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The Levels of Antinutritional Factors in *Moringa Oleifera* and *Vernomia Amygdalina* Leaves Found in Some Part of Plateau State, Nigeria

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Abstract

In this research work, various materials were used and they include; apparatus such as fume cupboard, measuring cylinder, spatula, centrifuge, pipette and reagent such as Na₂CO₃, CaCl₂, H₂SO₄, HCl, AgNO₃. The nutrient composition of *V. Amydalina* are: Phytate 11.9 \pm 0.01 (mg/100g), oxalate 244.02 \pm 0.57 (mg/100g), tannins 1.28 \pm 0.50 (mg/100g), alkaloid 1.66 \pm 0.01%, HCN 2036.00 \pm 0.58 (mg/100g) and *M. oleifera* are: phytate 10.58 \pm 0.01 (mg/100g), oxalate 334.33 \pm 0.67 (mg/100g), tannin 8.19 \pm 0.01 (mg/100g), alkaloid 1.72 \pm 0.01% and HCN 3998.30 \pm 0.49 (mg/100g). These results showed that *V. Amydalina* leaves could be a bio resources for Zn as a result of the low level of phytate. But they are not Cu bioavailable resources due to the high level of oxalate. Hence, people are encouraged to utilize *V. amydalina* and *M. oleifera* leaves as a good source of micronutrient particularly those prove to be bioavailable.

Introduction

Antinutrient refers to substance that reduces the nutrient utilization by binding with the mineral to form complexes that are readily indigestible. Antinutrients are found at Some level of antinutrients may be found in almost all food for many reasons. Nowadays, the levels may be reduced in modern crops due to process domestication (Geo, 2008). Anti-nutrients in food are responsible for deleterious effects related to the absorption of nutrients and micronutrients. However, some anti-nutrients may exact some beneficial effect (Welch and Graham, 2004). The basis of these anti-nutrients elicits harmful and beneficial biological active compounds which,

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Keywords:

Antinutrients; Oxalate; Phytate; *V. Amydalina. M. oleifera.* in recent times, revealed their functions in several biological compounds (Igile, 1996). It is generally recognized that plant hold antinutrients obtained from pesticides, fertilizers and chemicals with occur naturally (Igile, 1996). Secondary metabolites types of antinutrients are recognized to contain high biological activity (Zenk, 1991). Phytate, alkaloids, oxalate, saponins, flavonoids, hydrogen cyanide were shown to be present.

Moringa is being harvested for its high nutritious content. Both the edible leaves and flowers may be used as a source of food and medicine and may also be used as cosmetic oil or forage for livestock. Moringa have the height of about 5 to 10cm (Padayachee and Baijnath, 2012). Through research, the moringa was found to contain many essential nutrients, for instance, vitamin, amino acid, beta-carotene, antioxidant, anti-inflammatory nutrient and omega 3 and 6 fatty acids (Kasolo et al., 2010). Vernomia amygdalina is another common shrub which is harvested in typical Africa conutries. It has green leaves, odorous with a pungent taste (Akpaso et al., 2011). Vernomia amygdalina leaves should be washed, cooked to remove the bitter taste before consumption as soup (Hamza et al., 2013). The leaves may also be used in traditional treatment of the disease like infertility, malaria, gastrointestinal problem, diabetes and sexual stransmitted diseases (Farombi and Owoeye, 2011). The leaves have been show to be an additive to a horse feed to improve it strength or body builder (Hamza et al., 2013).

Materials and Methods

Collection and Preparation of Plant Materials

The leaves samples of *Moringa oleifera* and *Vernomia amygdalina* were obtained from farm. The leaves were planted and harvested, destalked and washed with a clean cold water. The leaves were dried under a sun shade and the dried leaves were pulverised in a porcelain mortar, and the sample were stored in plastic container. The powdered samples were used for the analysis.

Methods

Various methods were reported by different researchers for the quantification of the antinutritional factors in green vegetable leaves. Oxalates were determined according to Underwood and Day (1986) procedure, phytate, Maret and Sandstead (2006) method, tannins, (Aletor, 1993) procedure, alkaloids, Henry (1973) method and cyanogenic glucosides, alkaline titration method of AOAC (1990).

