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Mineral Composition and *in Vitro* Antioxidant activities of extracts of Bambara Groundnut (*Vigna subtereanean*) and Pigeon Pea (*Cajanus cajan*) Harvested in Ugep, Yakurr Local Government Area of Cross River State, Nigeria

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Abstract

The study evaluates the mineral and antioxidant content of Bambara groundnut (BG) and pigeon pea (PP) and their potential therapeutic benefits. Three different samples were taken: sample A was made up of Bambara groundnuts; sample B was made up of pigeon peas; and sample C was made up of 50% BG and 50% PP. Using accepted techniques, the samples were examined. The study shows that pigeon pea and the mixture (50%BG+50%PP), the mineral composition results indicated that Bambara groundnut had considerably greater levels of calcium, zinc, and potassium (P<0.05). Bambara groundnut and the combined sample, pigeon pea had substantially higher magnesium and iron levels at (p<0.05). Additionally, the mixed sample (50% BG+50% PP) demonstrated a notable increase in sodium when compared to Bambara groundnut and Pigeon pea. When compared to Bambara groundnut and pigeon pea, the combined sample (50%BG+50%PP) significantly increased at (p < 0.05) the ability to inhibit DPPH, H_2O_2 scavenging activity, and lipid peroxidation. The ferric reducing activity of Bambara groundnut, however, significantly increased at (p>0.05). These medicinal plants' bioactive components have a high concentration of minerals and antioxidants. As a result, BG and PP might be utilized to treat conditions including high blood pressure, osteoporosis, anemia, and arthritis. This study supports the in-vitro study's assertion that Bambara groundnut and pigeon pea are therapeutic in nature. These researches come to the conclusion that the medicinal plants contain antioxidant scavenging capacity that may be advantageous for human health.

Keywords: Vigna subterranean, Bambara groundnut, pigeon pea, Antioxidant, Mineral.Received: 15th Oct, 2024Accepted: 6th April, 2025Published Online: 15th April, 2025

Introduction

A legume known as Bambara groundnut (*Vigna subterranean* underground) may be traced back to Africa; there are several names for Bambara groundnut. It is the third significant legume in Africa. The Igbo tribe of eastern Nigeria consumes the most of this nutrient-rich bean (Nwadi, *et al.*, 2020). The

Yakurr people refer to it as Ajam Eteti, whereas the Igbo name it Okpa bean. It may be eaten by frying it, and eating it like a peanut or it can be consumed by utilizing it in food pottage. According to Jideani and Diederick (2014), its chemical makeup is comparable to that of soya beans. According to Brough *et al.* (1993), Bambara groundnut

milk is more palatable than milk made from soy bean and cowpea. Due to its probiotics content (antioxidant activity), Bambara groundnut has been used for medicinal purposes. Bambara groundnut has been referred to as a complete food (Yuonn and Victoria, 2013) because it has every nutrient required to create a diet that is balanced. Bambara groundnut is still underused despite its nutritional and medicinal benefits as well as the presence of antioxidants that are helpful in the fight against free radicals. In the chapter after, we'll delve more into its reputation as a complete food. An edible legume is the pigeon pea (Cajanus cajan), which belongs to the Fabaceae family. The nutritional and antioxidant content, which has been used for medicinal purposes, is to blame for this (Akinsulie et al., 2005). Pigeon peas are one of several plants being looked at as a potential substitute for animal-based protein because of the high cost (Akande et al., 2010). The pigeon pea, one of the main tropical pulse crops, is available in a variety of hues, including cream, red, yellow, and even black. In traditional medicine, different parts of this plant (pigeon pea) have been used to treat a variety of conditions affecting the skin, liver, lungs, and kidney (Adeomowaye et al., 2015). The several applications of the pigeon pea seed have made them popular. Pigeon pea is underused in Calabar, Cross River State, and Nigeria as a whole despite its various benefits. The plants are only used by a tiny community in one area of the nation. Igbos and Ugep residents of Cross River State Yakurr Local Government area. Therefore, the aim of this study is to clarify the nutritional significance of the mineral and antioxidant content of pigeon pea and Bambara groundnut. Since there is a need for a cheap source of nutrients with minimal to no adverse effects on human health, this study will disclose the nutritional value of two beans that are high in animal base protein.

