 600-800 mg/day. These results support the report of Ojo *et al.,* (2021), where the intake of these shells in feed could be safe without the potential risk of protein precipitation and mineral chelation associated with high antinutrient intake.

CONCLUSION

This study revealed that there were significant differences in the concentration of antinutrients and toxicants in the shells of *V. paradoxa* in the three geopolitical zones in Niger State. It is also pertinent to note that despite the relatively low concentrations of saponin, tannins, phytates, and nitrates in the samples of all the three zones being within the safe limits, Zone A had the highest concentration of tannins, oxalates, phytates, cyanides, and nitrates when compared with the other zones. There is a clear indication that there is a strong relationship between the secondary metabolites of the plant and its growth environment.

The higher amounts of nitrate and cyanide found in Zone A (Mokwa, Bida, and Lapai) could be attributed to the chemical composition of the soil in the Middle Belt region, where unique processes involved in the cycling of nitrogen or nitrogenous fertilization in adjacent agricultural farms might affect the cyanogenic capability of the plants within the vicinity. Moreover, the higher amounts of oxalates found in the samples in this particular zone would suggest higher amounts of calcium absorption from the soil because oxalic acid production serves as a means of regulating calcium balance in plants.

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CONFLICTS OF INTREST

The authors declare no conflicts of interest

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