Proximate Analysis and Minerals of Black Bean Seeds (*Phaseolus vulgaris* L.) Used to Manage Sickle Cell Disease in West Region of Cameroon

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Authors’ contributions

This work was carried out in collaboration between all authors. Author TCK conducted the study and assays, managed the literature searches and wrote the first draft of the manuscript. Authors ACP, GK and EF designed the study, co-directed the research work. Authors PJ and PN wrote the protocol. Authors PJ and TCK managed the analyses of the study. Author PA supervised the research. All authors read and approved the final manuscript.

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ABSTRACT

**Objective:** The purpose of this present study was conducted to determine the proximate composition, and minerals of black bean seeds (*Phaseolus vulgaris* L.) used to manage Sickle Cell Disease (SCD) in West Cameroon Region.

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Methods: The Proximate composition was estimated by the standard procedures of the AOAC. Mineral contents were determined by X-ray fluorescence spectrometry. Results: The results revealed that black bean seeds contained moisture (8.268%), ash (3.063%), crude fat (1.718%), total protein (29.169%), carbohydrate (58.107%), crude fiber (9.397%), total dietary fiber (21.833%) and energy value (276.994 Kcal/100 g). The minerals analysis showed that potassium has the highest value (51.648 mg/100 g), followed by Phosphorus (6.022 mg/100 g), Magnesium (3.867 mg/100 g), Chloride (0.425 mg/100 g) and iron (0.357 mg/100g), while Zinc was the least (0.099 mg/100 g). Calcium was not detected.

Conclusion: The results of the study revealed that black bean seeds used to manage Sickle Cell Disease in West Cameroon Region are a good source of important nutrients such as carbohydrate, protein, fat, fiber and minerals. This study concluded that black bean seed contained immense nutritional therapeutic importance in the management of Sickle Cell Disease.

Keywords: Proximate analysis; minerals; black bean seed; sickle cell disease.

1. INTRODUCTION

Finding a widely expectable cure for Sickle Cell Disease (SCD) still remains a challenge even one hundred years after its discovery as a genetically inherited disease [1]. Hydroxyurea has been recommended since the 1990s as a first therapy but there are some serious side effects include reduction of white blood cells, decreased sperm production and cancer. Bone marrow transplantation has recorded good curative results of some Central Africa SCD children living in Belgium [2,3] and France [4]. Its application in Africa is difficult because of its highest cost (approximately one hundred thousand Euros) and lack of technical equipment [5]. Researchers are beginning to look into alternative more natural therapies. While these alternative treatments will not cure the disease, they may have the potential to reduce its symptoms without significant side effects. One area researchers have looked at nutritional alternatives to decrease morbidity and to improve quality of life among sickle cell patients [1]. Products from plants have been used as popular medicine in several countries, especially in developing nations of the world, as alternative treatments or management regimes for various conditions including SCD [6,7].

People with SCD need to have a well-balanced diet because their red blood cells break down faster. They should have adequate calories, protein, fat (macronutrients), vitamins and minerals (micronutrient). It is highly recommended that people with SCD consume food that is rich in protein, vitamins like folic acid, vitamins E, C, A and minerals (Mg, Zn, Ca and K) because that may be helpful with having less pain crises a year [8]. A recent study has shown that SS patients present a deficiency in micronutrients like Ca, Mg, K, P, Fe, Zn and Cu [9].

Common bean (Phaseolus vulgaris L.) is a nutrient-rich food that contains nutrients essential for humans, such as proteins, minerals, vitamins, carbohydrates, and fiber. The protein quality in common bean is high and many cultivars available have sufficiently high levels of essential and nonessential amino acids to meet the daily nutritional needs [10], according to standards of the Food and Agriculture Organization [11]. For minerals, the main bioavailable sources are foods of animal origin. However, in view of the high cost, they are inaccessible to many people [12]. In view of the high mineral content in the relatively cheap seeds, the consumption of common beans is beneficial to health and can be a means of prevention of a number of mineral deficiencies [13,10,14].

Black seeded bean (Phaseolus vulgaris L.) cultivars (wild variety) is used to manage Sickle Cell Disease (SCD) in West Cameroon Region. This is the ethnomedical investigation that we carried out in this area to know how populations of this locality manage SCD. The study showed that these patients usually consumed black bean seeds and it means 3 times per week. This consumption reduced significantly the frequency of the crises [15].

