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Comparative Phytochemical Analysis of Bambara Groundnut (*Vigna subterranea*) and others Vigna spp. with Respect to its Nutritional, Antinutritional and Antioxidant Properties

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Authors' contributions

This work was carried out in collaboration among all authors. Author AMZ designed the study, wrote the protocol, wrote the first and finalised draft manuscript and performed the HPLC parameter and quantification. Authors INA, MR, KMI, MSMS and AF managed the literature search and wrote the first draft of the manuscript. Author AA performed the statistical analysis. Authors MNS, MYSA, MSNS and MFNN helped the extraction of bambara groundnut and performed the antioxidant activities and also performed the fatty acids composition. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Bambara groundnut is considered as underutilize and commercially forgotten legume in Malaysia. Few studies abroad showed interesting potential on its phytochemical and nutritional contents. This study was carried out to investigate and compare the nutritional, antinutritional, free radical scavenging assay, total phenolic content and fatty acids composition of Bambara groundnut (*Vigna subterranea*) with comparison with two others commercial legumes (Vigna spp.) red bean (*V. angularis*) and black-eyed pea (*V. unguiculata*) in Malaysia.
Study Design: Each sample was extracted three times (n=3) for the free radical scavenging assay, total phenolic content, fatty acids composition and antinutritional content. All the data were analysed using ANOVA and Tukey Pairwise tests.
Place and Duration of Study: Phytochemistry Laboratory, Kompleks MyGene Bank, Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia from April to December 2022.
Methodology: Bambara groundnut (BGN), red bean (RB) and black-eyed pea (BEP) were purchased from various local market situated in the northern state of Peninsular Malaysia. Each sample was dried in the oven at 40°C for 3 days. Samples were grounded and its proximate and minerals content were determined. Each sample was extracted for the free radical scavenging assay, total phenolic content, oxalates (total, soluble and insoluble) content using HPLC and fatty acids composition using GCMS.
Results: Bambara groundnut (BGN) was found to have higher total carbohydrate content (66.0 ± 0.01 g/100g) compared with RB and BEP ($P<0.05$) hence provides more energy (406.67 ± 1.25 kcal/100g) (14% higher than red bean and black-eyed peas). Further analysis on its mineral content revealed the legume is rich in potassium (24% higher than red bean and black-eyed peas) suggesting its potential to regulate body fluid and muscle contractions. The bambara groundnut's seed oil is rich in fatty acids; omega 3 and 6 (palmitic acid, α-linoleic acid and linolenic acid). BGN also have higher phenolic content (87.3 ± 2.05 g/100g) compared to RB and BEP ($P<0.05$). The free radical scavenging assay showed moderate antioxidant activity with inhibition concentration (IC_{50}) value of 0.57 ± 0.04 mg/ml (standard: ascorbic acid = 0.06 mg/ml). The underutilize legume also had undetectable oxalate content; hence it is safe for human consumption (lethal dose 660 mg/kg body weight).
Conclusion: The potential findings on this legume should elevate the importance of this crop to be commercialised locally and for the future crop that will lead to achieve food security particularly in Malaysia.

Keywords: Bambara groundnut; legumes; nutritional; Vigna.

1. INTRODUCTION

Bambara groundnut (BGN) or scientifically known as *Vigna subterranea* is originated from the Eastern Africa and can be found grown in the drier part of sub-Saharan Africa, certain parts of Latin America and Asia [1-3]. BGN was locally known as earth pea, jugo bean, nyimo bean or ditloo in Southern Africa. Its main centre of genetic diversity is in north-eastern region of Nigeria and Cameroon [4]. Bambara groundnut also can be found in Southeast Asia region in Thailand, Indonesia and Malaysia. BGN can be found cultivated in various places in northern part of Peninsular Malaysia and it is locally known as kacang poi [1]. According to the Food and Agriculture Organization (FAO), the production of BGN was increased from 2019 to 2020 with estimation of 0.22 million tons to 0.23 million tons [5]. Bambara groundnut holds a specialty quality attributes and resilience to the abiotic and biotic stress associated with the climate change. It is tolerant to drought stress and can be cultivated in various marginal soils with low input agronomic practices [4]. This underutilized legume known as complete food due to its beneficial and substantial nutritional compositions but it has not been commercially available in Malaysia.

