

ANTINUTRITIONAL AND PHYTOCHEMICAL EVALUATION OF RAW AND FERMENTED AFRICAN LOCUST BEAN (*PARKIA BIGLOBOSA*) SEEDS

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ABSTRACT

The anti-nutritional and phytochemical analyses of raw and fermented seeds of *Parkia biglobosa* were carried out. The unfermented seeds contained low levels of anti-nutrients (oxalates and phytates) and these were significantly reduced by fermentation. Fermentation also resulted in the release and detection of some bioactive phytochemicals (flavonoids and total phenolics) while reducing the levels of some saponins.

KEYWORDS: Fermentation, *Parkia biglobosa*, locust bean, phytochemicals, anti-nutrients

INTRODUCTION

Fermented locust bean, known to the Yorubas of south western Nigeria as *iru* and to the Hausas of northern Nigeria as *dawadawa* is a very popular condiment traditionally prepared from African locust bean (*Parkia biglobosa*). *Parkia biglobosa* is a wild legume and many of these wild legumes are known for their inexpensive proteins, high calorific value, essential amino acids, essential fatty acids, fiber and vitamins; but the presence of anti-nutrients in the seeds has limited their use (Bhat & Karim, 2009). Anti-nutrients or anti-nutritional factors are substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms which exert effects contrary to optimum nutrition such as inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed (Liener & Kakade, 1980), although some anti-nutrients may exert beneficial health effects at low concentrations. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties.

Seeds of legumes may account for up to 80% of dietary protein and may be the only source of protein for some groups (Aidoo, 1986). Their cooked forms are eaten as meals and are commonly used in fermented form as condiments to enhance the flavors of foods (Achi, 1992; Oniofiok *et al.*, 1996). With high contents of protein, legume condiments can serve as a tasty complement to sauces and soups and can substitute for fish or meat.

Many food flavoring condiments are prepared by traditional methods of uncontrolled solid substrate fermentation resulting in extensive hydrolysis of the protein and carbohydrate components (Fetuga *et al.*,

1973; Eka, 1980). Quite often seeds that are used for fermentation are inedible in their unfermented or uncooked state (Achi, 2005) and fermentation markedly improves the palatability, digestibility, and nutritive value of the raw seeds, in addition to increasing the shelf life and reducing the anti-nutritional factors (Odufa, 1985b; Reddy and Pierson, 1999; Barimalaa *et al.*, 1989; Achi and Okereka, 1999).

This work thus aims at investigating the effect of different degrees of fermentation on the phytochemicals and anti-nutrients of *P. biglobosa*.

EXPERIMENTAL METHODS

Sample Collection and Preparation: *Parkia biglobosa* seeds were obtained from Ilorin, and handpicked to remove stones and bad ones. The two types of fermented samples (partially and completely fermented) were obtained from a local market in Ilorin. All samples were ground to smaller particle size and the fermented samples were air-dried before use.

Reagents: All Chemicals used were of high purity and of analytical grade. All solvents were distilled before use.

Phytochemical Screening: Each sample was separately macerated in each of the following solvents: n- hexane, ethyl acetate, methanol, and water. The extracts were then filtered and the filtrates concentrated.

The methods of Trease and Evans, (1989); Sofowora, (1993); Ayoola, *et al.*, (2008) and Savithramma *et al.*, (2011) were used to identify the phytochemicals present in the extracts. The phytochemicals tested for are tannins, saponins,

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alkaloids, cardiac glycosides, anthraquinones, flavonoids, terpenoids, steroids, anthocyanins and coumarins.

Quantitative Determination of Anti Nutrients and Phytochemicals

Determination of Phytate Content: 4.0 g of each sample was soaked in 100 ml of 2% HCl for 3 hrs and 25 ml of the filtrate was titrated with a standard iron (II) chloride solution using 0.3% ammonium thiocyanate as indicator until a brownish yellow colour appeared which persisted for 5 mins (Ajayi, 2011).

Determination of Oxalate Content: 75 ml of 3.0 M H₂SO₄ was added to 1 g of ground sample, stirred and filtered. 25 ml of the filtrates (extract) was titrated hot (80-90°C) against 0.05 M KMnO₄ solution to the point when a faint pink colour appeared that persisted for at least 30 seconds (Jrand and Underwood, 1986).

Determination of Cyanide Content: 4 g of each sample was soaked in a mixture containing 40 ml of distilled water and 2 ml of orthophosphoric acid, mixed, stoppered and left overnight at room temperature to free all bounded hydrocyanic acid. 5 ml of the resulting mixture was distilled into 40 ml of distilled water containing 0.1 g of NaOH pellets. The distillate was made up to 50ml with distilled water and 20 ml of this was titrated against 0.01 M silver nitrate solution using 1.0 ml of 5% potassium iodide solution to an end point indicated by a faint but permanent turbidity (AOAC, 1990).

