

Full Length Research Paper

Breeding for nutritional quality for *Corchorus olitorius*, *Annona muricata* and *Pentaclethra macrophylla* 1: A study of their antinutritional contents

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Breeding for nutritional quality of food/feed crops had somewhat been neglected by plant breeders all through the years. The objectives had mainly been focused on disease resistance and yield. Current concerns about the global food security need to encompass the issue of breeding for nutritional quality of food plants. One of the issues that revolve around the nutritional quality is the presence of antinutrients. These substances reduce the bioavailability of nutrients such as proteins, vitamins and minerals which could result in malnutrition especially in developing countries. Designing breeding programmes for the enhancement of nutritional quality of food crops require information on the types and concentrations of these antinutrients in such crops. This study was thus initiated to obtain information on the concentrations of the antinutrients - cyanogenic glycosides, oxalates, phytic acids, tannins and alkaloids - of *Annona muricata* (a fruit), *Corchorus olitorius* (a vegetable/grain crop) and *Pentaclethra macrophylla* (a legume) which are common among the local people of South East of Nigeria. Results showed that mean contents of oxalate, phytic acid, tannins and alkaloids were within permissible limits while high contents of cyanogenic glycosides were observed in *C. olitorius* (0.551 ± 0.0165 mg/100 g) and *P. macrophylla*. Fermentation did not reduce the level in *P. macrophylla*. The values obtained in unfermented and fermented seeds were 0.577 ± 0.0004 mg/100 g and 0.575 ± 0.003 mg/100 g respectively. The results were discussed bearing in mind the need for plant breeders and nutritionists to design programmes that will balance the adverse and beneficial effects of these antinutrients since many of them have also been found to be pharmacologically beneficial.

Key words: Antinutritional, contents, breeding, quality.

INTRODUCTION

The major aims of plant breeding since its early days had been on plant performance, disease resistance and yield (Raboy, 2013). Very few programmes had dwelt on

nutritional quality. And even in recent times when concerns had mounted about the future of global food security, researchers and policy makers had routinely

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forgotten to mention the issue of nutritional quality (Raboy, 2013). The author, however, predicts that very soon “breeding programmes aimed at enhancing the nutritional quality and health-beneficial properties of staple food and feed crops may be central to and even critical to the sustainability of agriculture and food security world-wide”.

One of the issues that revolve around the nutritional quality of food crops is the presence of antinutrients otherwise known as secondary metabolites. Antinutrients are compounds which sedentary organisms (plants, fungi and bacteria) synthesize to protect them from herbivores, insects, pathogens or adverse growing conditions. When plants containing these compounds, however, are eaten by domestic animals or humans, adverse physiological effects usually result (Khokhar and Apenten, 2003). Examples of these antinutrients which occur in varying degrees include, oxalate, phytate, cyanogenic glycosides, tannins, flavonoids, saponins and alkaloids (Soetan, 2008; Musa et al., 2011).

These substances cause a reduction in the bioavailability of nutrients like proteins, vitamins and minerals. Consequently even when these nutrients are present in foods, the body does not fully make use of them (Kolawole and Obueh, 2013) thus resulting in malnutrition especially in developing countries. This effect occurs because of the interaction of these antinutrients with normal nutrients. Phytates and oxalates, for example, are strong chelators that react with minerals such as calcium, magnesium, zinc, copper, iron etc to form complexes that cannot be absorbed by the intestine (Akande et al., 2010). Such complexes are excreted thus reducing the bioavailability of the minerals to the body. Kidney stones are formed as a result of such complex formation between calcium and oxalate (Ogbadoyi et al., 2011). The phytates and oxalates also affect protein digestion.

Cyanogenic glycosides are hydrolysed by enzymes to give off hydrogen cyanide (HCN) which is a potent respiratory poison. The HCN is associated with many of the diseases of the central nervous system and it is very toxic even at low concentrations (Akande et al., 2010; Kolawole and Obueh, 2013). Tannins and Saponins can interfere with protein digestion (Dei et al., 2007) while alkaloids cause gastrointestinal and neurological disorders (Aletor, 1993). Reports showed that some plant alkaloids can cause infertility (Olayemi, 2007).