Legumes are vital for human diet as it provide sources of calories and protein [6]. According to the Malaysian Dietary Guidelines, recommended serving size for legumes is 0.5 – 1/day [7]. A study in Malaysia showed a poor obedience of dietary guidelines for legumes (0.143 serving size) and more than half of the adult women were overweight and their quality of diet was poor [8].

Several studies have been conducted to study its morphological and agronomic practices, nutritional and benefit importance and breeding or genetic improvement and many more. In Malaysia, assessment on multicriteria land suitability of BGN has been conducted in Peninsular Malaysia [1]. However, the nutritional, antinutritional, fatty acids composition and antioxidant studies have not been explored by local researchers in Malaysia. Thus, this study was conducted to investigate the nutritional, antinutritional and antioxidant capacity of BGN with comparison with others genus of Vigna (red bean (*V. angularis*) and black-eyed pea (*V. unguiculata*). These two beans are commercially available and commonly can be found in the market. Hence, this study also was conducted to study the potential of BGN compare to red bean and black-eyed pea.

2. EXPERIMENTAL DETAILS

2.1 Preparation of Sample

Bambara groundnut (BGN), red bean (RB) and black-eyed pea (BEP) were purchased from the local supermarket. Samples identification and authentication were carried out by botanist and plant genetic resources expert from Agrobiodiversity and Environment Research Centre, MARDI. All of the samples were washed in running tap water (room temperature) to remove any traces of soil dirt and dried in the oven (40°C) for 3 days (Memmert, Model 100-100, Germany). The samples were dehulled and the samples (seed and testa) were ground into fine powder using a mechanical grinder (IKA Werke MF 10 basic, Germany) and sieved using 1mm mesh size and the samples were kept in the chiller at -80ºC until needed for further analysis.

2.2 Nutritional Analysis

250g of dried powder samples were sealed in plastic bag, properly labelled and sent for nutritional analysis (proximate and minerals content) in the accredited laboratory. All the analysis were done in triplicates.

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

All the powder samples were extracted with 70% methanol and tested for their free radical scavenging ability as described earlier with minor modifications [9]. The test was carried out using 96 well plate. 5 mg of extract was prepared in one ml of 100 % methanol as stock solution. The stock solution was diluted accordingly into desired concentration for the working solution. The final volume obtained (7 µL) was mixed with 280 µL methanolic solution of (2,2-diphenyl-1-picrylhydrazyl) DPPH (Sigma, USA). The plate was covered with aluminium foil to avoid exposure with the sunlight and kept in the dark place for 30 minutes. Analysis was carried out using spectrophotometer at 517 nm. The results were expressed as inhibition concentration (IC_{50}) value (mg/mL) which is the inhibitory concentration at which DPPH radicals were scavenged by 50%. The ability of the sample to scavenge DPPH radical was determined from:

$$DPPH\ scavenging\ effect = \frac{Control\ OD\ -\ Sample\ OD}{Control\ OD} \times 100$$

2.4 Total Phenolic Content (TPC)

Total phenolic content of the crude extract was determined by the Folin–Ciocalteu method with some modifications [10]. Briefly, 50 µL of the crude extract were mixed with 100 µL of Folin Ciocalteau's phenol reagent (Merck, Germany). After 3 min, 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 min. The absorbance was measured at 725 nm and the total phenolic content was obtained from a calibration curve using gallic acid (0-10 µg/mL) as a standard reference. Estimation of the phenolic content was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg gallic acid equivalent per 100 g samples (mg GAE/100g) in dry weight (DW).

2.5 Fatty Acids Composition Using Gas Chromatography Mass Spectrometry (GCMS)

The fatty acids content in the BGN was determined using GCMS. The extraction of BGN oil was carried out using Soxhlet technique. The oils from the BGN were converted into fatty acid methyl ester (FAME) based from the previous

literature [11]. Sodium methoxide was used to produce methyl ester and saturated sodium bicarbonate was used to neutralise any trace of acid present in the mixture. The sample was then transferred into glass vial by using metal syringe to avoid plastic remnants such as phthalates. 1ml of the sample was transferred into GCMS glass vial. The capillary column used for the GCMS parameter was SolGel-Wax (30 m × 0.25m) with carrier gas was helium. The column gas temperature was set at 155.0 °C with injection temperature 250/0°C (split mode). Total run for the analysis was 30 minutes. The peaks similarity from the results were compared according to their retention time (t_R) with the provided library National Institute of Standards & Technology (NIST) and Flavour and Fragrance Natural and Synthetic Compounds (FFNSC).

