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Nutritional Quality, Phytochemical and Mineral Assessment from Various Accessions of Bambara Groundnut

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Authors' contributions

This work was carried out in collaboration among all authors. Author AMZ designed the study, performed the statistical analysis, wrote the protocol, and drafted the first version of the manuscript. Authors AF, KMI, MSMS and INA provided and identified the samples. Authors MNSA, MYSA, and YND managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate nutritional composition, polyphenols contents and antioxidant activity from various accessions of Bambara groundnut (BGN) (*Vigna subterranea*) in Malaysia.
Study Design: Different parts of BGN were extracted and investigated for its nutritional composition, total p0068enolic contents (TPC) and free radical scavenging testing. Each sample

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were extracted in triplicates (n=3). Tabulated data were statistically analyzed using Duncan's Multiple Range Test (DMRT).

Place and Duration of Study: Herbarium and Phytochemistry Laboratory. Agrobiodiversity and Environment Research Centre (Kompleks MyGene Bank), MARDI, Serdang, Malaysia from November 2023 to February 2024.

Methodology: Four accessions of BGN (BGN996, BGN997, BGN998 and BGN999) were morphologically characterized and determine its proximate and minerals composition while different parts (seed, pod and testa) from each of the accessions were extracted to evaluate its polyphenols content and antioxidant activity.

Results: The nutritional analysis revealed significant variations in carbohydrate, protein, and energy content among the accessions. BGN 999 exhibited the highest carbohydrate (65.2 g/100g) and energy contents (403.7 kcal/100g), while BGN 997 had the highest protein content (22.6 g/100g). Analysis of mineral composition showed potassium as the predominant mineral, with contents ranging from 1552.7 to 1712.3 mg/100g, surpassing other essential minerals like phosphorus, magnesium, and zinc. Meanwhile, extracts from the different parts of BGN (seed, pod and testa) from all of the accessions were subjected to the TPC and antioxidant testing. The TPC analysis showed significant differences in phenolic contents across different BGN parts, with the testa exhibiting the highest TPC (45.02 to 56.78 g GAE/100g DW). The free radical scavenging assay revealed that BGN 996 had the strongest antioxidant activity among the testas, with the lowest IC_{50} value (0.37 mg/ml).

Conclusion: This study underscores the nutritional and antioxidant potential of Bambara groundnut, highlighting its role as a functional food rich in phenolic compounds and essential nutrients beneficial for human health. Future research should further explore its applications in both the food and nutraceutical industries, leveraging its promising attributes for enhanced dietary and health benefits.

Keywords: Bambara groundnut; phytochemistry; antioxidant; proximate; underutilized crops; legumes.

1. INTRODUCTION

Bambara groundnut (BGN) (*Vigna subterranea*) belongs to the Fabaceae family and is considered one of the essential but forgotten legumes, originating from tropical African regions. BGN is also found in the Southeast Asian region, particularly in Thailand, Indonesia, and Malaysia. In northern Peninsular Malaysia, BGN is cultivated and locally referred to as "kacang poi" [1,2]. Its remarkable tolerance to drought and its comprehensive nutritional profile distinguishes it as a prime candidate among underutilized crops [2]. Underutilized crops play a crucial role in enhancing food and nutrition security, promoting agricultural diversification, minimizing environmental impacts, and mitigating the effects of climate variability [1]. Extensive studies on nutritional values of BGN have been carried out throughout the years. The carbohydrate content in BGN was reported in the range of 55.8–68.0 g/100g, with an average value of 61.7 g/100g [3]. The high carbohydrate and protein contents in BGN makes it a potential candidate as an alternative source of meal for tilapia, substituting wheat flour [4]. Furthermore, the high protein content in BGN has also made it one of the indigenous vegetables cultivated in Africa for both human and animal feed [5]. Additionally, due to its low maintenance cost and sustainability, this crop has been widely cultivated by those living in arid and semi-arid regions who are looking for alternative sources of carbohydrates, plant-based proteins, and essential minerals for their livelihood [6]. BGN seeds were also found to be rich in polyphenols compared with red beans. For example, red BGN was found to have 135.9 mg GAE/100g DW polyphenols content compared with red beans with only 84.5 mg GAE/100g DW. The study also revealed the identification of polyphenols profiling, namely caffeic acid hexoside, resveratrol glucoside, naringenin, and kaempferol glucosides [7]. In addition, our recent studies on BGN, in comparison with other Vigna spp. (red bean and black-eyed pea) discovered higher polyphenols content in BGN. The total phenolics content was comparatively higher in BGN with 87.3 ± 2.05 mg GAE/100g compared in red bean and black-eyed pea with 43.7 ± 0.47 and 60.7 ± 2.62 mg GAE/100g respectively [8]. Meanwhile, studies on the seed coat of BGN collected from Thailand have been carried out by Klompong *et al*. in 2015. The findings concluded the seed coat of BGN revealed astonishing total phenolic contents, free radical scavenging activity, and antimicrobial activity [9].