Materials and Methods

Collection and identification of plant materials

The plant materials were gathered from the Yakurr Local Government Area in Ugep, Cross River State. The samples were identified and authenticated by Dr. E. G. Amanke, a Plant Ecologist from the Department of Botany at the University of Calabar, Calabar. A voucher specimen was subsequently deposited in the herbarium of the same department, where it was carefully stored in a plastic bag to prevent decomposition.

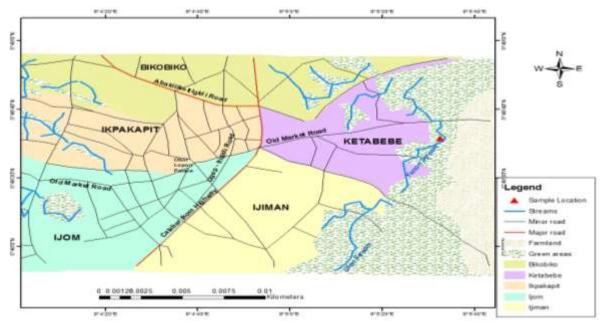


Figure 1: Map of Study area

Methods

Preparation of plant materials

The seeds of Bambara groundnut and pigeon pea were cleaned with clean water to eliminate sand and other debris, such as wood chips, that may function as pollutants, and then left out in the sun for two days to finish drying. The dried materials were then ground into a fine powder using an electric blender. When it was time for the proximate analysis, the crushed sample was sieved (0.2mm) and kept in airtight containers.

Preparation of extracts

Analytical grade ethanol and distilled water were combined in a 7:3 ratio to create the hydro-alcoholic solution that was used to extract each sample. For a week, the setups were macerated. After 24 hours, the bottles were shaken and maintained in a laboratory at room temperature with the foil-sealed bottles. The filtrate was then processed through many layers of muslin fabric.

The obtained filtrate was evaporated in a vacuum rotary evaporator under reduced pressure at 40°C until the filtrate was reduced to one-third of the starting filtrate volume, the obtained extracts were collected in stopper glass bottles, and the filtrate was then filtered through Whatman number 1 filter paper. The obtained filtrate was then evaporated in a vacuum rotary evaporator under reduced pressure at 40°C until the filtrate was reduced to one-third of the starting filtrate volume.

In Vitro Anti-oxidant evaluation of the extracts

Inhibition of lipid peroxidation

The different propolis extracts were lipid peroxidation inhibited using the prior technique described by Rajneesh *et al.* (2008). To 50 ml of egg yolk that had been produced in phosphate buffered saline (PBS) at a ratio of 1:4 (w/v), different quantities of each extract (1 ml) were added separately. Next, 0.5 ml of 24 mm ferrous sulfate and 0.5 ml of PBS were added. The mixture was strongly agitated and incubated for 15 minutes at 37°C. The mixture was then given 0.5 ml of 20% (w/v) trichloroacetic acid and 1 ml of 0.8% (w/v) thiobarbituric acid. The mixture was centrifuged at 2200 g for 20 min. at 25°C after boiling at 95°C and chilling for 30 min. The findings were represented as the concentration of each extract necessary to prevent lipid peroxidation by 50% (IC50), estimated by regression analysis, and the absorbance was measured at 532 nm.

