



## Investigation of phytochemical, anti nutrients and food toxicants composition of tender leaves of black-eyed bean for food

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### Abstract

This research aims to investigate the phytochemical, anti nutrients and food toxicants composition of tender leaves of fresh and shade dried Black-eyed bean. Chemical analyses were carried out using standard method of Association of Official Analytical Chemists, (2000). The analyses of phytochemical compositions showed that both fresh and shade dried tender leaves of Black-eyed bean contains considerable amount of Phenolic compounds, Flavonoids, Alkaloids, Tannins, Saponins, and Cardiac glycosides with the shade dried sample having higher values whose difference is statistically significant ( $p < 0.05$ ). The research also showed that both the fresh and shade dried leaves contained low values of oxalates, phytates, cyanides, with the shade dried leaves having higher values whose difference is statistically significant ( $p < 0.05$ ). The heavy metals analyses showed that both the fresh and the shade dried leaves contains a very low amount of cadmium and lead, however, there is no significant difference between the fresh and the shade dried leaves ( $p > 0.05$ ). Thus, this findings showed that this vegetable contains substantial amount of phytochemicals and low level of anti nutrients which are within the tolerable level of WHO/FAO exception of cyanide. However, simple food preparatory techniques can remove the cyanide contents to the safe limit.

**Keywords:** phytochemicals, anti nutrients, toxicants, black-eyed bean leaf

### Introduction

Black-eyed bean (*Vigna unguiculata*) a member of the family fabaceae, is an annual legume thought to have originated in Africa (Davis, *et al.*, 2007). The Black-eyed bean (cowpea) popularly known as egwa (Igala), ewa (Yoruba), akwa (Igbo) and wake (Hausa) has been referred to as a “poor man’s meat” due to high level of protein found in the seeds and the leaves (Nielsen, *et al.*, 1997) <sup>[19]</sup>, the leaves have a richer phytochemicals and nutritional compositions compared with the grains (Mamiro, 2012). The utilization of cowpea leaves for food has mainly been done in various dishes, soups, and sauces in some part of Africa as leafy vegetable (Imungi & Potter, 2013).

Over the past 25 years epidemiological studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables (Kearo, Popkin & Frison, 2010) <sup>[12]</sup>. Countries like South Korea, a high income country that have undergone rapid social change and economic development since the 1970s, still have lower rate of obesity and other non-communicable diseases than the countries with comparable average income. This is because South Korea has protected its traditional food systems. These foods are relatively high in vegetables and fruits (Lee, Popkin & Kim, 2002) <sup>[15]</sup>. Equally numerous empirical and investigative reports have indicated that current non communicable diseases (NCDS) trend in Africa can be attributed to rapid shift from traditional foods which contain mostly vegetables to western food products resulting in elevated intake of saturated fats and food preservatives with reduced intake of dietary fiber, vital nutrients and phytochemicals when compared to basic dietary guidelines (Nahurung, 1997; Gupta, 2011). The shift from traditional foods to western food products has been

dubbed the nutrition transition and is directly implicated in the rise of type 2 diabetes, cardiovascular and other NCDs (Uguru, 2005) <sup>[29]</sup>. Past generations whose diets consisted mainly of herbs, fruits, vegetables, nuts and starchy tubers lived longer than the present generation (Sathanaraynan, Thomas, Fashik & Sekher, 2009) <sup>[27]</sup>. They were not victims of the many health problems faced by the present population (Uguru, 2005) <sup>[29]</sup>. Life expectancy was better in the past because vegetables were a major component of the diet (Sathanaraynan, *et al.*, 2009) <sup>[27]</sup>. Vegetables should be adequately included in the diet to help fight against the deadly scourge diseases. According to Socrates, a Greek philosopher, Fruits and vegetables are the earliest source of food to mankind (Largen, 1984). Equally Tutare (2000) reported that there are over 200 varieties of vegetables to which majority of Nigerians are not accustomed to. The major reason for less exploitation and utilization of fruits and vegetables in Nigeria is due to ignorance of their contribution to adequate nutrition (Kubmarawa, *et al.*, 2009; Nnam, 2011) <sup>[13]</sup>.