Bambara groundnut is cultivated on a small scale by local farmers to provide a supplementary income and for daily consumption This underutilized legume is not as readily available in the market compared to other legumes such as groundnut, cowpea, red bean, soybean, and many other beans. Statistic from Department of Agriculture Malaysia in 2023 reported three major legumes and beans cultivated in Malaysia are long bean (*Vigna sinensis*), french bean (*Phaseolus vulgaris*) and four-angled bean (*Psophocarpus tetragonolobus*) with estimated planting area of 4,472 ha, 1,164 ha and 470 ha, respectively [10].

A survey conducted by Koo *et al*. in 2016 to study the eating patterns among Malaysian children in two age groups (7 to 9 years old and 10 to 12 years old) showed that the consumption of legumes was extremely low. Specifically, less than 10% of children from both age groups achieved the recommended legumes intake according to the Malaysian Dietary Guidelines [11]. Meanwhile, studies on Malaysians aged 35 to 70 years old show that, a daily recommendation of at least three servings of legumes helps to prevent cardiovascular disease [12]. Given the benefits of legumes in preventing cardiovascular disease and the low consumption rates among Malaysian children, it is recommended that efforts be made to increase legume intake among this younger population. This aligns with the strategic policy thrust 4 under the National Agrofood Policy of Malaysia 2021–2030, which aims to develop healthy and sustainable food systems. This strategy also seeks to provide Malaysians with access to a healthy and balanced diet [13]. One solution is to promote the consumption of underutilized legumes in Malaysia, such as bambara groundnut. This will ensure a wide variety of legume choices in the diet, reduce dependence on certain crops, enhance nutritional diversity, and support agricultural sustainability. Documenting the nutritional values of BGN in Malaysia will help elevate its potential as a future crop and broaden its marketability. To date, no studies have been conducted to investigate and differentiate the proximate and mineral compositions, polyphenols content and antioxidant activity of Bambara groundnut (BGN) collected from Malaysia and its different parts. Therefore, this study aims to investigate the proximate and mineral compositions of various BGN accessions collected from Malaysia, to quantify the total phenolic contents and its antioxidant activity of different BGN plant parts (seed, pod, and testa), and to assess the potential of BGN as a functional food ingredient and a sustainable nutritional source for the Malaysian population.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Sample

Four (4) accessions of BGN from Malaysia (BGN 996, BGN 997, BGN 998 and BGN 999) were morphologically characterized by a team of experts in the Agrobiodiversity and Environment Research Centre, MARDI. The morphological characteristics of each accession are listed in Table 1. Voucher specimens were deposited in the Kompleks My Genebank, Agrobiodiversity and Environment Research Centre, MARDI, Serdang, Malaysia. The samples were dehulled, and the seeds were separated from the pod and testa. The samples were dried in the oven for 3 days. All parts of the samples were ground into a fine powder using a mechanical grinder (IKA Werke MF 10 basic, Germany) and sieved using a 1mm mesh size. The samples were then kept in the chiller at -80ºC until needed for further analysis.

2.2 Proximate and Mineral Composition Analysis

A total of 250g of dried powder samples from seeds of BGN 996, BGN 997, BGN 998 and BGN 999 were sealed in a vacuum plastic bag to prevent contamination. The samples were appropriately labelled and dispatched to an accredited laboratory for proximate and mineral contents analysis. All analysis were conducted in triplicate.

2.3 Total Phenolic Contents (TPC)

The total phenolic contents of the sample was determined using the Folin–Ciocalteu method with some modifications [14]. A total of 2g of dried powder sample was extracted in 20 ml of 70% methanol. Crude extract (50 µL) was mixed with 100 µL of Folin Ciocalteau's phenol reagent (Merck, Germany). After 3 minutes, 100 µL of 10% sodium carbonate (Na_2CO_3) (Sigma Aldrich, USA) was added to the reaction mixture and allowed to stand in the dark for 60 minutes. The absorbance was measured at 725 nm, and the total phenolic contents was calculated from a calibration curve using gallic acid (0-10 µg/mL) as a standard reference. The estimation of phenolic contents was conducted in triplicate. The results were presented as mean values ± standard deviations and expressed as g gallic acid equivalent per 100 g of samples (g GAE/100g) in dry weight (DW).