DPPH radical scavenging ability assay

The extracts' capacity to neutralize the DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical assessed in accordance with was Senguttuvan's (2014) guidelines. Each extract will be combined with 1 ml of the 0.4 mm DPPH radical-containing methanolic solution, agitated, and then kept in the dark for 30 minutes. After measuring the solution's absorbance at 517 nm, the percentage of DPPH scavenging activity was estimated as follows: DPPH scavenging activity (%) = A_{blank} - $A_{sample} \times 100$

A blank

Where A is the absorbance

Regression analysis was used to determine the concentration of each extract that provided 50% DPPH scavenging activity (IC50), which was used to express the results. **Hydrogen peroxide** (H_2O_2) scavenging activity

Hydrogen peroxide is dissolved in 50 mm of phosphate buffer (pH 7.4) to create a 40 mm solution. The absorbance at 230 nm is then measured to assess the hydrogen peroxide's scavenging activity. Next, 2 ml of hydrogen peroxide solution and 1 ml of the sample extract or standard are added. The absorbance is calculated using a blank solution and measured after 10 minutes. With phosphate buffer and no hydrogen peroxide added, the blank solution is created. The amount of hydrogen peroxide that was scavenged may then be estimated (Nabavi et al., 2008). H₂O₂ scavenge (%) = [(A230 Control–A230 Sample)/A230 Control) ×100

Ferric reducing power capacity

The technique of (Athukorala *et al.* 2006) will be used to assess the reducing power of the extracts.20 minutes at 50°C were spent incubating 1ml of various extract concentrations (5-500 g/ml) with 2.5 ml of PBS and 2.5 ml ferrous cyanide. The mixture was mixed with 2.5 cc of TCA, and it was centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant was

combined with 0.5 ml ferric chloride and equal quantities of distilled water. Absorbance was measured at 700 nm, against blank and compared with standard (Catechin), increasing absorbance of the reaction mixture indicates stronger reducing power.

Mineral Analysis

Measurements of absorbance were performed using an atomic absorption spectrometer with a hollow-cathode lamp and an air or acetylene flame. Zinc, calcium, sodium, iron, and potassium were measured using wavelength, slit, and lamp current (Model AnA-135, OSK, and Japan).

ICP-OES (Hansen et al., 2009) was used to measure this using direct flame inductively coupled plasma optical emission spectrophotometry. All analytical methods must account for flaws in dilute solutions were utilized, and estimates were performed in triplicate. The temperature applied to the sample was high enough to generate large levels of ionization and collisional excitation of the sample atoms in addition to its normal breakup into atoms. Once the atoms are stimulated, they can move to lower states using thermal or emission energy. ICP-OES uses measurements of the light's intensity at particular wavelengths to calculate the amounts of the elements of interest (sodium, potassium, calcium, iron, zinc, and magnesium). The mineral content findings were given as $\mu g/g$ of sample.

The aforementioned techniques were used for pigeon pea, Bambara groundnut, and a 50% blend of both.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to assess quantitative data, and for significant results, a post hoc Duncan test was conducted. Microsoft Excel was used to plot the charts, and Social Science Application Software (SPSS) version 20 was used for statistical analysis. The mean \pm standard errors mean is used to express data.

Results

Comparison of Calcium composition (µg/g sample) in Bambara groundnut (BG), pigeon pea (PP) and mixture of 50% Bambara groundnut and 50% pigeon pea (BG+PP)

According to the data in Fig. 1, BG had a substantially greater calcium, zinc, and potassium content (P<0.05) than PP and the combined extract (50%BG+50%PP). In comparison to PP and the combined extract, there was a substantial drop (P < 0.05) in the composition of magnesium and iron and a minor rise in sodium. Comparing PP's results to BG and the combined extract, the same calcium, results for sodium, iron. magnesium, and zinc were considerably higher (P>0.05). In comparison to BG and the combined extract, there was a considerable drop in potassium content.