During the last decade the concept of health promotion using fruits and vegetables has become legitimate part of health care (Nielsen, 2010). There is an increasing preference expressed by many patients in recent time towards the popular use of alternative therapies that include food supplements and herbal/folklore preparation with antioxidant potentials. This is because of the much scientific evidence available to support their efficacies in the control of nutritionally related metabolic disorders and long term complications (WHO, 1980; Shittu, Bankole & Ashiro, 2007). Anemia and diabetes blog are all over research linking increased consumption of vegetables with protective health benefit. Treatment with diets has fewer side effects.

Moreover foods are cheap and readily available even in the rural community.

There is low level of information on the phytochemical/antioxidant potential of tender leaves of Black-eyed bean as remedy in the management of diseases. Hence, in a bid to create and step up awareness on the usage; this research sought to investigate the phytochemical, antinutrients and food toxicants composition of tender leaves of Black-eyed bean grown in Kogi State, Nigeria.

## Materials and Methods

### Materials

#### Chemical and reagents

All chemicals and reagents were of analytical grade obtained in Biochemistry laboratory, Kogi State University, Anyigba, Nigeria.

#### Collection of plants materials

The vegetable used for the study was tender leaves of Black-eyed bean. The tender leaves of Black-eyed bean were obtained fresh from farm in Ibaji, Kogi State in September 2020. The vegetable was identified in the Department of Biological sciences, Kogi State University Anyigba, Nigeria.

### Methodology

#### Preparation of sample

About 3kg of Black-eyed bean leaves was separately sorted by removing extraneous materials, washed with distilled water, and then, dried under shade for about 10 days at room temperature in Food Science laboratory, Kogi State University. The dried leaves and fresh parts were separately pulverized using Gallenkamp mixer Kenwood –MPR 201. About half of the pulverized leaves were used for chemical analysis.

#### Chemical analysis

The Alkaloids, saponins, tannins, cardiac glycosides, phytates, cyanide and oxalates compositions of fresh and shade dried tender leaves of Black-eyed bean were determined using standard methods of AOAC, (2005), phenolic compounds and flavonoids were determined using Folin-ciocalteu method (Lee, *et al.*, 2015)<sup>[16]</sup> and Dowd method (Avouet-Grant, *et al.*, 1994) respectively while heavy metals were done by stripping Chronopotentiometry as described by Loco, (2004)<sup>[17]</sup>.

#### Statistical analysis

Data were collected and the statistical analysis was run using Graphpad stat software. The result of the triplicate was pooled and analyzed using descriptive statistics (mean and standard deviation) and the difference between fresh leafy vegetable and the shade dried leafy vegetable were analyzed using t- test. The means were considered significant ( $p < 0.05$ ).

### Conclusion

Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties (Oguntona, 1986). The result of this study shows that phytochemicals were highly present in the fresh and shade dried leaves of black-eyed bean relative to the nutrients. The levels of the phytochemicals were higher in the shade dried sample compared to fresh sample ( $p < 0.05$ ). The reason could be due

to concentration effect. This is similar with literature report that phytochemicals are present in all vegetables in different amount (Nnam, 2011). The values of the total phenolic compound were lower than the values reported by Sushant, *et al.*, (2019) on sundried leaves of *Cassia tora*, but higher than the report by Krishna, *et al.*, (2019) on shade dried *Solanum nigrum* leaves. Flavonoids level was higher than that reported by Vidjay, *et al.*, (2014) on *Hibiscus tillaceus*. Phenolic compounds are known for their various functions in plants, pigmentation, growth and reproduction and for resistance against pathogens and fungi (Lactanzo, *et al.*, (2008). Phenolic compound act as an antioxidant, it presents in plants has an important role in the reduction of lipid oxidation in plants and animal tissues because when incorporated into human diet, it does not only conserve the quality of the food but reduces the risk of developing some diseases. The high levels of phenolic compounds and flavonoids attribute antioxidant action on black-eyed bean leaves by scavenging free radical donating hydrogen atom, electrons or chelate metal radicals thereby reducing the risk of cancer and cardiovascular diseases (Whitney and Relfe, 2015). Based on this report, the consumption of little quantity of the Black-eyed bean leaves as leafy vegetable could be of health benefit to human.