Table 1. Morphological characteristics of BGN 996, BGN 997, BGN 998 and BGN 999 collected in Malaysia

	BGN 996	**BGN 997**	**BGN 998**	**BGN 999**
Physical attributions				
Pod colour	Yellowish brown	Yellowish red	Yellowish red	Yellowish red
Pod shape	Ending in a point, round on the other side	Ending in a point, round on the other side	Ending in a point, round on the other side	Ending in a point, round on the other side
Pod texture	Little grooves	Little grooves	Little grooves	Smooth
Seed colour	Dark brown	Dark brown	Brown to dark brown	Dark red
Seed shape	Oval	Oval	Oval	Oval
Seed texture	Smooth	Smooth	Little to much pitted	Smooth to little grooves
Testa pattern	Black spots and stripes	Absent	Black spots	Absent
Testa eye pattern	Absent	Absent	Present	Absent

2.4 Free Radical Scavenging Assay (2,2-diphenyl-1-picryl-hydrazyl-hydrate, DPPH)

All the powder samples underwent extraction and were assessed for their free radical scavenging ability, following the previously described procedure with minor modifications [14,15]. The assay was conducted using a 96-well plate, with 2g of dried powder sample was extracted in 20 ml of 70% methanol. The stock solution was diluted to the desired concentration for the working solution. The final volume obtained (7 µL) was mixed with 280 µL of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, USA). The plate was covered with aluminium foil to prevent exposure to sunlight and was kept in a dark place for 30 minutes. Analysis was performed using a UV spectrophotometer (Eon Biotek Instrument) at 517 nm. The results were expressed as the inhibition concentration (IC_{50}) value in mg/mL, representing the concentration at which DPPH radicals were scavenged by 50%.

2.5 Statistical Analysis

Statistical analysis was performed on all triplicate samples using Duncan's Multiple Range Test (DMRT) (Mean ± SEM, n=3) with SAS Version 9.4.

3. RESULTS AND DISCUSSION

3.1 Proximate and Mineral Composition

The proximate and mineral composition for all seeds of BGN accessions can be found in Table 2 and Table 3. The Duncan's Multiple Range test for the total carbohydrate showed a significant difference ($p<0.001$) across all accessions, ranging from 59.5 to 65.2 g/100g with BGN 999 showing the highest carbohydrate content (65.2 ± 0.21 g/100g) while BGN 997 exhibited the highest protein content with 22.6 ± 0.33 g/100 g. However, BGN 999 exhibited the lowest protein content (16.6 ± 0.25 g/100g), a difference that was statistically significant ($p<0.001$) compared to other accessions. These findings and data are consistent with a previous study conducted by Atoyebi *et al.*, in 2017 on twenty accessions of bambara groundnut obtained from Nigeria. The study revealed a range of protein content, with the highest value of 24.91 g/100 g and the lowest of 15.88 g/100g [16]. Meanwhile, the total energy calculated for all accessions ranged from 392.3 to 403.7 kcal/100g, with BGN 999 providing the highest energy kcal/100g of sample (Table 2).

Table 3 showed the mineral composition (phosphorus (P), potassium (K), magnesium (Mg) and Zinc (Zn)) in four accessions of BGN. Potassium content across all the accessions

Table 2. Proximate and mineral contents in different accessions of bambara groundnut (BGN) (996,997,998 and 999)

	Ash (g/100g)	Fat (g/100g)	Protein (g/100g)	Total Carbohydrate (g/100g)	Energy (kcal/100g)
996	3.87 ± 0.05b	6.57 ± 0.05c	20.93 ± 0.21c	62.4 ± 0.16b	392.3 ± 0.47d
997	4.10 ± 0.05a	7.43 ± 0.17b	22.6 ± 0.33a	59.5 ± 0.27d	395 ± 0.82c
998	3.40 ± 0.08c	7.40 ± 0.12 b	21.7 ± 0.22b	61.2 ± 0.05c	398 ± 0.47b
999	3.33 ± 0.08c	8.51 ± 0.05a	16.6 ± 0.25d	65.2 ± 0.21a	403.7 ± 0.94a

*Means followed by the same letter within a column are not significantly difference at (P≤0.05) by Duncan's Multiple Range Test (DMRT) (Mean SEM, n=3). *, ** and *** significantly difference at P<0.05, 0.01 and 0.001 respectively and NS= not significant*

Table 3. Mineral composition in different accessions of bambara groundnut (BGN) (996,997,998 and 999) (mg/100g)