50% pigeon pea and 50% Bambara groundnut), yet the combined extract had considerably greater levels of sodium, iron, and zinc (P <0.05) than PP. It also had slightly higher levels of potassium. When compared to BG and PP, there was a substantial drop (p < 0.05) in the composition of calcium and magnesium. In conclusion, the composition of BG appeared to be richer in calcium, zinc, and potassium. The composition of BG was fairly rich in sodium and lower in magnesium and iron. The composition of PP was lower in potassium and higher in sodium, iron, calcium, magnesium, and zinc. Combination extract had higher sodium, iron, and zinc content. The potassium and calcium content of the combine extract was reasonably high, whereas the magnesium content was lower. Even yet, the combined sample and Bambara groundnut are both inferior sources of minerals to the pigeon pea. If you compare the Bambara groundnut to the mixed extract, it is a richer source of minerals. Comparable to BG and PP, combine extract had less mineral content.

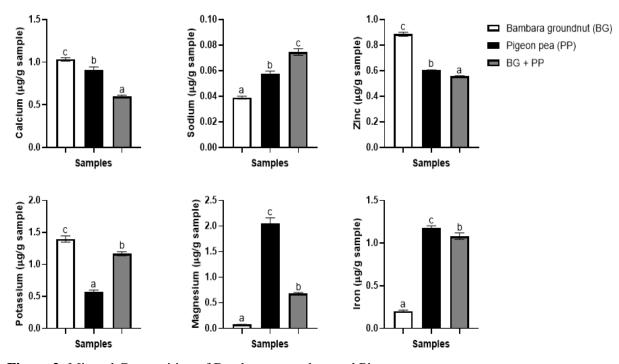


Figure 2: Mineral Composition of Bambara groundnut and Pigeon pea.

The result of the antioxidant composition of Bambara groundnut and pigeon pea

When compared to ascorbate PP and combination extract, the result in fig. 2 BG was substantially greater (p < 0.05) in the inhibition of DPPH, H₂O₂ scavenging activity, in ferric reducing activity, and was fairly high in the inhibition of lipid peroxidation. The combined effect of the extract and ascorbate significantly increased the inhibition of DPPH, H₂O₂ scavenging activity, and ferric reducing activity. According to the results, the combined extract considerably outperformed ascorbate in terms of DPPH inhibition, H_2O_2 scavenging activity, ferric reducing activity, and lipid peroxidation inhibition (P>0.05). In conclusion, BG was more effective at inhibiting DPPH, H_2O_2 scavenging activity, lipid peroxidation, and ferric reducing activity than PP, combine extract and ascorbate. However, PP was more effective at inhibiting DPPH, H_2O_2 scavenging activity, lipid peroxidation, and ferric reducing activity than PP.

In comparison to ascorbate, the combined extract had a somewhat high level of DPPH inhibition, H_2O_2 scavenging action, lipid peroxidation inhibition, and ferric reduction activity. According to the findings, PP is less rich in antioxidant activity than Bambara groundnut. PP had more antioxidant capabilities on its own than the combined extract, which was shown to have somewhat fewer antioxidant qualities.

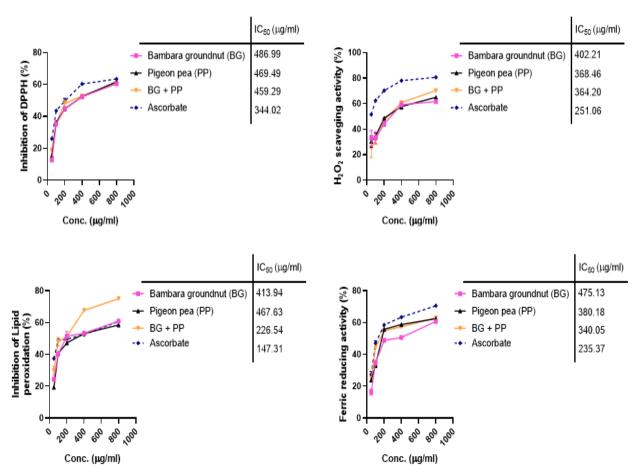


Figure 2: Antioxidant Composition of Bambara groundnut and pigeon pea

Discussion

Although certain legumes offer medicinal and nutritional properties that would benefit the body and help avoid malnutrition if consumed, there has been a significant problem with underutilization (Hillocks et al., 2012). This issue could be brought on by a lack of accurate understanding about the nutritional and medicinal properties of such legumes, their therapeutic uses, and knowledge gaps in their production, processing, and use (Denis et al., 2015). Despite having a high nutritional value, several legumes, like pigeon pea and Bambara groundnut, are underused in Nigeria, Africa, and globally. This research was done to look at the antioxidant properties and mineral content of Bambara groundnut, Pigeon pea, and a mixture of 50% Bambara groundnut and 50% Pigeon pea. Minerals are biomolecules that the body needs in minute amounts to operate (Mayes et al; Hillocks et al., 2012). They are divided into necessary and non-essential

