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## Anti-nutrient Composition and Bioavailability Prediction as Exemplified by Calcium, Iron and Zinc in *Melocia corchorifolia* Leaves

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**Abstract:** Plants are the major sources of microelements to populace of the developing world. However, presence of antinutritional factors limit their optimal utilization. In this paper, antinutritional content and their effect on bioavailability of Ca, Fe and Zn in *Melocia corchorifolia* leaves was investigated. The result indicated that the plant leaves had high level of tannin (4,689.06±2.60 mg/100 g dry weight, DW). The concentration of other antinutritional factors per/100 g DW is as follows: phytate (88.57 mg), total oxalate (585.00 mg), soluble oxalate (217.50 mg), cyanide (16.02 mg) and nitrate (74.41 mg). The predicted Ca, Fe and Zn bioavailability showed that [Oxalate]/[Ca] and [Oxalate]/[(Ca + Mg)] are below the critical level of 2.5 known to impair calcium bioavailability. Furthermore, [Phytate]/[Ca], [Phytate]/[Fe] and [Phytate]/[Zn] are below the critical level of 0.5, 0.4 and 1.5 respectively. However, [Ca][Phytate]/[Zn], 16.72, is above the critical level of 0.5, which indicates significant effect of phytate on Zn bioavailability. From the results it can be concluded that *M. corchorifolia* leaves could be an important bioresource for Ca and Fe but not for Zn considering the predicted bioavailability.

**Key words:** Vegetables, antinutrient, bioavailability, calcium, iron, zinc, *Melocia corchorifolia*

### INTRODUCTION

Micronutrients encompasses essential mineral elements and vitamins which are essential food nutrients needed by the body purposely as protective agent against diseases; thus necessary for health and growth (Seshadri, 2001; Ertan *et al.*, 2002; Falade *et al.*, 2003). Micronutrients deficiency is a serious form of malnutrition affecting the entire world population. Inadequate intake of micronutrients otherwise known as "hidden hunger" particularly the microelements contribute to the global burden of disease through increased rates of illness and death from infectious diseases and of disability such as mental impairment (Black, 2003). It was estimated by the WHO that out of about 10.8 million children deaths annually, the number attributed to zinc, vitamin A and iron deficiencies is 2.082 million (19%) far greater than one million child deaths caused by malaria (Black, 2003). Similarly, Ali and Tsou (1997) and Hart *et al.* (2005) reported that over 2 billion people worldwide (mainly in developing countries) are micronutrients deficient.