2.6 Oxalates Determination Using High Performance Liquid Chromatography (HPLC)

The extraction for the total oxalate (TOX), soluble oxalate (SOX) and insoluble oxalate (IOX) contents using HPLC were determined based from previous literature review with minor modifications [10,12-13]. Total oxalate content (TOX) from BGN was determined through the extraction of the sample powder with hot sulphuric acid (H_2SO_4) (2 M) (80°C). The procedure was based from the previous published work by Savage in 2007 with minor modifications [14]. Approximately 0.5g powder sample was added into 50 ml of sample tube containing 20 ml of hydrochloric acid (HCl) (R&M Chemicals, Malaysia). The sample was extracted in the water bath (80°C) for 15 minutes. The extract was cooled in the room temperature before adding the remaining 2M HCl to make up the total volume of 50 ml. The sample tube then was centrifuged for 15 minutes (2889 rpm). The supernatant was filtered through cellulose nitrate membrane (0.45 µm) and subjected to the separation process using High Performance Liquid Chromatography (HPLC). Total oxalate content in the samples were expressed as mg in 100 g of dried weight of sample (mg/100g DW). Soluble oxalate content (SOX) was determined through extraction with distilled water. The procedure followed the TOX extraction method. Meanwhile, insoluble oxalate content (IOX) was determined by the following formula:

Insoluble oxalate content (IOX) = Total oxalate (TOX) – Soluble oxalate (SOX)

2.7 Standard Calibration for TOX and SOX

Two sets calibration curve were prepared by using oxalic acid (Sigma Aldrich 658537) as standard in five different concentrations 6.25, 12.5, 25, 50, 100 ug/ml. Oxalic acid was dissolved in 2M HCl and water for the analysis of TOX and SOX.

2.8 Parameter of HPLC for the Oxalates Determination

A 5 µl filtered sample from acid and water extracts were injected through HPLC system with Diode-Array Detection at 210 nm (Agilent chromatography System 1200 Infinity Series). The oxalate determination using HPLC was based from the previous literature with minor modification [10]. The separation was done using Rezex ROA ion exclusion organic acid column, 300 × 7.8 mm (Phenomenex, Torrance, CA, USA) attached to a cation H+ guard column. The column temperature was set at 50°C. The isocratic elution selected for the separation was 60% of 0.005 N sulphuric acid (H_2SO_4), 40% acetonitrile (ACN) at the flow rate of 0.5 mL/min for 20 minutes.

3. RESULTS AND DISCUSSION

Fig. 1 showed the proximate analysis of BGN with comparison with RB and BEP. The proximate analysis consists of evaluation of ash, protein, fat and total carbohydrate contents (g/100g).

The ash content in the BGN was found to be higher with 3.83 ± 0.08 g/100g and significantly different ($F= 56.27$, $P<0.05$) to RB and BEP with 2.93 ± 0.08 g/100g and 3.10 ± 0.07 g/100g respectively. Ash content determination refers to the process of ignition or complete oxidation of organic matters in any food samples to leave the inorganic residue. The inorganic residue mainly consists of mineral present in the food samples [15]. It is also indicating the samples is high in micro and macro elements [6]. The protein content in the three samples was in the range of 17.1 – 22.5 g/100g with BGN showed the lowest with 17.1 ± 0.01 g/100g as compared with BEP and RB ($F=1486.19$ $P<0.05$). The protein content was parallel to the previous studies with range of 15-25 % [16]. Study by Saanu in 2017 also showed the percentage of protein in the commercial BGN was 18.5 % [6]. However, the fat content value showed the highest in the BGN