Alkaloid levels recorded was similar to the report by Vijay, *et al.*, (2014) on *Hibiscus tillaceus* in India. Alkaloids are nitrogen containing naturally occurring compounds. They are commonly found to have antimicrobial properties due to their ability to intercalate with DNA of micro organisms (Kasolo, *et al.*, 2010). This could be responsible for their medicinal values. Cardiac glycoside levels observed were comparably higher than the reported by Nwankwo, (2014)<sup>[22]</sup> on *Hibiscus canibinnus* fresh leaves. Cardiac glycoside battles against cancer and heart diseases due to their antioxidant properties. Several studies have reported beneficial effect of the therapy with antioxidant phytochemicals against cardiovascular consequence of diabetes (Ruhe, 2001). Glycosides present in the leaves are known to protect the body by decreasing the risk of heart diseases, stroke and certain types of cancers (Nnam, *et al.*, 2011). This study suggest that the use of this vegetable in the preparation of diet for healthy and convalescents could reduce the risk of diseases.

Saponin levels in the vegetable have the potential of lowering cholesterol levels in humans due to their hypercholesteralmic effect (Nnam, 2011). Saponins are bitter and could reduce the palatability of food when present in high amount. However, simple food preparatory techniques like blanching, sun drying other than shade drying could reduce their bitterness (Nnam, *et al.*, 2011). Recent research depicts that tannins when present in small amount could lower serum cholesterol and combat cancer (Whitney and Rolfes, 2005)<sup>[30]</sup>. The levels present in Black-eyed bean leaves were higher than that reported by Chikwendu (2014)<sup>[7]</sup> on fresh tender cowpea leaves. Despite the antioxidant actions of saponins and tannins, considerable amount of it play a role in the body, as such this vegetable could be use in the preparation of diet when subjected to proper processing methods like sun drying and blanching other than shade drying.

The phytate levels of the fresh and shade dried sample were reasonably low, but the shade dried sample has higher value which is significant at ( $p < 0.05$ ). The phytate levels of the vegetable were below the safe limit (5.00mg/100g)

(Bamishaiye, *et al.*, 2011)<sup>[5]</sup>. The levels of phytate observed here were comparable to the value reported by Agomuo, (2016)<sup>[1]</sup> on normal squeeze washed bitter leaf. However, the value were higher than the report by Nwankwo, (2014)<sup>[22]</sup> on *Hibiscus cannabinus* fresh leaves, but lower than the value reported by Chiwendu, (2014) on tender fresh and shade dried cowpea leaves.

The levels of the oxalate in both the fresh and the shade dried sample were low. The higher value observed in shade dried sample could be due to concentration effect. The values observed were higher than the values reported by Chiwendu, (2014) on fresh cowpea leaves. However, the values were within the safe limit (40-50mg/day) (www.tetrapak.com/substitute/food). The levels of phytate and oxalate in vegetables are of interest because studies have shown that, at lower dosage the antinutrient shows phytochemical beneficial effects. Phytate and oxalate in large amount forms insoluble salts with mineral elements such as zinc, calcium, iron and phosphorus to prevent their availability and utilization (Sariyan, *et al.*, 2010). The high levels of oxalate in black-eyed bean leaves could limit the absorption and utilization, hence, this research suggests that other traditional methods of processing like sun drying and blanching other than shade drying could be used to reduce the oxalate and phytate content of the vegetable in order to enhance proper mineral element utilization.