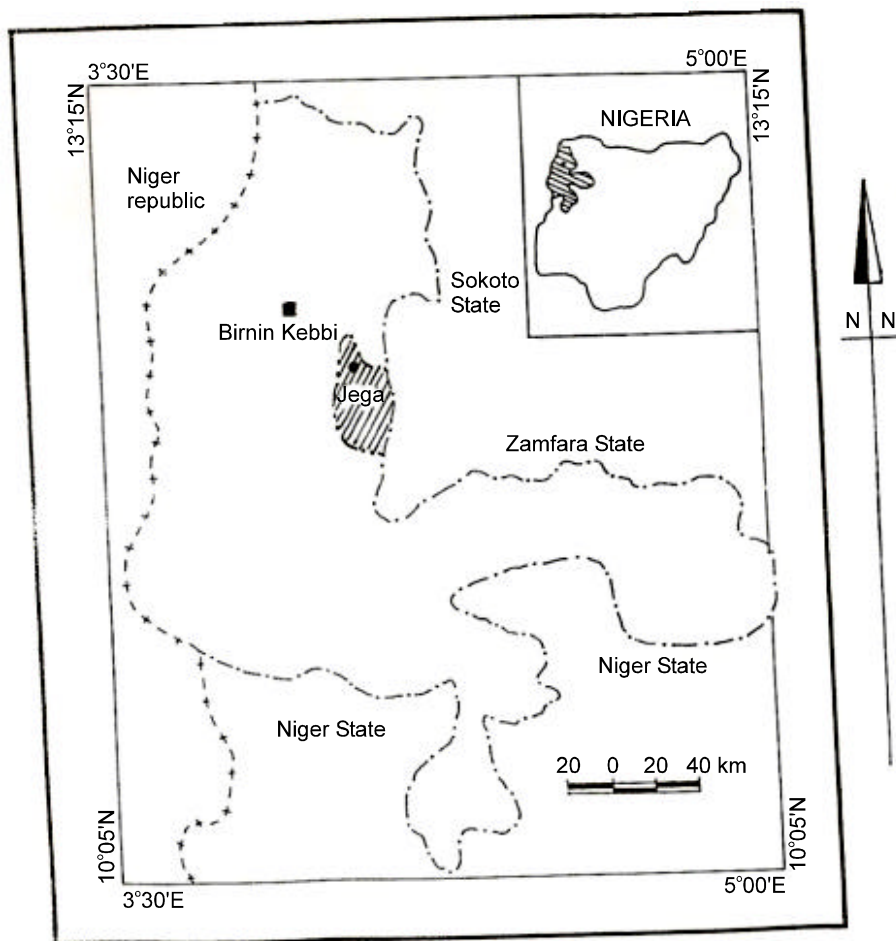
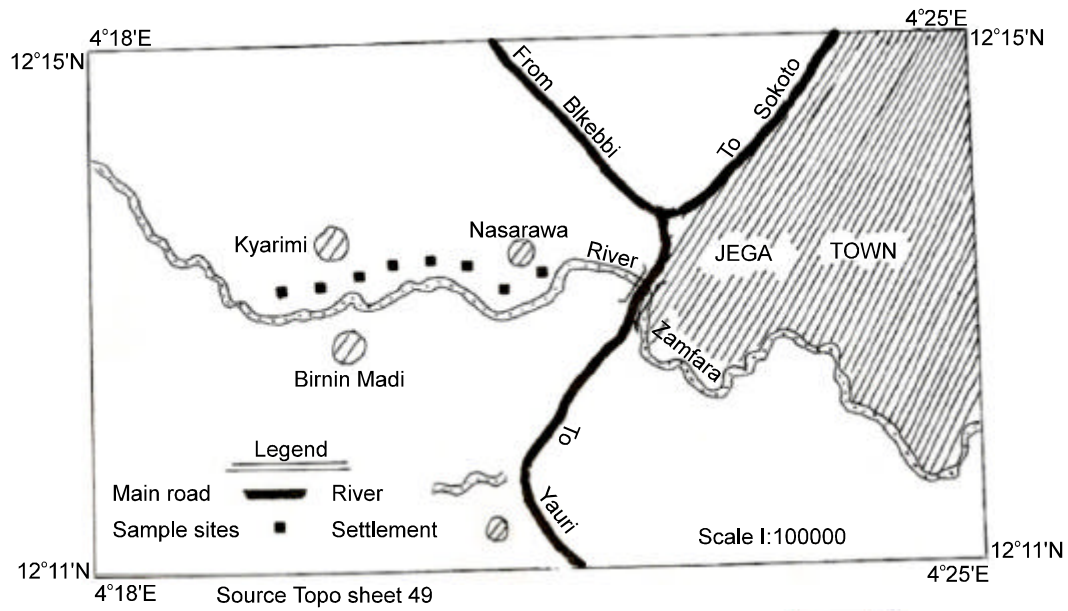
The Nigerian food consumption and nutrition survey highlighted that 34% of the Nigerian children under the age of five are anaemic while 40-60% children (6-24 months old) are at risk of disrupted brain development due to iron deficiencies (Bilbis, 2007). Furthermore, Seshadri (2001) reported that 46% of Nigerian pregnant women are anaemic while 48% were iron deficient. For zinc, the Nigerian National Food Survey reported that 20,

28.1 and 43.8% of the Nigerian children (under 5 years), mothers and pregnant women are zinc deficient respectively (Maziya-Dixon *et al.*, 2004).

Wild leafy vegetables are another food resource that was neglected even though, available literature has indicated that they are rich sources of micronutrients, fibre and antioxidants. Thus, integration of wild leafy vegetables will no doubt boost the micronutrients in the diet. *Melocia corchorifolia*, a wild leafy vegetable, is relish by the majority of populace where the plant grown. Umar *et al.* (2007) reported the nutritional content of *Melocia corchorifolia* leaves and shown that the plant leaves is rich in crude protein (23%) and some mineral elements (per 100 g dry matter) which include calcium (750.37 mg), Fe, (19.91 mg) and Zn (6.73 mg). In their work no attempt was made to evaluate the antinutritional factors and predict the divalent metals bioavailability. Thus, this paper addresses these issues.

### MATERIALS AND METHODS

**Samples collection and transportation:** Tender leaves of *Melocia corchorifolia* were randomly sampled from different locations along River Zamfara at Jega, Kebbi State, Nigeria (Fig. 1). Prior to analyses, the sample were identified and authenticated at the Herbarium of the Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria. The leaves were separated from the stalk, washed with distilled water, put in separate large paper envelopes and oven dried at 60°C to constant weight



Source:- Dept. of lands and survey Birnin Kebbi

Drawn by Abubakar Salah 2010

Fig. 1: Map showing study area and sampling sites

(Fasakin, 2004). The dried leaves were pulverized in a porcelain mortar, sieved through 20-mesh sieve and stored in plastic containers. The powdered samples were used for the analyses.