them on its own or receives them from the food we eat that is mineral-rich. The two categories of necessary minerals are trace or microminerals and macrominerals. These mineral categories are all equally significant, yet the body needs different amounts of each group. While micro minerals are only needed in little amounts, macro minerals are needed in enormous amounts. In contrast to trace minerals, which include iron, manganese, copper, iodine, zinc, cobalt, fluoride, and selenium, macro minerals include calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur (Banks et al., 1998). The body cannot produce these minerals, thus they must be obtained through the ingestion of foods high in minerals. According to (Bamigboye and Adepoju, 2015), Bambara groundnut has a considerably greater calcium content (P<0.05) than pigeon pea and a mixture of 50% Bambara groundnut and 50% Pigeon pea. According to

minerals based on whether the body produces

clinical research (Banks et 1998; al., Bamigboye and Adepoju, 2015), calcium is crucial for hormone production and secretion as well as bone development, bone metabolism, muscle function, intracellular signaling, and transmission. Because nerve Bambara groundnut has a greater calcium content than pigeon pea, which has a relatively high calcium level, Bambara groundnut, pigeon pea, or a combination of the two might be used to treat osteoporosis, osteomalacia, rickets (a condition in which the bones are weak), arthritis, and other conditions.

This study also demonstrates that the sodium composition of the combined extract was greater than that of pigeon pea, which demonstrated a notable rise in sodium concentration above that of Bambara groundnut. This outcome was consistent with the findings of Kunyanga et al. (2013), who claim that pulses often contain little sodium, and Seeling and Romanoff (2004), who claim that pigeon peas also contain little sodium. Sodium plays a key role in the management of bodily fluid, electrolyte balance, and blood pressure as well as the preservation of proper cellular homeostasis in the extracellular fluid. However, excessive ingestion of foods high in salt can cause kidney failure, cirrhosis, and heart failure. In order to relieve disorders brought on by a lack of salt, pigeon pea and Bambara groundnut can be ingested or utilized medicinally to balance the body's need for salt, it might also be consumed in moderation.

According to Mayes *et al.* (2019), Bambara groundnut was high in zinc composition. In this study, Bambara groundnut's zinc composition was considerably (P<0.05) greater than that of the combination sample and non-significantly (P>0.05) higher than that of Pigeon pea.

Consumption of Bambara groundnut might balance the body's demands for zinc and also cure diseases linked to zinc deficiency. Zinc plays a crucial part in cell mediated immunological activity, and it has been shown that its deficiency is connected with an increased risk of cancer (Bois, 2016).However, it must be done in moderation since a large amount might raise the level of zinc, which has been linked to colon and bronchogenic cancer (Marathe *et al.*, 2011). According to the study, magnesium was discovered to be the mineral that was most plentiful in pigeon pea, although it was substantially lower (P < 0.05) in Bambara groundnut and relatively low in the combined extract. Pigeon pea's high magnesium concentration was consistent with (Arise et al., 2017). Magnesium plays a crucial role in the cell cycle, and deficiencies are linked to certain diseases such the development of precancerous cells. Magnesium and zinc cations are necessary for the production of nucleic acids and the action of enzymes. The investigation also revealed that potassium levels were considerably (P<0.05) greater in Bambara groundnut than in the combined extract and non-significantly (P>0.05) higher in pigeon pea than in the combined extract. Potassium is essential for keeping cells functioning properly, and a lack of it can cause hypokalemia. Consuming Bambara groundnut can help the body get the potassium it needs while also treating conditions caused by low potassium levels.