Several authors have noted that these antinutrients are usually reduced or eliminated through various processing techniques such as cooking, drying, blanching etc. (Akwaowo et al., 2000; Akinyeye et al., 2011). Unfortunately, these techniques cause concomitant decrease in protein, fat, ash, various vitamins and minerals contents of those plants (Shokunbi et al., 2011; Ogbadoyi et al., 2011).

According to Akande et al. (2010) for one to authenticate the nutritional potential of a plant,

information on the type, nature and concentration of antinutrients present in that particular plant should be obtained. This assertion is corroborated by the report of Nwanjo et al. (2006) who concluded from their experiments that *P. macrophylla* is nutritional but its nutritive value is slightly hindered by the presence of antinutritional factors present in the seeds. The information thus obtained on the concentrations of the antinutrients will be valuable to plant breeders for designing breeding programmes. Breeding genotypes with low levels of these antinutrients is the best option in the long run for solving the problem of antinutrients. Lott et al. (2011) for example advocated for the breeding of crop genotypes with low levels of phytate. According to the authors, this will contribute to a global effort to enhance the appropriate utilization of phosphorus in agricultural production.

This research was, therefore, undertaken to assess some of the antinutrients present in the seeds of three nutritious plants (Okeke et al., 2008) that are common among the local people of the South East region of Nigeria. The species have been listed as part of the underutilized crop species in Africa. These are: (1) *Annona muricata* (Soursop, family Annonaceae). Compositional analyses have shown that the seeds contain 21.43 to 27.34% protein (Awan et al., 1980; Fasakin et al., 2008), carbohydrate (4.36%), fat (22.57%), magnesium (53.3%), iron (63.2%) and so on (Fasakin et al., 2008). The seeds along with the bark, leaves and stem of this species are very popular components of local preparations for handling cancer cases. They contain chemicals known as Annonaceous acetogenins. These chemicals are found only in the Annonaceae family and they have been reported to have antitumorous, antiparasitic, insecticidal, and antimicrobial activities. Numerous studies have shown that they selectively inhibit enzymes present only in the membranes of cancerous cells and thus they are toxic to cancer cells and non-toxic to normal cells (Morton, 1987; Oberlies et al., 1995; Gupta et al., 2011; Gajalakshmi et al., 2012). The seeds and roots, however, have been found to possess some alkaloids that have shown some neurotoxic effects. (2) *Corchorus olitorius* (Bush Okro, family Sparrmaniaceae). It has abundant levels of β -carotene, iron, calcium, vitamin C. It has been listed as one of the seven highly valued indigenous leafy vegetables in Nigeria (Adebooye et al., 2003) and one of one hundred orphan crops whose genome will soon be sequenced, assembled and annotated (Mars Incorporated, 2013). Its seeds are used medicinally as a purgative (Gupta et al., 2003) and they also have a broad spectrum of antibacterial activity (Pal et al., 2006). (3) *Pentaclethra macrophylla* (African oil bean, Family, Fabaceae) popular for its oil-rich seeds. The seeds, however, are inedible when raw and very bitter until the final stage of fermentation. They have been reported to contain high quantity protein (9.31%) and the 20 amino

acids (Achinewhu, 1982; Ikhuria et al., 2008). One of the plants used in this study had earlier been listed by IPGR, 2002 as among the neglected and underutilized crop species of Africa so as much information as possible are needed on them to guide their improvement.

MATERIALS AND METHODS

The seeds of *P. macrophylla* and the fruits of *A. muricata* were sourced from Nsukka town in Enugu State of Nigeria while the seeds of *C. oltorius* were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation, Ibadan, Nigeria. The samples were authenticated by Mr Alfred Ozioko of Biodiversity and Conservation programme/International Centre for Ethnomedicine and Drug Development located at No. 110 Aku Road Nsukka. The seeds of *A. muricata* were separated from the flesh and oven-dried. The method of Amadi et al. (2011) was used to induce fermentation for 2 days in some of the seeds of *P. macrophylla*. Cooked dehulled and oven-dried samples were used as the unfermented sample. All the respective seeds were separately ground into powder with the aid of a blender. For assessing the levels of oxalate and alkaloid, one gram (1 g) of each powder was used respectively while 0.5 g was used for cyanogenic glycosides, phytate and tannins. Pearson's (1976) methods were used for all the assessments. The procedures were as follows:

1. Determination of phytic acid (Pearson 1976):

- a. 0.5 g of the sample was weighed into a test tube.
- b. This was macerated with 20mls of 1.2% Hydrogen Chloride + 10% Sodium sulphate.
- c. The tube was shaken vigorously for 10 min and allowed to stand for 2 h with intermittent shaking every 10 min.
- d. The solution was subsequently filtered.
- e. 5 ml of the filtrate were transferred into triplicate tubes.
- f. 5 ml of water and 6 ml of 2 g ferric chloride + 17 ml hydrogen chloride per litre were added and these were:
 - i. Mixed and boiled for 75 min in a water bath.
 - ii. Cooled for one hour at room temperature
 - iii. Centrifuged for 15 min at 3000 r.p.m.
 - iv. The supernatant was decanted and
 - v. The residue was washed with 0.6 hydrogen chloride + 2.5% sodium sulphate.
 - vi. The residue after washing was centrifuged again and decanted.
 - vii. To the residue, 5 ml of concentrated Nitric acid and 4 ml of concentrated sulphuric were added and
 - viii. Transferred to a girdal flask.
 - ix. The mixture was heated on a hot plate for 30 min until only the sulphuric acid remained.
 - x. It was allowed to cool after which 5 drops of Hydrogen peroxide were added and the mixture heated for further 10 min.
 - xi. It was cooled and 3 ml of normal hydrogen chloride were added and the mixture was heated for 5 min.
 - xii. It was neutralized with 5 normal sodium hydroxide and made up to 10 ml with water and the phosphorus content determined.

2. Determination of phosphorous (Pearson, 1976):

- a. 5 ml of the digest was transferred into triplicate tubes.
- b. 2 drops of nitric acid and 2.5 vanadate molidate reagent was added.
- c. These were mixed and 2.5 ml of water added and
- d. The absorbance was taken at 470 nm against a blank.

3. Determination of oxalate (Pearson, 1976):

- a. 1 g of the sample was weighed and put in a test tube.
- b. 47.5 ml of water and 2.5 ml of 6 normal hydrogen chloride were added to the tube.
- c. The mixture was boiled for an hour and made up to 62.5 ml with water.
- d. The tube was cooled at room temperature and the contents subsequently filtered.
- e. 12.5 ml of the filtrate was taken and the pH was adjusted to fall between 4.0-4.5 with dilute ammonia (NH₃).
- f. This was heated up to 90°C and subsequently filtered.
- g. The mixture was heated again up to 90°C
- h. 5 ml of calcium chloride was added with constant stirring.
- i. The tube was allowed to stand overnight.
- j. Centrifuged for 5 min and the supernatant decanted off.
- k. The precipitate was dissolved with 5 ml of 20% sulphuric acid.
- l. And the mixture heated until about to boil.
- m. This was titrated with 0.05 normal standard potassium permanganate (KMnO₄) until pale pink colour that persists for 30 s.

4. Determination of alkaloid (Pearson, 1976):

- a. 1 g of the sample was weighed and put in a test tube and macerated with 10 ml of 20% sulphuric acid and 10 ml of ethanol for 10 min. The tube was allowed to stand for an hour with intermittent shaking and subsequently centrifuged for 5 min.
- b. 0.5 ml of the supernatant was transferred in triplicate tubes.
- c. 2.5 ml of 60% sulphuric acid was added and the two were mixed.
- d. 2.5 ml of 0.5% formaldehyde was subsequently added and the test tubes were allowed to stand for 3 h.
- e. The absorbance was taken at 565 nm against a blank.