**Determination of phytate:** The method reported in Ola and Oboh (2000) was adapted for phytate quantification. Powdered sample (4 g) were soaked in 100 cm<sup>3</sup> of 2% HCl (v/v) for 3 h and filtered. To 25 cm<sup>3</sup> of the filtrate in a conical flask, 5 cm<sup>3</sup> of 0.3% NH<sub>4</sub>SCN<sub>(aq)</sub> and 53.5 cm<sup>3</sup> of distilled water were mixed together and titrated against standard FeCl<sub>3(aq)</sub> solution containing 0.00195 g Fe/cm<sup>3</sup> until a brownish yellow colour persisted for five minutes. Blank was treated in a similar manner. Phytin-Phosphorus (1 cm<sup>3</sup> Fe = 1.19 mg Phytin-Phosphorus) was determined and the phytate content calculated by multiplying the value of Phytin-Phosphorus by 3.55.

**Determination of total and soluble oxalate:** The method of Krishna and Ranjhan (1980) was used. For total oxalate, 2 g sample were put into a 250 cm<sup>3</sup> volumetric flask containing 190 cm<sup>3</sup> of distilled water and 10 cm<sup>3</sup> of 6 M HCl (for total oxalate only). The content was digested for one hour in a boiling water bath, cooled, made up to the volume and then filtered. Three 50 cm<sup>3</sup> aliquots of the sample were taken into beakers and to each; 20 cm<sup>3</sup> of 6 M HCl were added. The mixtures were then evaporated to about half of the original volume and filtered. The precipitates were washed several times with warm distilled water. To the filtrate, 3 drops of methyl red indicator were added to each beaker followed by the addition of concentrated ammonia solution until the solution turned faint yellow. The solutions were then heated at 95±5°C, cooled and filtered to remove precipitates containing ferrous ions. The filtrates were brought to boiling and to each, 10 cm<sup>3</sup> of 5% CaCl<sub>2</sub> solution were added with constant stirring and allowed to stand overnight. The precipitates were filtered, washed several times with distilled water and the filter papers containing the residues were transferred into the original beakers and dissolved using H<sub>2</sub>SO<sub>4</sub> (1:4v/v). The beakers were then heated to near boiling in water bath and titrated against standard 0.004 M KMnO<sub>4(aq)</sub> until first pink colour persisted for more than 30 sec. Blank was treated in a similar manner.

1 cm<sup>3</sup> of 0.004 M KMnO<sub>4</sub> = 2.25 mg anhydrous oxalic acid

**Determination of tannins (Allen et al., 1974):** Powdered sample (0.1 g) was put into a 100 cm<sup>3</sup> conical flask and 50 cm<sup>3</sup> of distilled water added. The flask was gently heated to boiling for 1 h, filtered hot and the filtrate collected in a 50 cm<sup>3</sup> volumetric flask. The residue was washed several times and the combined solution made to the volume with distilled water. To 0, 1, 2, 3, 4 and 5

cm<sup>3</sup> of the standard tannic acid and 10 cm<sup>3</sup> of the sample solution in a 50 cm<sup>3</sup> volumetric flask, 2.5 cm<sup>3</sup> Folin-Denis reagent and 10 cm<sup>3</sup> Na<sub>2</sub>CO<sub>3</sub> solution were added and made to volume with distilled water. The flasks were allowed to stand for 20 min after which optical density was measured at 760 nm. The calibration curve was plotted from which the concentration of tannic acid (X) in the sample was extrapolated and tannin content in the sample was calculated as:

$$\text{Tannic acid (mg/100g)} = \frac{X(\text{ppm}) * \text{Extracted volume (50 cm}^3\text{)}}{\text{Aliquot (10 cm}^3\text{)} * \text{Sample weight (0.1g)}} * 100$$

**Determination of hydrocyanic acid:** The method of AOAC (1990) was used for the analysis. 10 g sample were put into a 800 cm<sup>3</sup> distillation flask and about 200 cm<sup>3</sup> distilled water added. The flask was left to stand for 3 h before being connected to the macro-Kjeldahl distillation apparatus and steam distilled. The distillate (150-160 cm<sup>3</sup>) was collected into a 250 cm<sup>3</sup> volumetric flask containing 50 cm<sup>3</sup> of 2.5% NaOH solution. The flask was made up to the mark with distilled water. 100 cm<sup>3</sup> of the aliquot were put into a 250 cm<sup>3</sup> conical flask and 2 cm<sup>3</sup> of 5% KI added. The solution was titrated with standard 0.02 M AgNO<sub>3(aq)</sub> to the end point recognized by the appearance of a permanent faint turbidity.