Determination of Saponin Content: 5 g of each sample was dispersed in 100 ml of 20% ethanol. The suspension was heated and filtered, and the residue was re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was extracted with 20 ml of diethyl ether and the ethereal layer was discarded. The aqueous layer was extracted twice with n-butanol and washed twice with 10 ml of 5% aqueous sodium chloride. The solution was then evaporated over a water bath and dried to a constant weight.

Determination of Total Phenolics: 2 g of each sample was soaked in 100 ml n-hexane for 4 hours to remove all fats. The residue was extracted with 50 ml diethyl ether, and the ether extract was extracted into 50 ml of

10% NaOH solution. The aqueous layer was acidified to pH 4.0 with 10% HCl solution and then extracted into 50 ml dichloromethane (DCM). The organic layer was finally collected, dried and then weighed (Bohm and Kocipai, 1994).

Determination of Flavonoid Content: 10 g of each sample was extracted with 100 ml of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered and the filtrate evaporated to dryness over a water bath. (Bohm and Kocipai, 1994).

RESULTS AND DISCUSSION

The result of the various determinations carried out on unfermented seed (ALBS), partially fermented (PFALB) and completely fermented (CFALB) seeds of African Locust beans are presented below.

Result of Phytochemical Screening.

The results of phytochemical screening of aqueous, methanolic, ethyl acetate and n-hexane extracts of the samples are presented in Table 1 below

n-Hexane Extracts: The result showed the presence of glycosides, terpenoids and anthraquinones in all three samples, flavonoid was detected in the two fermented samples, while saponins, alkaloids, steroids, anthocyanins, coumarin and tannins were not detected in any of the three samples. The non-detection of flavonoids in the raw unfermented sample may be because flavonoids were present in the unfermented sample in a combined form (probably as part of a giant compound), and was only released during fermentation by a certain action of the microorganism responsible for fermentation.

Ethylacetate Extract: the screening revealed the absence of alkaloids, tannins, coumarin and anthocyanins; the presence of saponins, steroids, glycosides and terpenoids; while flavonoids and anthraquinones were only found in the two fermented samples.

Methanolic Extracts: the result showed the presence of alkaloids, saponins, terpenoids and anthraquinones in the methanolic extract of the three samples. Tannins, flavonoids, glycosides, steroids and anthocyanins were not detected in the sample extracts. Coumarin was detected only in the two fermented samples.

Table 1: Phytochemical Screening

Phyto	Ethylacetate			Methanol			N- Hexane			Water		
	1	2	3	1	2	3	1	2	3	1	2	3
Alkaloids	-	-	-	+	+	+	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	-	-	-	+	+	+
Tannins	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	+	+	-	-	-	-	+	+	-	-	-
Glycosides	+	+	+	-	-	-	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	-	-	-
Steroids	+	+	+	-	-	-	-	-	-	-	-	-
Anthraquinones	-	+	+	+	+	+	+	+	+	+	+	+
Coumarin	-	-	-	-	+	+	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-

Key: 1= ALBS 2=PFALB 3= CFALB

Aqueous extracts: Saponins, glycosides and anthraquinones were found to be present in the aqueous extracts of the three samples; while other phytochemicals were not detected in the aqueous extract of the three samples.

The result of the phytochemical screening showed that the fermented samples contain more bioactive compounds than the unfermented sample. The presence of these bioactive agents means the samples also have therapeutic value in addition to the already established nutritional value.

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Saponins have been reported to have both deleterious and beneficial effects including membranolytic effects, toxic and fungitoxic effects, adverse effects on animal growth and

performance and hypocholesterolemic effects (Price *et al.*, 1987). Steroids found only in the ethyl acetate extract, are of importance and interest in pharmacy due to their relationship with sex hormones (Santhi, *et al.*, 2011). Terpenoids have also been shown to decrease blood sugar levels in animal studies. Flavonoids are natural phenolic compounds and well known antioxidants. Phenolic compounds are ubiquitous bioactive compounds and a diverse group of secondary metabolites universally present in plants.

Quantitative Anti Nutrient and Phytochemical Contents

Oxalate: The oxalate contents of the samples were found to be 0.13, 0.05 and 0.04 mg/100 g respectively, which mean that the process of fermentation leads to a great reduction in the oxalate contents.

Table 2: Quantitative determination of Anti nutrients and Phytochemicals

Sample	Oxalate (mg/100 g)	Phytate (mg/100 g)	Cyanide	Flavonoid (g/100 g)	Saponin (g/100 g)	Total Phenolics (g/100g)
ALBS	0.13 ^a ±0.00	2.19±0.10 ^a	-	-	6.19±0.54 ^a	-
PFALB	0.05 ^b ±0.00	2.00±0.03 ^a	-	14.51±0.17 ^a	2.36±0.13 ^b	6.17±0.17 ^a
CFALB	0.04 ^b ±0.00	1.50±0.02 ^b	-	9.27±0.06 ^b	2.20±0.11 ^b	4.75±0.33 ^b

Values are mean±S.D of triplicate determination. Values followed by same alphabet along the columns are not significantly different at $p = 0.05$