As the legume, common beans have a prominent role in human health and we must know about its composition. The present study was, therefore, initiated to know the proximate composition and minerals content of black bean seeds to evaluate its sickle cell patient’s health nutritional importance.
2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The seeds of black beans sample (Fig. 1) were obtained from sickle cell patients families and authenticated under (PNN) at the Agricultural Institute of Research for the Development of Foumbot station, Cameroon. In Agroprocessing and Natural Product Division of CSIR-National Institute for Interdisciplinary Science and Technology, Kerala-India, the raw whole bean seeds (1 kg) were weighted (Sartorius 15DGA balance) and pulverized using an electrical grinding machine (Microfluidics M-110P). The powdered material was passed through a 40-mesh sieve to a uniform size (1.5μm) and conserved in cold room at 6°C for further analysis.

Fig. 1. Photo of black bean seeds (Phaseolus vulgaris L.) PNN wild variety

2.2 Proximate Analysis

Moisture, ash, fat, protein, carbohydrate and crude fibre were estimated by the standard procedure of the AOAC [16] as described below in Agroprocessing and Natural Product Division.

2.2.1 Determination of moisture

Moisture was determined by oven drying method. About 3 g of powder seed sample was accurately weighed in a pre-weighed Petri dish and dried in a hot air oven at 105°C for 12 – 24 h. The dish with the sample was cooled in desiccators and weighed. This exercise was repeated till the difference in weight between two successive weighing becomes constant. From the weight loss during drying, amount of moisture was calculated using the following formula and the moisture can be represented in percentage.

\[ \text{Moisture (\%)} = \left( \frac{W_1 - W_2}{W} \right) \times 100 \]

\( W \) = Weight of sample with petridish before drying
\( W_2 \) = Weight of sample with petridish after drying
\( W \) = Weight of sample

2.2.2 Determination of ash content

For the determination of ash, 1 g of powder seed sample accurately weighed into pre-weighed were oven dried at 105°C for 24 hr. The dried samples were weighed and then transfer in clean crucibles. The crucibles were heated to the point of charring of the samples on hot plates. The crucibles with the carbon residue obtained as a result of ignition were placed in a muffle furnace at a temperature maintained at 650°C until the carbon residue disappears (6 hrs). The samples are allowed to cool and then weighed. From the difference in weight obtained the ash content was calculated using the formula:

\[ \text{Total ash content (\%)} = \left( \frac{\text{Weight of crucible with ash (g)}}{\text{Weight of crucible with sample (g)}} \right) \times 100 \]

2.2.3 Crude fat estimation

Crude fat was determined by hexane extract method using Soxhlet apparatus. Approximately 10 g of dried powder sample was taken in a thimble and plug the top of the thimble with a wad of fat-free cotton. Drop the thimble into the fat extraction tube of a Soxhlet apparatus. Attach the bottom the extraction tube to a Soxhlet flask. Pour approximately 75 mL or more of hexane through the sample in the tube into the flask. Attach the top of fat extraction tube to the condenser. Extract the sample for 6 h or longer on a heating mantle at 40°C. At the end of the extraction period, remove the thimble from the apparatus and concentrate the extract at rota evaporator at 40°C. Dry at 100°C for 1 h, cool and weigh. The difference in weights gives the ether-soluble material present in the sample.

\[ \text{Crude Fat (\%)} = \left( \frac{\text{Weight of hexane soluble material}}{\text{Weight of sample}} \right) \times 100 \]

2.2.4 Determination of total protein

Protein in the sample was determined by Kjeldahl method (Kjeltron KDJIGB 4M). The samples were digested by heating with
concentrated sulphuric acid (H$_2$SO$_4$) in the presence of digestion mixture. The nitrogen content in the plants mainly appears as proteins and amino acids and thus the total amount of nitrogen indicates the amount of total proteins and amino acids.