1 cm<sup>3</sup> of 0.02 M AgNO<sub>3</sub> = 0.108 mg HCN

**Determination of nitrate:** The method described by IITA (1988) was adopted in which 100 mg of the powdered sample was weighed into a 15 cm<sup>3</sup> centrifuge tube and 10 cm<sup>3</sup> of distilled water added. The content was incubated in water bath at 45°C for one hour, cooled and centrifuged at 5000 revolution per minute for 15 min. The clear supernatant was put into a clean test tube, stoppered and stored in a refrigerator prior to nitrate analysis.

Nitrate stock solution (100 ppm) was prepared by dissolving KNO<sub>3</sub> (1.63 g) with distilled water in a 100 cm<sup>3</sup> volumetric flask up to the mark. To prepare series of standard solutions of 0, 1, 2, 3, 4 and 5 ppm, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 cm<sup>3</sup> of the stock solution were added to six 20 cm<sup>3</sup> volumetric flask. Similarly, 0.2 cm<sup>3</sup> of the extract was put into another 20 cm<sup>3</sup> volumetric flask. To the flasks, 0.8 cm<sup>3</sup> of 5% (w/v) salicylic acid-sulphuric acid reagent was added and mixed thoroughly. The contents were allowed to stand for 20 min and followed by the addition of 2 M NaOH solution (to raise the pH to above 12) to the mark. The contents were cooled to room temperature and its absorbance measured at 410 nm with spectrophotometer. The calibration curve was plotted from which the concentration of nitrate (X) in the samples was extrapolated and nitrate content in the sample was calculated:

$$\text{NO}_3^- (\text{mg}/100\text{g}) = \frac{X(\text{ppm}) * \text{Solution volume (cm}^3)}{\text{Aliquot (cm}^3) * \text{Sample weight (g)}} * 100$$

## RESULTS AND DISCUSSION

The result of antinutritive content of *M. corchorifolia* leaves was presented in Table 1. Tannin was the most abundant antinutritive factor in the leafy vegetables followed by oxalate. Phytate and nitrate were moderate in abundant, while HCN was the list. Table 2 shows the calculated nutrient to antinutritive ratio, which indicates the mineral bioavailability. The results indicated good calcium and iron bioavailability but zinc bioavailability was poor.

Table 1: Antinutritive content of *M. corchorifolia* leaves

Antinutrient	Concentration (mg/100 g DW)*
Phytate	88.57±18.63
Total oxalate	585.00±56.62
Soluble oxalate	217.50±19.84
Tannin	4,689.06±2.60
Cyanide	16.02±2.38
Nitrate	74.41±1.16

\*The data are mean value ± standard error of three replicates.  
DW = Dry weight

Table 2: Antinutrients to nutrients molar ratios of *M. corchorifolia* leaves

Antinutrient/mineral ratio	Value	Critical value*
[Oxalate]/[Ca]	0.13	2.5
[Oxalate]/[(Ca + Mg)]	0.10	2.5
[Ca]/[Phytate]/[Zn]	16.72	0.5
[Phytate]/[Zn]	0.89	1.5
[Phytate]/[Fe]	0.26	0.4
[Phytate]/[Ca]	0.01	0.5

Concentrations (mg/100 g DW) of Ca (750.37), Mg (108.33), Fe (19.91) and Zn (6.73) were sourced from Umar *et al.* (2007).

\*Source: Umar (2005), Frontela *et al.* (2008), Mitchikpe *et al.* (2008) and Obah and Amusan (2009)

**Antinutritional factors and predicted mineral bioavailability:** The phytate content of the *M. corchorifolia* was 788.57±18.63 mg/100 gDW, which is low compared to 1,214±15.56 mg/100 g reported for *Tribulus terrestris* leaves (Hassan *et al.*, 2007). High amount of phytate was also reported in some leafy vegetables such as *Tralinum triangulare* (2341.1 mg/100 g), *Vernonia amygdalina* (1466.7 mg/100 g) and *Basella alba* (2030.8 mg/100 g) (Akindahunsi and Oboh, 1999; Oboh *et al.*, 2005). Oboh *et al.* (2005) reported the phytate content of eggplant as 40.4±0.3 mg/100 g wet weight equivalent to 392.23 mg/100 g dry weight. The phytate content in the leaves was within the range of 0.1-6% reported in food items (Mohammed *et al.*, 2002).