5. Determination of cyanogenic glycoside (Pearson, 1976):

- a. 0.5 g of the sample was weighed and put in a test tube.
- b. The sample was macerated with 20 ml of phosphate buffer pH 6 for 10 min.
- c. The test tube was allowed to stand for an hour with shaking every 10 min interval.
- d. It was later centrifuged for 5 min.
- e. 1 ml of the supernatant was transferred into triplicate tubes.
- f. 4 ml of alkaline picrate was added and boiled for 5 min in a water bath.
- g. The tube was cooled in cold water and the absorbance was taken at 470 nm against a reagent blank.

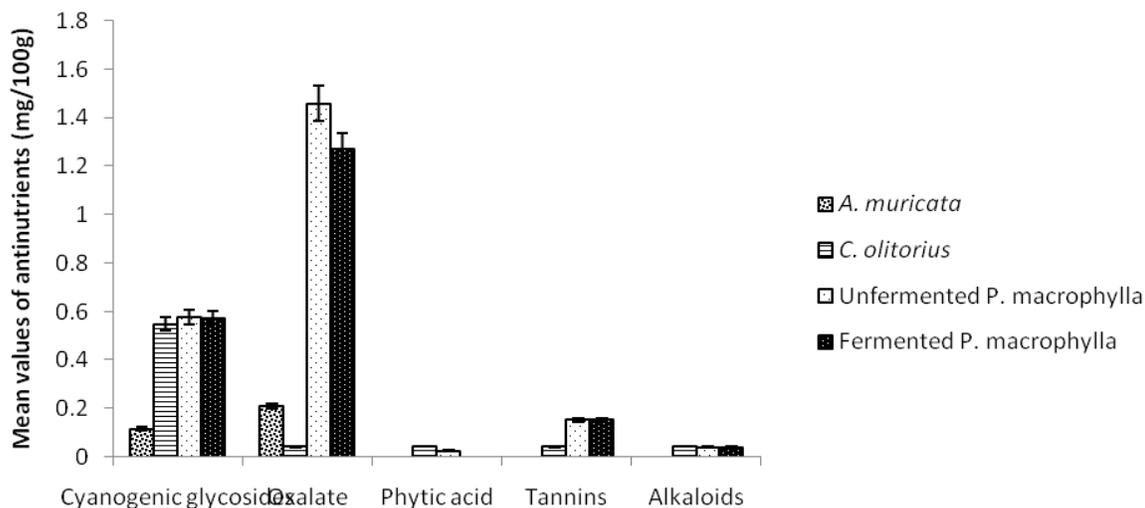
6. Determination of tannin (Pearson, 1976):

- a. 0.5 g of the sample was weighed and put in a test tube.
- b. The sample was macerated with 20 ml of methanol for 10 min and centrifuged for 5 min at 3000 r.p.m.
- c. 5 ml of the supernatant was transferred into triplicate tubes.
- d. 0.3 ml of 0.1 M ferric chloride in 0.1 M hydrogen chloride were added.
- e. The solution was mixed.
- f. 0.3 ml of 0.0008 M potassium ferricyanide was added.
- g. The solution was mixed.
- h. The absorbance was taken after 5 min at 720 nm against a blank.

The design of the experiments is as shown in Table 1. The means of the antinutrients for each species were calculated and subsequently used to plot a bar chart to depict graphically the contents of the antinutrients in each of the species.

Table 1. Design of experiments – randomised complete block design with three replications for each antinutrient per species was employed.

Antinutrients/species	<i>A. muricata</i>	<i>C. olitorius</i>	<i>P. macrophylla</i>	
			Unfermented	fermented
Cyanogenic glycosides	1	1	1	1
	2	2	2	2
	3	3	3	3
Oxalate	1	1	1	1
	2	2	2	2
	3	3	3	3
Phytic acids	1	1	1	1
	2	2	2	2
	3	3	3	3
Tannins	1	1	1	1
	2	2	2	2
	3	3	3	3
Alkaloids	1	1	1	1
	2	2	2	2
	3	3	3	3

**Figure 1.** Comparison of the antinutrients of the species.

RESULTS AND DISCUSSION

All the antinutrients were found in the species but at varying levels. A combined comparison of the antinutrients for all the species is depicted in Figure 1. Oxalate was found to be the highest antinutrient and this high content was found in unfermented and fermented seeds of *P. macrophylla*. The content in unfermented seeds was, however, higher than that of fermented seeds. The presence of cyanide, oxalate and saponins in