	Phosphorus (P)	Potassium (K)	Magnesium (Mg)	Zinc (Zn)
996	486.2 ± 4.53b	1712.3 ± 2.87a	169.6 ± 1.56ab	2.6 ± 0.05a
997	528.2 ± 7.00a	1700.0 ± 26.3a	180.0 ± 0.45a	2.74 ± 0.10a
998	430.1 ± 1.19c	1552.7 ± 24.4c	153.2 ± 3.46bc	2.68 ± 0.12a
999	329.4 ± 5.98d	1636.0 ± 16.3b	140.6 ± 1.20c	3.2 ± 0.09a

*Means followed by the same letter within a column are not significantly difference at (P≤0.05) by Duncan's Multiple Range Test (DMRT) (Mean SEM, n=3). *, ** and *** significantly difference at P<0.05, 0.01 and 0.001 respectively and NS= not significant*

predominantly higher in the seeds of BGN compared to the other minerals with the range of 1552.7 - 1712.3 mg/100g. Our finding was in a good agreement by previous studies conducted by Adebiyi *et al.,* in 2019 and Ndidi *et al*., in 2014 with potassium was found as the highest mineral in the BGN with 831 – 979 mg/100g and 187 mg/100g compared to others minerals tested [17,18]. The elevated potassium content in these legumes falls within the recommended intake levels outlined by the Dietary Reference Intakes (DRIs) for individuals aged 1 to 51 years. Adequate intakes range from 2000 - 3400 mg for males and 2000 - 2600 mg for females [19].

3.2 Total Phenolic Contents (TPC)

Different parts of BGN (seed, pod and testa) were extracted and the total phenolic contents (TPC) from all of the parts shown in Table 4. The trend of TPC in different parts of BGN as follows; seed< pod< testa. Seed from BGN 999 showed the highest total phenolic contents (1.37 ± 0.06 g GAE/100g DW) while seed from BGN 997 has the lowest total phenolic contents (0.35 ± 0.02 g GAE/100g DW). The pod of BGN 997 exhibits the highest total phenolic contents (14.18 ± 0.66 g GAE/100g DW) which is significantly different (p<0.001) compared to BGN 996 and BGN 998 with values of 5.12 ± 0.07 and 4.40 ± 0.13 g GAE/ 100g DW, respectively. Among all the parts of BGN being tested, the testa demonstrates the highest TPC ranging from 45.02 to 56.78 g GAE /100g DW across all accessions, and there is no significant difference (p>0.05) observed. The elevated phenolic contents in the testa are primarily attributed to its colour characteristics. This finding also was in a good agreement by the previous study conducted by Marathe *et al.* in 2011 [20]. The outer layer of seed coat from different legumes collected in India showed the highest polyphenols content. Morphological identification has confirmed that the testa, also known as the seed coat of bambara groundnut, exhibits distinctive coloration (Table 1). The testa from all accessions displayed hues ranging from dark brown to dark red, primarily due to the presence of anthocyanins, which belong to the phenolic class of compounds. The investigation into the seed coat colour variations among different types of legumes revealed an impact on total phenolic contents. Research indicates that red pigments are associated with higher phenolic content and increased antioxidant activity. A study conducted by Adedayo *et al*, in 2021 revealed the phenolic contents in BGN mainly influenced by the shade of their seed coat. It was observed that the phenolic content rises as the colour deepens and declines as it lightens. The black-coloured BGN seeds exhibited the highest total phenolic contents, followed by red, brown, and mixed-colour seeds, in descending order [21]. These findings align well with our results, showing that the testa of BGN 996, attributed to its dark brown colour, has the highest phenolic content (Table 1). In addition, studies on the colour variations of seed coats in legumes such as chickpeas, cowpeas, soybeans, and peanuts have also shown significant differences in total phenolic content. Red, black, and brown seed coats exhibit the highest phenolic contents compared to beige and cream-colored seeds [20]. The study also revealed that seed size plays an important role in the total phenolic contents. It was found that the indirect proportionality of seed coat to seed size influences the total phenolic contents. Larger seeds of legumes have a smaller surface area of seed coat, providing lesser phenolic contents.

3.3 Free Radical Scavenging Testing

Ascorbic acid was used as the standard measurement for the free radical scavenging testing from the methanolic extracts of BGN (Table 5). The lower the IC_{50} values indicates stronger the antioxidant activity [15]. Seeds from BGN 997, BGN 998, and BGN 999 exhibited IC_{50} values below 1.0 mg/ml, ranging from 0.4 - 0.71 mg/ml. Among these, BGN 997 showed the lowest IC_{50} value, indicating the highest antioxidant activity compared to the other accessions. Meanwhile, pods from BGN 996 and BGN 997 showed IC_{50} values<1.0 mg/ml and significantly differences (p<0.001) with values of 0.38 ± 0.1 and 0.83 ± 0.06 mg/ml, respectively. Due to contamination, extraction of pods from BGN 999 was not possible, potentially affecting the free radical testing. The same trend was also observed from the testas of BGN 996 and BGN 997 with IC_{50} values<1.0 mg/ml. The significance different in the free radical scavenging from the testas in all the accessions of BGN are shown in the Table 5.