About 0.5 g each of sample and digestion mixture (copper sulphate + potassium sulphate) was weighed into a Kjeldahl flask and 10 mL of concentrated H$_2$SO$_4$ was added. The Kjeldahl flask was then heated on a mantle (in slanting position) until colour of solution changes to pale blue-green. This clear solution was made up to 25 mL under cold condition. The Kjeldahl apparatus was set up for protein estimation. 20 mL of 4% boric acid and 1 mL of mixed indicator (bromocresol green) was taken in a conical flask and placed under the condenser. 5 mL of sample with 20 mL of 40% NaOH and 10 mL water were added to distillation tube through funnel. When water starts boiling inside the round bottom flask, steam produced then passes into distillation tube. NH$_3$ evolved in distillation tube is trapped in boric acid. Upon ammonia evolution, the colour of boric acid changes to blue. For maximum ammonia evolution, the process is continued for 20 min. The solution was then titrated with standard HCl (0.01N) till blue colour of the solution disappears.

Amount of nitrogen in the samples was calculated by the following equation

\[
\text{\% of Nitrogen} = \frac{14 \times \text{Normality of HCl} \times \Delta V \times 100}{\text{Weight of Sample} \times 1000}
\]

\[
\text{\% Protein} = \text{\% of Nitrogen} \times 6.24
\]

2.2.5 Determination of carbohydrate

Carbohydrate is found by difference method and expressed as percentage of carbohydrate.

\[
\text{Carbohydrate (\%)} = 100 - [\text{Moisture + Ash + Fat + Protein}]
\]

2.2.6 Determination of crude fiber

About 1 g of sample was weighed into the beaker. 200 mL of boiling sulfuric acid were added. The unit was connecting with the digestion apparatus (Fibrotron FRB-6). After having boiled for exactly 30 minutes, the solution was filtered through filtering cloth and washed with hot water until it is free from acid. The residue transferred on the cloth into the flask with 200 ml of boiling sodium hydroxide solution. Immediately the flask was connected with the digestion apparatus and boil further for exactly 30 minutes. The flask was removed and immediately filters through Gooch crucible. The residue was washed with hot water until it was free from alkali and then with 10 ml of alcohol. It was dried at 105-110°C in an air oven for about 2 hours and cooled to room temperature in desiccator and weigh. The process was repeated of 30 minute drying, cooling and weighing until the difference between two successive weightings' was less than 1 mg. The lowest weight was noted which shall be considered as the weight of crude and contents after drying. The contents and the crucible were incinerated in the electric muffle furnace at 600+20°C for about 30 minutes. The unit was cooled to room temperature in desiccator and weigh. The process was repeated of 30 minute incinerating, cooling and weighing until the difference between two successive weightings in less than 1 mg. The lowest weight was note which shall be considered as the weight of crude and ash after incinerating. The difference between the two weightings' is the weight of crude fiber.

\[
\text{Crude fiber (\% by weight)} = \frac{(W_1 - W_2) \times 100}{W}
\]

Where,

- W is weight of sample, g
- W$_1$ is weight of crucible and contents after drying, g.
- W$_2$ is weight of crucible and ash after incinerating, g

2.2.7 Total Dietary Fiber

The total dietary fiber from our sample was isolated according to Bureau of Indian Standard Method (IS:11062, 1984). Briefly, 3 g of defatted, moisture free sample was mixed with 50 mL distiller water and autoclaved at 120°C for 20 min. It was then cooled and the pH was adjusted to 1.5 with 5 M HCl followed by the addition of 50 mg pepsin and 200 mL of chloroform. It was incubated at 37°C for 20 hrs with mild stirring. After incubation the pH was adjusted to 6 with 3 N NaOH and 25 mL of 0.1 M phosphate buffer pH 6, 100 mg pancreatin and 20 mg glucoamylase were added. This mixture was incubated for 18 h at 37°C with mild stirring. After incubation the contents were centrifuged at 3000
g for 30 min, the residue was collected and washed with acetone and diethyl ether and dry in an oven at 105°C to constant weight to obtain the insoluble dietary fiber. To the supernatant, ethanol was added in 1:4 ratio and again centrifuged for 30 min at 3000 g. The residue was collected and washed with alcohol, acetone and diethyl ether and dry in an oven at 105°C to constant weight to obtain the insoluble dietary fiber.

