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Effects of Traditional Processing on the Antinutrient-Phytochemical Contents of *Vernonia Amygdalina*

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Abstract
Antinutrient-phytochemical contents of raw and blanched bitter leaf (Vernonia amygdalina) were determined using standard analytical techniques. Vernonia amygdalina is a shrub or small tree belonging to the family Asteraceae. It is a popular African vegetable which grows in several parts of tropical and subtropical Africa. The results showed that phytate and saponin were the most abundant among the antinutrient-phytochemicals with values ranging from 2600-2760 mg/100g (phytate) and 2580-3140mg/100g (saponin). Other antinutrients of significant concentrations were (mg/100g) oxalate (156-217), alkaloid (1210-1270) and flavonoid (385-485) whereas tannin (4.51-4.80) and total phenol (4.26-4.65) had low concentrations. The results of [Ca] x [Phy]: [Zn] molar ratios in both samples (0.050-0.057) were lower than 0.5 and would therefore enhance Zn bioavailability. The statistical analysis showed positively high correlation coefficient, significant at n-2 (7-2) = 5 and $r_{=0.05}$ level. Generally, the concentrations of antinutrient-phytochenmicals were higher in the raw sample than the blanched bitter leaf which could be as a result of leaching of part of the antinutrient-phytochemicals into the hot water.

Keywords: Traditional processing, antinutrient-phytochemical, bitter leaf

INTRODUCTION
The importance of green leafy vegetable as food for population of people across the globe cannot be over-emphasized. Leafy vegetables are composed of nutrients which can protect the body against various ailments. They occupy an important place among the food crops as they provide adequate amount of many vitamins and minerals for humans (Fasuyi, 2006). Leafy vegetables represent inexpensive but high quality nutritional sources for the poor segment of the population, especially where malnutrition is widespread (Nnamani *et al*., 2007). Leafy vegetables contain low calories; hence, they are ideal for obese people who can satisfy their appetite without consuming much carbohydrate. *Vernonia amygdalina* commonly known as bitter leaf is a shrub or small tree of 2-5 meters, which grows under a range of ecological zones in Africa and produces large mass of foliage and is drought resistant. The leaves are green with a characteristic odour and a bitter taste. *Vernonia amygdalina* mostly occurs wild, though it could be cultivated. It is widely consumed in mostly Central African countries both by humans and animals. It has varieties of names across different tribes such as 'Chusar-doki' in Hausa, 'Ewuro' in Yoruba, 'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Oriwo' in

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Edo, 'Etidot' in Ibibio, and 'Grawa' in Amharic. Elsewhere in Africa, it is called muop or ndole (Cameroon), tuntwano (Tanzania), and mululuza (Uganda) (Owolabi *et al*., 2008).

The plant is well known for its antidiabetic and antihypertensive properties, and also used in the treatment of headache and fever (Oboh, 2003). It contains phosphorous, ascorbic acid, iron, and phosphorous, ascorbic acid, iron, β –carotene, calcium, water, and fibre as well as other nutrients. It contains phytochemicals much as alkaloids, saponins, tannins, sesquiterpere lactone, steroid glucosides flavonoids, glycosides and sterols which cause its bitterness. Some of these phytochemicals are considered as anti-nutrients, capable of precipitating deleterious effects in man and animal and reducing nutrient bioavailability at certain levels but some exhibit health promoting activities in managing chronic diseases (Chinma & Igyor, 2007).
Blanching is one of the major traditional processing methods carried out on bitter leaf before consumption in an attempt to reduce its level of bitterness. This study is therefore aimed at investigating the antinutrient-phytochemical contents of fresh and blanched bitter leaf (*Vernonia amygdalina*). This is to ascertain the effects this processing method would have on the antinutrient-phytochemicals of the sample.

MATERIALS AND METHODS

Sample collection and preparation

Fresh leaves of *Vernonia anygdalina* were obtained from a local farm in Azare, Katagum Local Government Area of Bauchi State. The leaves were sorted, de-stalked and rinsed with distilled water to remove dust and dirt, left to drain and were later divided into two parts. One part was immersed inside hot water for 10 min, removed and allowed to drain while the other part was left untreated. The two samples were air dried to prevent loss in nutrients. The samples were separately milled into fine powder using type 8 K 31 Kenwood blender and packed in plastic bags to prevent moisture absorption and contamination, labelled and kept in a dry, cool place prior to use for various analyses.