Oxalates may be present in plants as soluble salts such as potassium, sodium or ammonium oxalate. Oxalic acid is a weak reducing agent that is readily oxidized to carbon dioxide and water by potassium permanganate in H_2SO_4 solution. It reduces calcium availability both in man and in non-ruminants, at higher dose of 1g to 2 g/kg of body weight. Oxalic acid is toxic to the kidney and heart. Symptoms of mild oxalate poisoning include abdominal pains and gastroenteritis. In severe cases, it can cause diarrhea, vomiting,

convulsions, non coagulability of blood, coma and kidney disease (Akpabio *et al.*, 2012). Higher oxalate content contains more than 10mg per serving, while low content has less than 2mg per serving. The analysis of variance showed that the oxalate content of the unfermented seeds vary significantly ($p < 0.05$) from those of the fermented samples. On the other hand, oxalate contents of the two fermented samples did not vary significantly, ($p > 0.05$). Generally the oxalate content of the samples, especially the fermented

samples is low and do not pose any threat on consumption.

Phytate Content: The phytate contents of the samples are 2.19, 2.00 and 1.5 mg/100g, for the unfermented seed, the partially and the completely fermented seed samples respectively. Phytic acid, a hexaphosphate or inositol is an important storage form of phosphorus in plants. It is insoluble and cannot be absorbed in the human intestines. Phytic acid has 12 replaceable hydrogen atoms with which it can form insoluble salts with metals such as calcium, iron, zinc, and magnesium. The formation of these insoluble salts renders the metals unavailable for absorption into the body (Aregheore and Agunbiade, 1991; Akpabio *et al.*, 2012). Aregheore and Agunbiade (1991) reported that, cooking does indeed destroy anti-nutritional factors which are toxic to health and make dietary minerals available for absorption. The phytate content is seen to reduce with fermentation and further reduction is expected with cooking. The minimum amounts of phytic acid to cause negative effect on iron and zinc absorptions are 10 – 50mg per meal (Sanberg, 1991). The analysis of variance showed that the phytate content of the unfermented seeds vary significantly ($p < 0.05$) from that of the completely fermented sample. On the other hand, phytate contents of the partially fermented and the unfermented samples did not vary significantly, ($p > 0.05$).

Cyanide content: cyanide was not detected in any of the three samples.

Flavonoids content: The amount of flavonoid found in the two fermented (partially and completely) samples are 14.51 and 9.27 g/100 g respectively. Flavonoid was not detected in the unfermented sample. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis. The presence of flavonoids in the intestine lowers the risk of heart diseases (Okwu, 2004). The flavonoids content of the two fermented samples vary significantly ($p < 0.05$).

Saponin content: the saponin contents of the samples (6.19, 2.36 and 2.20 g/100 g for the unfermented, partially and completely fermented samples respectively) showed a gradual reduction in the saponin contents from the unfermented, through the partially fermented, to the completely fermented samples. Some of the popularly known characteristics of saponins include formation of foams in aqueous solution and hemolytic activities (Okwu and Emenike, 2006). Saponins have natural tendency to ward off microbes which makes them good candidates for treating fungal and yeast infections. These compounds serve as natural antibiotics, helping the body to fight infections and microbial invasions (Okwu 2004, 2005, Okwu and Emenike 2006). Plant saponins help humans to fight fungal infections, combat microbes and viruses, decrease blood lipids, lower cancer risks and lower blood glucose response (Shi *et al.*, 2004). They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospect for

saponins is how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells. Cancer cells have more cholesterol-type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus interfere with cell growth and division (Okwu 2005, Okwu and Emenike, 2006). The saponin contents of the unfermented seeds vary significantly ($p < 0.05$) from those of the fermented samples. On the other hand, saponin contents of the two fermented samples did not vary significantly, ($p > 0.05$).

Total Phenolics: the phenolic content of the two fermented samples are 6.17 and 4.75 g/100 g of wet sample for the partially and the completely fermented samples respectively. Phenols protect plants from oxidative damage and perform the same functions for humans (Okwu, 2005). The outstanding phytochemical feature of phenols is their ability to specifically block enzymes that causes inflammations. They also modify the prostaglandin pathways, thereby protecting platelets from clumping (Okwu and Omodamiro, 2005). Phenolic compounds can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels (angiogenesis) that is necessary for tumour growth. They also attenuate adhesiveness and invasiveness of cancer cells thereby reducing their metastatic potential (Wahle *et al.*, 2010). The phenolics content of the two fermented samples vary significantly ($p < 0.05$).

CONCLUSION AND RECOMMENDATION

This work has shown that fermentation leads to appreciable reduction in the amount of anti nutritional factors present in the seeds, while increasing the availability of phytochemicals like flavonoids, which was not detected in the unfermented sample, but found in the two fermented sample extracts. Fermented locust beans can thus be seen as functional foods, having high medicinal values in addition to the well-known and well established nutritional values.

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