\[ \text{Total Dietary Fiber} = \left( \frac{\text{Mass of soluble fraction} + \text{Mass insoluble fraction}}{\text{Mass of the sample}} \right) \times 100 \]

2.3 Mineral Contents Analysis

Mineral contents were determined in Material Science Division of CSIR-National Institute for Interdisciplinary Science and Technology by X-ray fluorescence spectrometry analysis (Panalytical M-1743 Epsilon 3 X-rays) equipped with a 50 kV – 3 mA Rhodium anode X-rays tube, 6 filters, helium purge, high resolution SDD detector, spinner and a 10-position removable sample changer, according to the methods of practical manual [17]. The basis of the method is the interaction of X-ray photons from the analyzer’s excitation source with atoms of the elements present in the filter deposit.

About 10 g of each sample powder were oven dried at 105°C for 24 hr. The dried samples weighed into crucibles and dry ashed in a muffle furnace maintained at 650°C for 6 hr. The ash was cooled in desiccators and then weighed. After weighing, the samples were transferred into a P1 cup assembled with a high prolene (4 μm) supporting foil. Subsequently, all ash were lightly compressed using a hand stamp tool. The analysis was performed in a helium environment and the total counting time was 15 min. Elements were quantified using fully deconvoluted spectra. Accurate spectrum peak show sets of spectra of the elements present at ppm levels from ash of black bean seed. The results expressed in mg/100 g referred in table 2 show the main minerals contained in black bean seed. The highest level of mineral was potassium (51.648 mg/100 g), followed by phosphor (6.022 mg/100 g); magnesium (3.867 mg/100 g); chloride (0.425 mg/100 g); iron (0.357 mg/100 g) and zinc (0.099 mg/100 g). In general, it was observed that black bean seed had higher potassium, phosphor and magnesium.

Table 1. Proximate composition of black bean seed (%)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Whole-grain of black bean seed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>8.268 ± 0.3</td>
</tr>
<tr>
<td>Ash content (%)</td>
<td>3.063 ± 0.6</td>
</tr>
<tr>
<td>Crude fat estimation (%)</td>
<td>1.718 ± 0.0</td>
</tr>
<tr>
<td>Total proteins (%)</td>
<td>29.169 ± 0.6</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>58.107 ± 0.9</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>9.397 ± 0.5</td>
</tr>
<tr>
<td>Total dietary fiber (%)</td>
<td>21.833 ± 0.2</td>
</tr>
</tbody>
</table>

3. RESULTS

3.1 Proximate Analysis

The Table 1 below showed that proximate analysis of black bean seed revealed that it contained crude protein (29.169%), crude fat (1.718%), crude fiber (9.397%), moisture (8.268%), ash (3.063%), and carbohydrate (58.107%). Total dietary fiber is estimated to 21.833%.

3.2 Mineral Analysis Results

The Fig. 2 arising from Epsilon 3 spectrum peak shows sets of spectra of the elements present at ppm levels from ash of black bean seed. The results expressed in mg/100 g referred in table 2 show the main minerals contained in black bean seed. The highest level of mineral was potassium (51.648 mg/100 g), followed by phosphor (6.022 mg/100 g); magnesium (3.867 mg/100 g); chloride (0.425 mg/100 g); iron (0.357 mg/100 g) and zinc (0.099 mg/100 g). In general, it was observed that black bean seed had higher potassium, phosphor and magnesium.
Fig. 2. Epsilon 3 spectrum peak show sets of spectra of the elements present at ppm levels from the ash of black bean seed

Table 2. Minerals content in the crude ash of black bean seed (mg/100g)