Phytic acid was determined by soaking 4 g of the sample in 100ml 2% HCl for 3 hours and then filtered. 25ml of the filtrate was placed in a 100ml conical flask and 5ml of 0.03% NH_4SCN solution was added as indicator. 50ml of distilled water was added to give it the proper acidity (pH 4.5). This was titrated with ferric chloride solution which contained 0.005mg of Fe per ml of $FeCl_3$ until a brownish yellow colour persisted for 5minutes. Phytin phosphorus (Pp) was determined and the phytic acid content was calculated by multiplying the value of Pp by 3.55 (Young & Greaves, 1940). Each milligram (mg) of Fe is equivalent to 1.19 mg of Pp.
Iron equivalence = titre value x 1.95
Pp = titre value × 1.95 × 1.19
Therefore, phytic acid = titre value x 1.95 x 1.19 x 3.55 mg

Tannin was determined by weighing 200mg of the sample into a 50ml sample bottle. 10ml of 70% aqueous acetone was added and properly covered. The bottles were shaken by orbital shaker for 2 hours at 30^0C. Each solution was then centrifuged and the supernatant stored in ice. 0.2ml of each solution was pipetted into test tubes and 0.8ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5mg/ml stock and the solution made up to 1ml with distilled water. 0.5ml folin reagent was added to both sample and standard followed by 2.5ml of 20% Na_2CO_3. The solutions were then vortexed and allowed to incubate for 40 minutes at room temperature after which absorbance was read against a reagent blank concentration of the sample from a standard tannic acid curve (Makkar & Goodchild, 1996).
Determination of oxalate was done following the procedure of Day and Underwood (1986). 1g of the sample was weighed into 100ml conical flask. 75ml of 1.5NH_2SO_4 was added and the

solution was carefully stirred intermittently with a magnetic stirrer for about 1 hour and then filtered using Whatman filter paper. 25ml of sample filtrate was collected and titrated hot (80-90^{0}C) against 0.1N$KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 seconds.

Alkaloid determination was carried out following the procedure of Harborne (1973). 5.0g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Total phenol was determined by taking 1 ml of the extracted sample into 10ml glass tube and made up to 3ml with distilled water. 5ml of Folin reagent (1:1) with water and 2ml 20% Na_2CO_3 were added sequentially in each tube. Blue colour was developed in each tube. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm the reagent, used as blank. A standard calibration plot was generated at 650 nm a concentration of catechol. The concentration of phenol was calculated from the calibration plot and expressed as mg catechol equivalent of phenol/100g of sample.

For saponin determination, the method used was that of Obadoni and Ochuko (2001). 5g of the sample was put into a conical flask and 100mlof 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{o}C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated as percentage.

The method of Boham and Kocipai-Abyazan (1974) was followed in the determination of flavonoid. 5g of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper (125ml). The filtrate was later transferred into a crucible and evaporated into dryness and weighed to a constant weight.

Statistical analysis

The mean, standard deviation, coefficient of variation percent, linear correlation coefficient, coefficient of alienation and index of forecasting efficiency were determined.

All analyses were carried at the Central Laboratory of the Federal University of Technology, Akure, Ondo State.

RESULTS AND DISCUSSION

The concentrations of the various antinutrient-phytochemicals of *Vernonia amygdalina* leaves are depicted in Table 1. Tannin and total phenol were (mg/100g): 4.80 and 4.65 (raw); 4.51 and 4.26 (blanched); phytate and oxalate recorded (mg/g): 27.6 and 2.17 (raw); 26.0 and 1.56

(blanched) whereas alkaloid, saponin and flavonoid were (g/100g): 1.27, 3.14 and 0.485 respectively (raw); 1.21, 2.58 and 0.385 respectively (blanched).

Table 1: Antinutrient-pyytochemical contents of *Vernonia amygdalina* leaf samples

Parameters	RWS	BLS	Mean	SD	CV%
Tannin (mg/100g)	4.80	4.51	4.66	0.205	4.40
Total phenol(mg/100g)	4.65	4.26	4.46	0.276	6.19
Phytate (mg/g)	27.6	26.0	26.8	1.13	4.22
Oxalate (mg/g)	2.17	1.56	1.87	0.431	23.0
Alkaloid (g/100g)	1.27	1.21	1.24	0.042	3.39
Saponin (g/100g)	3.14	2.58	2.86	0.396	13.8
Flavonoid (g/100g)	0.485	0.385	0.435	0.071	16.3

RSW: raw sample, BLS: blanched sample, SD: standard deviation, CV%: coefficient of variation percent

In the statistical analysis of results in Table 1 as shown in Table 2, the mean, standard deviation and coefficient of variation percent for the various parameters determined were 6.30, 9.53 and 151 respectively for raw sample and 5.79, 9.04 and 156 respectively for blanched sample. The values of correlation coefficient, coefficient of alienation and index of forecasting efficiency between the two samples were 0.999, 0.032 and 0.968 respectively.

Table 2: Statistical analysis of results from Table 1

Statistics	RWS		BLS
r_{xy}		0.999	
r^2_{xy}		0.999	
Mean	6.30		5.79
SD	9.53		9.04
CV%	151		156
CA		0.032	
IFE		0.968	
Remark	*		

r_{xy}: correlation coefficient, r_{xy}^2: coefficient of determination, C_A: coefficient of alienation, IFE: index of forecasting efficiency, *: results significantly different at n–2 and $r_{=0.05}$ (critical value: 0.755)

The calculated Phy: Zn, Ca: Phy and [Ca] [Phy]/[Zn] molar ratios of raw and blanched *Vernonia amygdalina* leaves are depicted in Table 3. The calculated values for the parameters above were 4.18, 0.287 and 0.050 respectively (raw); 5.21, 0.277 and 0.057 respectively (blanched leaf). The results for the two samples were very close as shown by the levels of coefficient of variation percent, CV% (2.48-19.7).