The strength of the free radical scavenging activity (from low to high) of the testa from all accessions can be denoted as follows: BGN 996 > BGN 997 > BGN 999 > BGN 998. BGN 996 has the lowest IC_{50} value (0.37 ± 0.03 mg/ml), indicating stronger antioxidant activity, while BGN 998 has the highest IC_{50} value (1.30 ± 0.1 mg/ml). The strong antioxidant activity observed in all the testas was directly proportional to the amount of phenolic contents: BGN 996 > BGN 997 > BGN 999 > BGN 998 (Table 4). These findings also were aligned with previous work conducted by Klompong *et al.*, in 2015. They studied the correlation of phenolic content and various antioxidant testing from seed coat of different extraction method from 9 different samples of bambara groundnut. The study found that the high amount of phenolic contents exhibited the strongest DPPH free radical scavenging activity [9].

Table 4. The total phenolic contents of different accessions of bambara groundnut (BGN) (996,997,998 and 999) (g GAE/100g DW)

	Seed (p< 0.001)	Pod (p< 0.001)	Testa (p>0.05)
996	0.81 ± 0.03b	5.12 ± 0.07b	56.78 ± 0.72a
997	0.35 ± 0.02c	14.18 ± 0.66a	51.26 ± 2.48ab
998	0.85 ± 0.03b	4.40 ± 0.13b	45.02 ± 1.36b
999	1.37 ± 0.06a	n.a	48.66 ± 4.91b

*Means followed by the same letter within a column are not significantly difference at (P≤0.05) by Duncan's Multiple Range Test (DMRT) (Mean SEM, n=3). *, ** and *** significantly difference at P<0.05, 0.01 and 0.001 respectively and NS= not significant*

Table 5. Free Radical Scavenging Assay from Different Accessions of BGN (996,997,998 and 999) (IC_{50} mg/ml)

	Seed (p< 0.001)	Pod (p< 0.001)	Testa (p< 0.001)
996	1.32 ± 0.11a	0.38 ± 0.1c	0.37 ± 0.03d
997	0.40 ± 0.1c	0.83 ± 0.06b	0.47 ± 0.04c
998	0.65 ± 0.13b	1.31 ± 0.1a	1.30 ± 0.1a
999	0.71 ± 0.17b	n.a	1.18 ± 0.09b
Standard (Ascorbic Acid)	0.06 ± 0.01		

*Means followed by the same letter within a column are not significantly difference at (P≤0.05) by Duncan's Multiple Range Test (DMRT) (Mean SEM, n=3). *, ** and *** significantly difference at P<0.05, 0.01 and 0.001 respectively and NS= not significant*

4. CONCLUSION

In conclusion, the comprehensive analysis of bambara groundnut (BGN) across various accessions has illuminated significant nutritional and antioxidant characteristics. The study revealed substantial variations in carbohydrate, protein, and energy contents among different BGN accessions, with BGN 999 standing out for its highest carbohydrate and energy levels, and BGN 997 showing the highest protein content. These findings are consistent with existing literature, underscoring the nutritional diversity of BGN. Moreover, potassium emerged as the predominant mineral across all BGN accessions, surpassing levels of phosphorus, magnesium, and zinc. The seeds of BGN 999 exhibited the highest total phenolic contents, while BGN 997 pods displayed the highest among all pods tested. The testa consistently showed the highest total phenolic contents across accessions, attributed to the presence of anthocyanins. These results reinforce previous findings indicating that darker-coloured seeds generally exhibit higher phenolic contents and stronger antioxidant activity. Additionally, the free radical scavenging assay using ascorbic acid as a standard demonstrated that BGN 996 had the strongest antioxidant activity among testas, followed by BGN 997 and BGN 999. This correlation underscores the direct relationship between phenolic content and antioxidant potency in BGN. Overall, these nutritional attributes underscore the potential of bambara groundnut as a valuable dietary resource, meeting recommended intake levels and offering health benefits associated with its nutrient profile.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author(s) hereby declare that generative AI technology (ChatGPT based on the GPT-4 architecture) was used during the writing and editing of this manuscript. ChatGPT was employed to check for grammatical errors and typos that occurred during the drafting process.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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