(8.27 ± 0.12 g/100g) and significantly different (F=1143.8, P<0.05) compared to RB and BEP with (1.07 ± 0.12 g/100g) and (2.30 ± 0.22 g/100g) respectively. Meanwhile, the total carbohydrate content in the BGN was comparable higher (66.0 ± 0.01 g/100g) (F=193, P <0.05) with RB and BEP with (63.0 ± 0.02 g/100g) and (60.7 ± 0.05 g/100g) respectively. The energy provided (kcal) in 100g of BGN was 13-14% higher (406.7 ± 1.25 kcal/100g) (F=1392.45, P<0.05) compared to RB and BEP with 349.0 ±1.41 and 353.3 ± 0.94 kcal/100g respectively. Our findings were in agreement with previous report [16-18] with the value of carbohydrate content in the range of 49– 65 (g/100g) and by Boateng et al. 2013 with the amount energy calculated 3617-414 kcal/100g [19]. The carbohydrate composition in BGN also has been studied as one of the alternative ingredients to wheat flour, dextrin and corn starch for tilapia, *Oreochromis niloticus*. The experiment showed the potential of BGN as one of the carbohydrate ingredients in the preparation of tilapia's diets. The nutritional documented suggested the groundnut to be highly nutritious

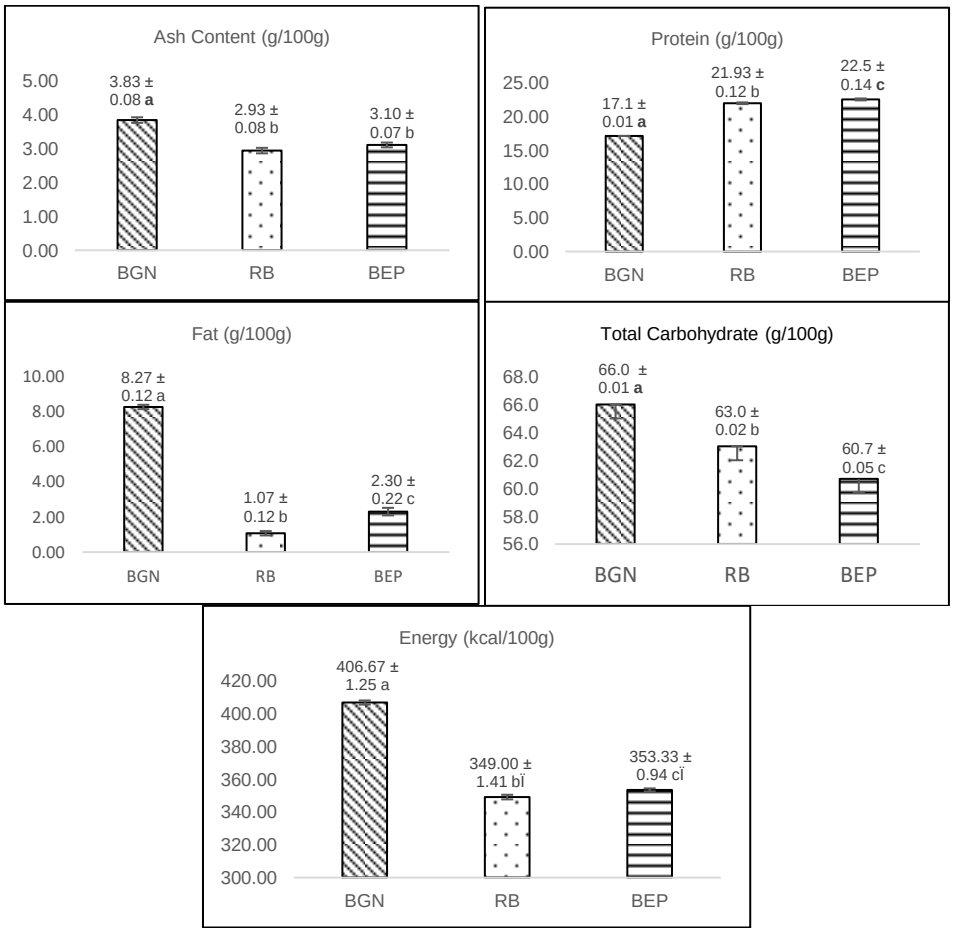

Fig. 1. The proximate analysis of Bambara groundnut (BGN), red bean (RB) and black-eyed pea (BEP)

Table 1. Mineral composition (mg/100g) in the bambara groundnut (BGN), red bean (RB) and black-eyed pea (BEP)

Legume/Mineral	Magnesium (Mg)	Phosphorus (P)	Potassium (K)	Zinc (Zn)
BGN	163.33 ± 4.7a	316.67 ± 23.57a	1516.67 ± 23.5a	2.0
RB	126.7 ± 4.7c	396.67 ± 12.47b	1116.67 ± 23.6b	2.0
BEP	216.67 ± 0.01b	463.33 ± 17.0c	1150.0 ± 40.8b	4.0