Table 3: Concentration of Zn, Ca, Phy and calculated Phy:Zn, Ca:Phy and [Ca]*[Phy]/[Zn] molar ratios of *Vernonia amygdalina* leaf samples

Parameter	RWS	BLS	Mean	SD	CV%
Zn (mg/100g)	65.4	49.4	57.4	11.3	19.7
Ca (mg/100g)	48.2	43.7	46.0	3.18	6.91
Phy (mg/100g)	2761	2596	2679	117	4.37
Phy:Zn	4.18	5.21	4.70	0.728	15.5
Ca:Phy	0.287	0.277	0.282	0.007	2.48
[Ca]*[Phy]/[Zn]	0.050	0.057	0.054	0.005	9.26

The antinutrient-phytochemical contents of *Vernonia amygdalina* leaf samples showed that saponin had the highest concentration in raw sample and phytate in the blanched sample. Total phenol was least abundant in both samples. The Phy levels in this study fell within the range (390-6210mg/100g) reported for 13 spices obtained in Nigeria (Adeyeye & Fagbohun, 2005) and seven varieties of Nigerian garden egg fruits (507-2788mg/100g) (Adeyeye & Fagbohun, 2006). The following values were reported for Basil plant leaf, cam wood plant leaf

and cocoyam leaf respectively (mg/ 100g): Phy (1.30; 1.00; 1.19), oxalate (0-08; -0.90; 0.07); tannin (1.10; 3.08; 1.28), flavonoid (6.20; 6.70; 2.90) and saponin(1.03; 1.20; 0.00) (Opega *et al*., 2016). The oxalate contents in this report (156-217mg/100g) were much higher than 4.08-6.42mg/100g reported for groundnut seed flour (Adeyeye, 2011) and 0.2 mg/100g for sweet potato leaf (Opega *et al*., 2016). High level of oxalate is undesirable; it negatively affects Ca absorption and utilization. It combines with Ca to form calcium oxalate, which passes through the intestine without being absorbed. Calcium oxalate is responsible for most of the kidney stones, either alone or mixed with the salts of calcium phosphate and calcium carbonate. Formation of these stones could result in chronic alkalinity of bladder (White *et al*., 1973). The results of this study showed that boiling water treatment (blanching) fairly reduced the levels of antinutrient-phytochemicals in *Vernonia amygdalina.*

The calculated Phy: Zn, Ca: Phy and [Ca] [Phy]: [Zn] molar ratios are presented in Table 3. Phy: Zn was the most varied among the ratios with CV% level of 15.5. Oberleas and Harland (1981) reported that foods with Phy: Zn molar ratio of values below 10 showed adequate availability of Zn and values greater than 15 presented some nutritional problems. Phy: Zn molar ratio of 15:1 was reported to have been associated with reduced Zn bioavailability (Turnlund *et al*., 1984). The levels of Phy: Zn molar ratios in this study were less than 10 and would therefore promote Zn bioavailability. It had been suggested that phytate precipitation is incomplete until dietary Ca: Phy molar ratio attains a value of 6:1 Any value below this would reflect incomplete phytate precipitation, such that some of the dietary Zn remains in solution and the proportion remaining in solution increases with decreasing Ca : Phy ratios (Wise, 1983). The calcium contents in both samples are very small and therefore Ca: Phy values were below the critical value of 6 hence might interfere with Zn bioavailability. It is interesting to note that another parameter had been suggested to be a better predictor of Zn bioavailability. Davis and Warrington (1986) and Ellis *et al*., (1987) showed that Zn bioavailability is better indicated using [Ca] x [Phy]: [Zn] molar ratio and noted that if the ratio is greater than 0.5mo/kg^{-1}, there would be reduction in Zn bioavailability. In this study, Ca x Phy: Zn ratios were lower than 0.5 mo/kg^{-1} in both samples therefore would enhance Zn bioavailability.

Statistical analysis of antinutrient-phytochemical results (Table 2) showed positively high correlation coefficient, significant at n-2 (7-2) = 5 and $r_{=0.05}$ (critical value = 0.669). The high value of IFE indicated that the biochemical functions of the antinutrient-phytochemicals in the raw (untreated) sample could also be carried out by those in the blanched (treated) sample.

CONCLUSION

The results showed high levels of most of the antinutrient-phytochemicals determined especially phytate and saponin. Total phenol and tannin were generally low in both samples. The results also showed that raw sample contained higher levels antinutrients than blanched sample. This indicates that traditional processing of *Vernonia amygdalina* leaf (blanching) results in loss of antinutrient-phytochemicals which could be as a result of leaching away into hot water. Blanching should therefore be encouraged, but not in an extremely high temperature and too long time in other to avoid loss of valuable minerals.

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