Cyanide levels in both fresh and shade dried samples were high, with shade dried sample having higher value ( $p < 0.05$ ). The high cyanide contents in both the fresh and shade dried sample implies that shade drying is not a better method of reducing antinutrients in vegetables and it also implies that cyanide content of vegetables varies from one location to another. The cyanide content observed in Black-eyed bean leaves was above the safe limit of 0.2 mg/100g (WHO, 2008) but, lower than the safe level by Oke, (1969) who reported that the safe level of cyanide in the role of hydrocyanide acid in nutrition is 35mg/100g. Evidence has shown that several factors such as abiotic factors (Temperature, soil components) and biotic factors (pest, herbivore, microbial infestation etc) affects cyanide concentration in plant (Boening, 1999). Also developmental stage or maturity stage (endogenous) as well as ecological factors (exogenous) determines cyanide levels. Cyanide when consumed in large quantity over a long period may prove toxic. However, blanching, sun drying and other simple food preparation methods would remove cyanide content of the vegetable to a safe limit.

Both the fresh and the shade dried sample contain trace of toxic metals (cadmium and lead). The result shows that there is no significant difference between cadmium and lead content of fresh and shade dried vegetable ( $p > 0.05$ ). The levels of cadmium and lead in the fresh and shade dried sample were within the safe levels of (0.2mg/100g) allowed by World Health organization standard for substances. Similarly, the values observed were lower than the value (0.03mg/100g) reported by Nwankwo, (2014)<sup>[22]</sup> on *Hibiscus cannabinus* fresh leaves. Cadmium and lead are inorganic metals (heavy metals) that are naturally present in the environment. Excess of cadmium and lead in the body causes toxicity and heart diseases. Leafy vegetables when processed and cooked are often free of food toxicants (Bokenga, 1994). This study suggests that the small quantity of the heavy metals in the leaf studied would be removed during food preparation.

**Table 1:** Phytochemical compositions of tender leaves of fresh and shade dried Black-eyed bean

| Photochemical composition (mg/100g) | FBBL <sub>1</sub>          | SBBL <sub>2</sub>          |
|-------------------------------------|----------------------------|----------------------------|
| Phenolic compounds                  | 127.50 <sup>b</sup> ± 0.24 | 201.42 <sup>d</sup> ± 0.36 |
| Flavonoids                          | 19.72 <sup>b</sup> ± 0.078 | 133.11 <sup>d</sup> ± 1.55 |
| Alkaloids                           | 20.83 <sup>b</sup> ± 0.23  | 80.75 <sup>d</sup> ± 0.11  |
| Tannin                              | 5.96 <sup>b</sup> ± 0.021  | 74 <sup>d</sup> ± 0.0070   |
| Cardiac glycoside                   | 0.11 <sup>b</sup> ± 0.001  | 0.46 <sup>d</sup> ± 0.002  |
| Saponin                             | 5.54 <sup>b</sup> ± 0.42   | 20.74 <sup>d</sup> ± 0.14  |

Mean ± SD, n= 3. Values in the same row with different superscripts were significantly different ( $p < 0.05$ ).

**Table 2:** Anti nutrients and food toxicant contents of tender leaves of fresh and shade dried Black-eyed bean

| Anti nutrients and food toxicants (mg/100g) | FBBL <sub>1</sub>           | SBBL <sub>2</sub>          |
|---------------------------------------------|-----------------------------|----------------------------|
| Phytate                                     | 0.085 <sup>a</sup> ± 0.0021 | 0.43 <sup>d</sup> ± 0.0014 |
| Oxalate                                     | 15.0 <sup>a</sup> ± 0.0035  | 43.0 <sup>d</sup> ± 0.0063 |
| Cyanide                                     | 1.10 <sup>a</sup> ± 0.077   | 1.78 <sup>d</sup> ± 0.0070 |
| Cadmium                                     | 0.011 <sup>a</sup> ± 0.001  | 0.010 <sup>a</sup> ± 0.001 |
| Lead                                        | 0.004 <sup>a</sup> ± 0.001  | 0.003 <sup>a</sup> ± 0.001 |

Mean ±SD, n= 3. Values in the same row with different superscripts were significantly different ( $p < 0.05$ ).

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