Phytate Content Determination

The method reported by Maret and Sandstead (2006) was adapted for phytate quantification. Powdered sample (4g) were soaked in 100 cm³ of 2% HCI (w/v) and allowed to stand for over 3 hours before filtration. From the filtrate, 25 cm³ was taken and placed in a conical flask, 5 cm³ of 0.3% NH₄SCN (aq) and 53.5 cm³ of distilled water were mixed together and titrated against standard FeCl₃ (aq) having 0.00195g Fe/cm³ and observed the formation of brown-yellow color which may persist for 5 minutes. Blank was treated in a similar manner.

Determination of Hydrogen Cyanide

The methods proposed by AOAC (1990) were adapted. 100cm³ of the leaves has undergone steam-distillation using sodium hydroxide solution (NaOH). KI solution was used for treatment the distillate. The distillate was titrated against 0.02N AgNO₃ solution. The end point was recorded immediately when the color changed to more turbid solution. Determination of HCN content was evaluated by measuring 1ml of 0.02N AgNO₃ as equivalent of 1.08mg hydrogen cyanide.

Oxalate Determination

Day and Underwood (1987) proposed a method for determination of oxalate. The leaves sample should be (1g) and put inside a 100cm³ flask. 75cm³ of $3MnH_2SO_4$ was measured, placed into the same conical flask and stirred for about 1 hour. The filtration of the solution was carried out using a Whattman No 1 filter paper. 25cm³ of the filtrate was measured and titrated over 0.05N potassium per manganite (KMnO₄) solution till the appearance of pale-pink color. 1ml of 0.05m KMnO₄ was used to calculate the oxalate content.

Tannins Determination

0.1g of the powdered leaves was placed inside 100cm³ conical flask and 350cm³ of distilled water added (Aletor, 1993). The flask was gently heated to boiling for 1 hour, filtered hot and the filtrate was collected in a 50cm³ volumetric flask. The residue was washed severally, and distilled water was used

top the volume of the combined solution. To 1, 2, and $3cm^3$ of the standard tannic acid and $10cm^3$ of the sample solution in a 50cm³ volumetric flasks, 2.5cm³ Folin-Denis reagent and $10cm^3$ of Na₂CO₃ solutions has been added and distilled water was used to top the volume of the solution. The flask was allowed to stand for 20 minutes after which optical density was measured at 760nm.

Results

Table 1: Antinutrient in the Moringa oleifera leaves

Antinutrients	Composition (Mg/100g)
Oxalate	334.33±0.67
Phytate	10.58±0.01
Tanins	8.19 ±0.01
Alkaloids	1.72±0.01
Hydrogen cyanide	3998.30±0.49

Table 2: Antinutrients in the Vernomia amygdalina leaves

Antinutrients	Composition (Mg/100g)
Oxalate	334.33±0.67
Phytate	10.58±0.01
Tanins	8.19 ±0.01
Alkaloids	1.72±0.01
Hydrogen cyanide	3998.30±0.49

Discussion

From table 1 and 2 values are calculated as Mean ± SEM (n=3)

From the Table 1 and 2 above, the Oxalate content of Vernomia amygdalina and Moringa oleifera, 244.02±0.57 mg/100g and 339.33 ± 0.67 mg/100g were high compare to 202.50 ± 6.50 mg/100g reported for *A. viridis* leaves (Umar *et al.*, 2007). Studies revealed that antinutrients were observed to chelate several elements like Zn, Fe and Ca with strong binding affinities and therefore diminishes their bioavailability. High levels of some antinutrients e.g oxalate and phytates in human food are considered undesirable. High level of oxalate, decreases Cu bioavailability by binding with it to form insoluble salt (Umar *et al.*, 2007). The phyate content of Vernomia amygdalina and Moringa oleifera leaves are 11.19±0.01 mg/100g and 10.58±0.01 mg/100g respectively, the results were very low compare to 1326 mg/100g reported for Amaranthus viridis (Umar et al., 2007) and 1214 mg/100g for Tribulus terestris leaves (Umar et al., 2007). This showed that the phytate content may not have cause any nutritional problems when consumed. Animal protein in the food is the cause of zinc bioavailability and increased calcium levels have been observed to reduce it (Borguaye et al., 2017). The formation of calcium-zinc-phytate complexes in the intestines may be as a results of inhibitory effect of calcium over zinc (Borguaye et al., 2017). Consumption of animal proteins in Africa is so low that it may not significantly alter the zinc bioavailability (Borquaye et al., 2017). Undesirable levels of oxalate and phytates were recorded from these spices (Borquaye et al., 2017). Oxalates cause nephrotic lesions in the kidney (Kumar Jha et al., 2013). Anti-nutrients like tannins, oxalate, and phytates may be harmful when consumed in unrefined food. Presence of plants with some anti-nutritional content like cyanogenic glycosides and oxalates may reduce the bioavailability of the essential nutrients in plant foods. When soluble oxalate content is present in higher amount in the body, it blocked the absorption of soluble calcium ions because the oxalate and the calcium ions bound together to form insoluble calcium oxalate complexes (Kumar Jha et al., 2013). Consequently, people were advised to avoid food that are rich in oxalate as it has a propensity to cause the formation of kidney stones (Kumar Jha et al., 2013).