<table>
<thead>
<tr>
<th>Mineral (mg/100g)</th>
<th>Whole-grain of black bean seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>3.867 ± 0.0</td>
</tr>
<tr>
<td>Fe</td>
<td>0.357 ± 0.0</td>
</tr>
<tr>
<td>Zn</td>
<td>0.099 ± 0.0</td>
</tr>
<tr>
<td>Cl</td>
<td>0.425 ± 0.0</td>
</tr>
<tr>
<td>K</td>
<td>51.648 ± 0.0</td>
</tr>
<tr>
<td>P</td>
<td>6.022 ± 0.0</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Black bean seed (*Phaseolus vulgaris* L.) from West Cameroon Region had higher crude protein, crude fiber and total carbohydrate content (29.169 g/100 g; 9.397 g/100 g and 58.107 g/100g, respectively) than the black beans seed from Ogbaru Main market Onitsha, Nigeria (14.00 g/100 g; 4.90g/100 g and 53.60 g/100g). Wherever, moisture (8.268 g/100 g); ash (3.063 g/100 g) and fat (1.718 g/100 g) in the black bean seed from West Cameroon Region had less than moisture (8.268 g/100 g); ash (4.90 g/100 g) and fat (1.718 g/100 g) in the black beans seed from Ogbaru Main market Onitsha, Nigeria [18]. Carbohydrate and protein indicated the high nutritional value of the seed. Presence of Carbohydrate revealed that the seed was a good source of energy (144.856 Kcal/100 g) while protein indicated that it can help in physical and mental growth and development. A study with sickled mice showed that a diet with high protein can improve weight gain and a decrease of the level of inflammation with sickle cell mice [1]. However, black bean seeds are considered the best quality protein resources.

Dietary fiber intake provides many health benefits. A generous intake of dietary fiber reduces risk for developing the diseases including coronary heart disease [19], hypertension [20], diabetes and certain gastrointestinal disorders [21], improve immune function [22]. The percentage of the crude fiber and total dietary fiber in black bean seeds from West Cameroon Region suggested that the consumption could help to maintain movement of food through the gut to provide energy and ensure break down of the food. Intake of dietary fiber reduces certain gastrointestinal disorders thus suffers SCD patients [21].

The crude fat or lipid provides a very good source of energy and aids in the transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cells processes [23,24].

Minerals are quantitatively minor compounds essential for the life because they contribute to multiple and different vital functions in the organism, like bone structure, homeostasis, muscular contraction, metabolism via the enzymatic systems, etc. [25]. The ash content of black bean seeds of *Phaseolus vulgaris* L. showed that they contained some quality of mineral element like Mg, Fe, Zn, Cl, K and P which are essential in our diet. The mineral content of black bean seeds from West Cameroon Region had higher potassium (51.648 mg/100 g) and phosphor (6.022 mg/100 g) than the black bean seed collected from a farmer in Bokkos town of Plateau State, Nigeria (potassium 14.1 mg/100 g and phosphor 2.4 mg/100 g). Wherever magnesium (3.867 mg/100 g), iron (0.357 mg/100 g) and zinc (0.099 mg/
100 g) in the black bean seeds from West Cameroon Region had less than magnesium (60.1 mg/100 g) iron (10.5 mg/100 g) and zinc (1.4 mg/100 g) in the black beans seed collected from a farmer in Bokkos town of Plateau State, Nigeria [26]. Several studies have reported a reduction in red cell Mg$^{2+}$ content in SCD patients. Thus, oral Mg supplementation with the aim of increasing red cell Mg$^{2+}$ levels and inhibiting K-Cl cotransport activity may represent a possible therapeutic strategy for ameliorating SCD red cell dehydration. Dietary magnesium supplementation in transgenic sickle cell mice has demonstrated that increasing erythrocyte Mg$^{2+}$ content can ameliorate red cell dehydration [27,28]. Zinc deficiency in patients with sickle cell disease was first reported in 1975. Zinc supplementation has shown to decrease in frequency of bacterial infections and hospitalization from painful crises [29]. Iron plays a central role in erythropoiesis and many other intracellular processes in all the tissues of the body. Iron deficiency, complicating SCD, is likely to worsen the clinical state of the disease [30]. Thus iron homeostasis requires tight regulation [31]. Other minerals found in the black bean can help Sickle Cell patients to regulate homeostasis.

5. CONCLUSIONS

This study has provided better understanding off the chemical composition and links to sickle cell patient's health benefits of black bean seed (*Phaseolus vulgaris* L.) PNN wild variety used to manage Sickle Cell Disease in West Cameroon Region. From these results, black bean seeds from this locality contain an appreciable amount of macro- and micro-nutrients thus will be beneficial in the management of Sickle Cell Disease in order to improve healthy lifestyle and focus on its therapeutic approach as future perspective.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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