The study also shown that pigeon peas contain more iron than Bambara groundnuts when compared to the combination extract, pigeon pea had considerably more iron (P < 0.05). Iron has a crucial role in how the central nervous system works. It performs a number of crucial processes, including transporting bodily oxygen from the lungs to the body's tissues via the hemoglobin of red blood cells. Most of which are found in hemoglobin as erythrocytes. Iron is a crucial component of several enzyme systems in the body's tissues. Pigeon pea consumption and use as a supplement may help to balance and cure symptoms linked to iron deficiency.

Antioxidants are compounds that stop or delay the cellular damage caused by free radicals, which are unstable molecules the body produces in response to its environment. Although it is not a new concept, some countries are unaware of the benefits of medicinal plants in the treatment of sickness. An antioxidant may be synthetic or natural. antioxidants Natural are essential for maintaining good health and preventing chronic and degenerative illnesses. Due to the harmful effects of free radicals in dietary and biological systems, this medicinal plant's

antioxidant qualities are crucial. The oxidation of fat in food is accelerated by excessive free radical production, lowering food quality. According to this investigation, BG was more efficient at scavenging DPPH free radicals than PP and the combined extract. This was consistent with a research by Nyau et al. (2015), which examined the DPPH free radical scavenging capabilities in a variety of widely consumed legumes and identified pigeon pea as one of the most effective. To have high to moderate scavenging activity compared to other legumes. An established method by which antioxidants reduce lipid oxidation is by scavenging DPPH free radicals. This demonstrates that pigeon pea eating or a combination of both samples may boost the body's ability to scavenge DPPH.

This study also demonstrates that Bambara groundnut has better hydrogen peroxide scavenging activity than the two PP and the combined extract. In contrast to ascorbate, the mixed sample had a modest level of hydrogen peroxide activity. Reactive oxygen species like hydrogen peroxide often have no negative effects but instead serve a crucial role in controlling how the body's cells react to signals from outside the cell (Nyau et al., 2015). However, too much of it could be harmful to cells. In other words, eating 50% Bambara groundnut and 50% pigeon pea has the potential to enhance the body's ability to scavenge hydrogen peroxide more effectively than ingesting either food alone, helping to avoid illnesses brought on by hydrogen peroxide free radicals.

Additionally, Bambara groundnut showed greater suppression of lipid peroxidation activity as compared to PP and the combined extract, indicating that the BG sample had a stronger antioxidant impact than ascorbate. According to Mboso *et al.* (2020), lipid peroxidation is the oxidative breakdown of lipid in a chain reaction. The findings indicate that eating Bambara groundnut and pigeon pea or a combination of the two might aid in reducing the likelihood of lipid peroxidation due to free radicals.

This investigation demonstrates that the combination extract and PP had lower ferric reduction activity than the BG. Comparing the

three samples, combine extract showed the lowest ferric reduction activity. This was contrary to (Nyau *et al.*, 2015), who claimed that Bambara groundnut had a similar level of ferric reducing antioxidant capacity to certain peas but lower than others. According to the report, consumption of the class of Bambara groundnuts combined with a diet that could increase ferric reducing activity in the body could prevent and protect the body from the harmful effects of free radicals was responsible for the low ferric reducing antioxidant power.

Conclusion

Despite having a strong nutritional profile, pigeon pea and Bambara groundnut are two examples of important plants that have long gone unutilized in various parts of the world. This study indicates various bioactive plant components that, despite their differences in amount, are quite rich in antioxidant and mineral content. Because of this, combining Bambara groundnut and pigeon pea in your diet may help you meet your body's needs for minerals while also fending against the harmful effects of free radicals. These results also suggest that Bambara groundnut and pigeon pea, or a combination of the two, might be utilized to maintain health and other physiological functions.

Conflict of Interest

The authors declare no conflict of interest.

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