Bind dietary protein and decreased animal's consumption of feed are caused by the presence of tannins and may result digestive enzymes which forms indigestible complexes. Tannins may also slow the rate of growth and decreased palatability (Kumar Jha *et al.*, 2013). Tannins content of *V. Amydalina* and *M. oleifera*, $1.28 \pm 0.00 \text{ mg}/100g$ and $8.19\pm0.01 \text{ mg}/100g$ were very low compare to 7530.20 mg/100g for *A. viridis* (Umar *et al.*, 2007). These indicated that low levels of tannins in both samples will not exert negative effect on the bioavailability of Cu and Zn.

The susceptibility to hydrogen cyanide toxicity in ruminants is higher than non-ruminants. Hydrogen

cyanide (HCN) is converted to thiocyanate (SCN) by detoxification of the absorbed hydrogen cyanide in the liver by the enzyme Rhodanese. Cytochrome oxidase could be inhibited by excess cyanide ion. Thus stopping ATP formation, while the tissues deprived of energy with lead to rapid death. For sheep and cattle, the sufficient limit to cause death is is 2.0 to 4.0mg/kg body weight (Kumar Jha et al., 2013). Cyanide can cause goitrogenic effects due to During thiocyanate production by detoxification, CN can cause an effect called goitrogenic (Kumar Jha et al., 2013). Hydrogen cyanide (HCN) content of V. Amydalina and M. oleifera, 2036.00±0.58 mg/100g and 3998.30±0.49 mg/100g were very high compare to 13.07 ± 2.38 mg/100g reported for A. viridis (Umar et al., 2005), (Whitney and Rolfes, 2010) reported HCN content in some raw leaves such as Celosia argentea (200 mg/100g), Tarlinum triangulare (75mg/100g) and Celosia laxa (300 mg/100g). These showed that the level of HCN were above the permissible range for human consumption, hence will lead to serious health problem. Neurological disorder and gastrointestinal disorder are all cause by alkaloids. Chaconine, solanine and glycoalkaloids found in potato and Solanum spp. Were reported to be haemolytically active and are harmful to fungi and human beings. Gastrointestinal and neurological disorders are the toxicological consequences of potato glycoalkaloids involved more especially when the doses is above 20 mg/100g sample (Kumar Jha et al., 2013). Alkaloids content of V. Amydalina and M. oleifera,

 1.66 ± 0.01 % and 1.72 ± 0.01 % were very low compare to 20 ± 6.5 % reported for *Solanum spp* (Saeto, 2008). These showed that the alkaloid content is safe for consumption.

Conclusion

It has been revealed that, on the general basis, plants contained anti-nutrients obtained x pesticides, fertilizer and various acquiring chemicals that are found in nature. It is also known that high level of these antinutritional factors leads to bad effects upon the bioavailability of many minerals when consumed.

However, it can be inferred from the results above that, *V. Amydalina* and *M. oleifera* leaves could be a bioresources for Zn, because of the low value of phytate content. But they are not Cu bioavailable resources due to the high level of oxalate. Hence, populace are encouraged to utilize *V. Amydalina* and *M. oleifera* leaves as a good sources of micronutrient particularly those proves to be bioavailable.

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Conflict of interest.

No conflict of interest declared.

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