To predict the bioavailability of Ca, antinutrients to nutrients ratios were calculated. From the result, it was observed that [oxalate]/[Ca] and [oxalate]/[(Ca+Mg)] ratios in the sample are below the critical level of 2.5 (Table 2) known to impair calcium bioavailability (Hassan *et al.*, 2007).

Phytate is known to decrease the bioavailability of minerals, especially Ca, Mg, Fe and Zn (Agte *et al.*, 1999; Oatway *et al.*, 2001; Anyum *et al.*, 2002; Bhandari and Kawabata, 2004). Hurrel *et al.* (1992) reported that a phytic acid intake of 4-9 mg/100 g DM decreases Fe absorption by 4-5 folds in human. This indicates the consumption of the leaves could hinder Fe bioavailability.

To predict the effect of phytate on the bioavailability of Ca, Fe and Zn, phytate to nutrients ratios were calculated. The calculated molar ratios of phytate to iron, calcium and zinc of *M. corchorifolia* were below the critical level of 0.4, 0.5 and 1.5 respectively as outlined by Frontela *et al.* (2008) and Mitchikpe *et al.* (2008). [Ca]/[phytate]/[Zn] ratio was found to be a better measure of zinc bioavailability than [phytate]/[Zn] ratio (Obah and Amusan, 2009). The sample had [Ca]/[phytate]/[Zn] ratio of 16.72 which is above the critical level. This implies that the phytate may hinder zinc bioavailability (Umar, 2005). Mineral Protein and starch solubility digestion and absorption was also reported to be affected by phytate. On the other hand, phytate was an anti-carcinogen that protects against colon cancer and it is known to be a potent antioxidant that inhibits Fenton reactions leading to lipid peroxidation and inhibition of polyphenol oxidase (Agte *et al.*, 1999).

*M. corchorifolia* also have total oxalate content of 585.00±56.62 mg/100 g in which 217.50±19.84 mg/100 g was in soluble form and is responsible for interference of divalent metals absorption particularly calcium by forming insoluble salts with them (Hassan and Umar, 2004). The oxalate content of the sample was low compared to 0.6%-15.1% reported in some edible leafy vegetables (Badifu, 2001). Consumption of oxalates may result in kidney disease (Hassan *et al.*, 2007). However, the level of oxalate in the sample is not a major concern for normal healthy person as toxic level for humans was set as 2-5 g (Hassan and Umar, 2004).

The hydrocyanic acid (HCN) content in *M. corchorifolia* is 16.02±2.38 mg/100 g DW. Badifu (2001) reported HCN content in some raw leaves such as *Celosia argentea* (20 mg/100 g) *Tralinum triangulare* (75 mg/100 g) and *Celosia laxa* (30 mg/100 g). Consumption of high levels of cyanide is associated with a serious health problem, spastic paraparesis known as *Konzo*. In Nigeria, a neurological disease known as Tropical Ataxic Neuropathy (TAN) was also linked to consumption of high level of cyanide in cassava-based diet (Hassan and Umar, 2004). The HCN levels in the studied leafy vegetables are well within the permissible range for human consumption. Only plants with more than 200 mg of HCN equivalent per 100 mg fresh weight are considered dangerous (Betancur-Ancona *et al.*, 2008). This shows that leaves are safe for consumption as far as HCN is concerned.

The concentration of nitrate in the sample (74.41±1.16 mg/100 gDW) was below the Acceptable Daily Intake (ADI) of 3.7 mg/kg body weight equivalent to 220 mg for 60 kg person (Hassan and Umar, 2004). Furthermore, boiling was reported to reduce nitrate to the level of about 60-70% as observed in vegetables (Hassan and Umar, 2004). Fytianos and Zarogiannis (1999) reported that spinach contains high nitrate concentrations (1000-3000 ppm) while as high as 6000 ppm has been reported in lettuce. Cabbage contains nitrate at concentrations ranging from several hundreds to over 1000 ppm. Studies have indicated that nitrates generally cause methaemoglobinaemia in young infants, but not in adults. However when reduced to nitric oxide it plays an important role in the body as it provides host defense against numerous micro-organisms (Benjamin, 2000).

**Conclusion:** From the results it can be concluded that *M. corchorifolia* leaves could be an important bioresource for microelements tested with good predicted Ca and bioavailability. However, low level of Zn couple to reciprocal level of antinutrients makes the leaves not a better zinc bioavailable resource. Hence populace are encourage to utilize *M. corchorifolia* leaves as sources of microelements particularly those proves to be bioavailable.

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