** Grouping Information Using the Tukey Method and 95% Confidence. Means that do not share a letter are significantly different, Mg (F=184.33, P<0.05) ;P (F=32.36, P<0.05), K (F=106.4, P<0.05*)

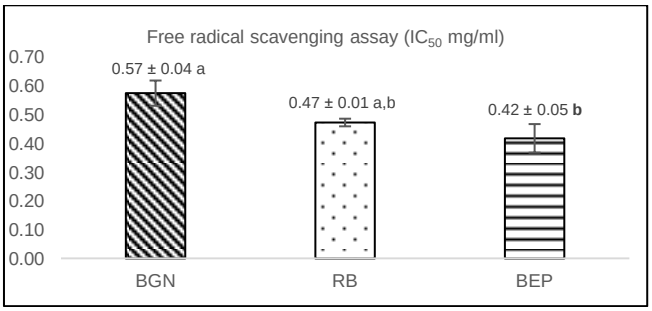

Fig. 2. Free radical scavenging assay of bambara groundnut (BGN), red bean (RB) and black-eyed pea (BEP) (IC_{50} mg/ml)

and kindly relevant for those people who cannot afford an expensive carbohydrate source [20].

Table 1 showed the mineral composition in the BGN, RB and BEP with the highest amount of potassium (K) detected in the BGN (1516.67 ± 23.5 g/100g), 26% higher and significantly different ($F=106.4$, $P<0.05$) to RB and BEP, respectively. The substantial amount of K in the BGN was also found in the previous report by Adebiyi et al., in 2019 which the mineral showed the highest level (831 – 979 mg/100g) compared to others minerals [21]. A study in Nigeria also showed potassium has the highest mineral composition with 187.07 mg/100g [22]. Potassium is an essential mineral that play a key role for the resting membrane potential and the intracellular osmolarity and helps to reduce the blood pressure by reducing the sodium intake in the body [23]. The high content of K in these legumes including BGN also was found to be more substantial compared to banana, which is known to have high level of potassium. The potassium in banana was found to be in the range of 358 – 400 mg/100g [24]. Our studies also in agreement with the previous data for others minerals such as P (247 – 316 mg.100g) [21].

Meanwhile, Fig. 2 showed the free radical scavenging assay of methanolic extracts of BGN, RB and BEP. The IC_{50} value in the BGN was 0.57 ± 0.04 mg/ml showing moderate antioxidant activities while RB showed the lowest IC_{50} value, indicates stronger antioxidant activity (standard ascorbic acid = 0.06 mg/ml). The IC_{50} value lower than 10 mg/mL is indicative of the effectiveness on its antioxidant activity [25]. Previous finding has shown the processed and cooked BGN has increased the free radical scavenging activity up to 10fold from uncooked BGN [26].

Meanwhile, the total phenolic content in BGN, RB and BEP shown in Fig. 3. The total phenolic content in BGN was comparable higher and significantly different ($F=256.49$, $p<0.05$) to RB and BEP with 87.3 ± 2.05 mg GAE/100g. The high amount of phenolics content in BGN mainly due to the red testa colour. A study done by Adedayo in 2021 showed that the six varieties of BGN with different testa colour has different polyphenols content with black to red testa colour varieties showed the highest polyphenols content as compared to brown testa colour variety [27].

Analysis of fatty acids from BGN was carried out using GCMS. The oils from BGN showed presence of various unsaturated and saturated fatty acids (Fig. 4). The oil from BGN was high in linoleic acid (C18:2), palmitic acid (C16:0), oleic acid (C18:1), linolenic acid (C18:3), stearic acid (C18:0), eicosanoic acid (C20:0) and behenic acid (C22:0). Our finding was in a good agreement with recent studies by Ntombokulunga et al. 2022, with palmitic, stearic, linoleic, oleic , α-linolenic are majors saturated and unsaturated fatty acids in Bambara groundnut [28]. Palmitic, oleic and linoleic acids also were found to be abundant in the Bambara samples in the Nigeria with the monounsaturated fatty acid (oleic) was the highest composition of fatty acid [6].