P. macrophylla were also observed by Onwuliri et al. (2004).

The minimum lethal dose of cyanogenic glycoside orally taken by man has been reported to be between 0.5 mg and 3.5 mg/kg body weight (Wobeto et al., 2007; Fowomola, 2010) while the lethal dose for cattle and sheep is 2.0 to 4.0 mg per kg body weight (Kumar, 1991). The concentrations obtained in this study were 0.551 ± 0.0165 mg/100 g for *C. olitorius*, 0.577 ± 0.0004 mg/100 g for unfermented seeds of *P. macrophylla*, 0.575 ± 0.003

mg/100 g for fermented seeds of *P. macrophylla* and 0.1183 ± 0.3687 mg/100 g for *A. muricata*. Thus *C. olitorius* and *P. macrophylla* had high concentrations of cyanogenic glycosides which were above the acceptable limits. Fermentation did not really reduce the concentration in *P. macrophylla*. This is not in consonance with the report of Amadi et al. (2011) who observed a reduction in the concentrations of phytates, tannins, saponins and complete elimination of flavonoids, alkaloids and cyanogenic glycosides in *P. macrophylla* following fermentation. The antinutrient, cyanogenic glycosides have been linked to many of the diseases associated with the central nervous system (Kolawole and Obueh, 2013).

Reports have shown that the lethal dose of oxalate is between 200 and 500 mg/100 g (Pearson, 1976). Noonan and Savage (1999) noted that the intake of 4 to 5 g of oxalate is the minimum dose that can result to death in an adult human. They further reported that a number of authors had showed that 10 to 15 g could be lethal. The levels obtained in this study were within acceptable limits. They ranged from 0.044 ± 0.0443 to 1.460 ± 0.002 mg/100 g.

The tannin content of the species studied ranged from 0.0039 ± 0.0004 to 0.155 ± 0.002 mg/100 g. These values were very low when compared with values reported by several researchers. Aletor and Adeogun (1995) reported that high level of tannins (76 to 90g kg DM⁻¹) could be very lethal if consumed. Sheep that consumed 0.9 g hydrolysable tannins kg/body weight showed signs of toxicity in 15 days (Kumar, 1991).

Large amounts of phytic acids have been reported to be present in fiber-rich foods. Such food, however, are pharmacologically recommended because they protect humans from cardio vascular diseases and some forms of cancer (Ensminger and Ensminger, 1996; Norhaizan and Nor-Faizadatul-A, 2009). In spite of this advantage, phytic acid reduce bioavailability of minerals because it has strong binding affinity to them. This chelation process increases the incidence of mineral deficiency diseases because the minerals are made unavailable for absorption by the intestine (Ekholm et al., 2003). Fortunately, the phytic acid contents observed in this study were quite low.

From this study, it can be concluded that cyanogenic glycosides are the antinutrients to consider while designing programmes for the improvement of *C. olitorius* and *P. macrophylla*. Some authors (Khokhar and Apenten, 2003; Soetan, 2008), however, made some observations concerning the complete removal of antinutrients through classical breeding or through biotechnological techniques. They noted that:

1. Since the antinutrients are critical for the survival of the plants that harbor them, complete removal may lead to reduction in growth of the plants as well as reduction in yield.
2. Complete removal will also eliminate the

pharmacological and medicinal properties of these compounds. Many of them have been reported to possess anticarcinogenic activity (for example, phytates, saponins, phenolic acids etc), antimicrobial activity (saponins, flavonoids, tannins), anthelmintic activity (tannins, saponins), hypocholesterolaemic activity (saponins) and pharmacological applications (tannins, saponins and flavonoids are constituents of several drugs).

The authors, therefore, suggested that both the adverse and beneficial properties of the compounds should be borne in mind by both the plant breeders and nutritionists while designing programmes for the improvement of the quality of the species.

Conflict of Interests

The authors wish to declare that there are no conflicts of interests in this work.

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