The antinutritional factor (ANF) in BGN has been determined using HPLC. The presence of ANF can disrupt the digestion and bioavailability of essential nutrients [29]. The selected HPLC conditions show a linear relationship ($r^2=0.999$) for acid and water prepared curve using five different concentrations of oxalic acid as standard. Extractions and analysis of BGN found no trace of total, soluble and insoluble oxalates. However, in contrary, few studies showed traces of oxalate in BGN. The oxalate content in raw

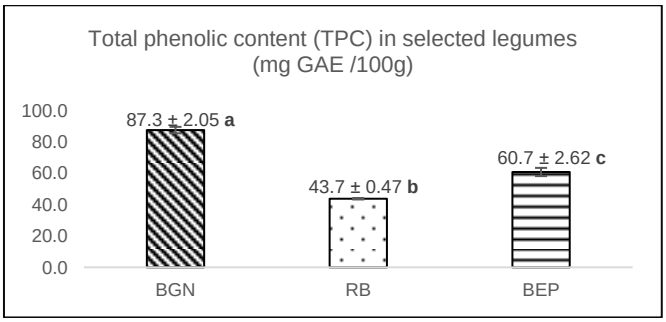

Fig. 3. Total phenolic content in BGN, RB and BEP (mg GAE/100G)

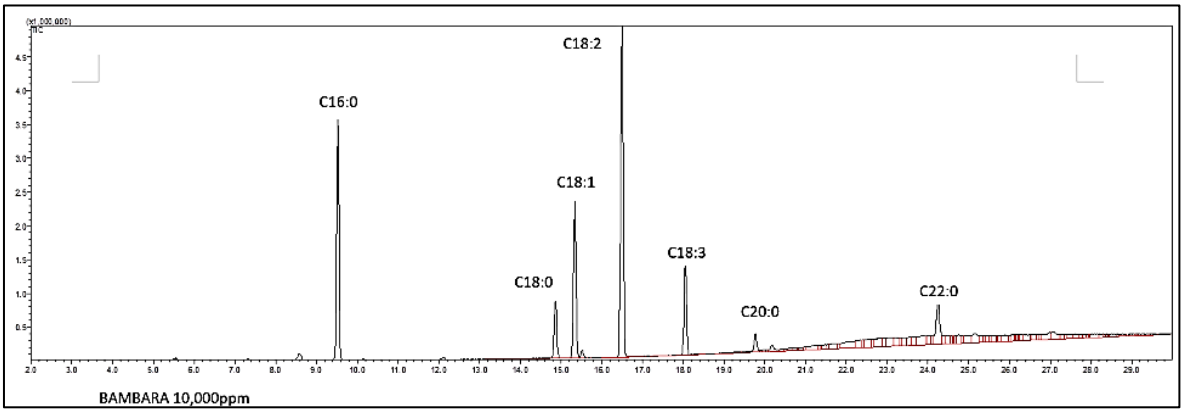

Fig. 4. GCMS chromatogram of fatty acid from BGN

BGN showed traces of oxalate content (above permissible levels (5mg/kg)). However, the oxalate contents were reduced when the samples were boiled and roasted [22]. The difference of oxalates contents may varies depending to the genetic and location distributions, varieties of samples and method of analysis. For example, study on oxalates content on taro (*Colocasia esculenta*) collected from various state in Peninsular Malaysia showed different in the oxalates content [30]. Apart from that, studies by Judprasong in 2006 to determine the oxalates content in different Thai vegetables, cereal grains and legume seeds from three different market in Bangkok had also indicated different oxalates content [31].

4. CONCLUSION

Comparison study on nutritional values on BGN with RB and BEP showed interesting finding on the total carbohydrate content with 66.0 ± 0.01 g/100g, higher than RB and BEP. BGN also provides more energy (kcal/100g) compare to RB and BEP. Meanwhile, substantial amount of potassium also was found in BGN. The free radical scavenging assay showed moderate inhibition from the extract of BGN but surprisingly higher in polyphenols. Fatty acids compositions from the oils of BGN showed presence of linoleic acid, palmitic acid, oleic acid, linolenic acid, stearic acid, eicosanoic acid and behenic acid. The antinutritional content showed no presence of total, soluble and insoluble oxalate content. These wide-ranging findings show the importance of this underutilize legume to be elevated for its commercial values.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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