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Morphological characterization and nutrient assessment of wild pepper, *Piper umbellatum* L. (Piperaceae) grown in Sarawak, Malaysia

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ABSTRACT

Wild pepper, *Piper umbellatum* L. is traditionally consumed as a leafy vegetable by the indigenous Kenyah tribesmen of Belaga, Sarawak, Malaysia. The shoots are normally harvested from secondary forest floors. The present study was carried out to determine the morphological and nutritional characteristics of *P. umbellatum*, i.e., proximate, mineral, total phenolic content (TPC), total flavonoid content (TFC), vitamin C, and anti-nutrients, i.e., oxalate and phytate. The results showed that *P umbellatum* possesses an ovate, alternate, entire and non-glandular trichome on its leaf surface. The inflorescence comprises an oblongoid spike attached to a peduncle and the ripened berries were orange and red in color. The results also revealed that the leaves of *P. umbellatum* had a high moisture content (63.27%), other recorded nutrient values were ash (8.62%), crude fiber (19.32%), K (1280.20 mg/100 g), Ca (570.60 mg/100 g), Mg (323.80 mg/100 g) and P (291.14 mg/100 g), TPC (510.63 mg/100 g), TFC (377.82 mg/100 g) and phytate (411.67 mg/100 g). Thus the consumption of the *P. umbellatum* as a leafy vegetable supplies a good dose of various essential nutrients.

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INTRODUCTION

The *Piperaceae* family is widely distributed in tropical regions and comprises over 1000 species (Parthasarathy et al., 2006). Piper umbellatum L. (syn. Pothomorphe umbellata (L.) Mig., Lepianthes umbellata (L.) Raf., Heckeria umbellata (L.) Kunth and *Peperomia umbellata* (L.) Kunth) is a species which grows extensively in Malaysian rainforests. This species is also known by several common names such as umbelled pepper, cowfoot, Segumbar urat (Peninsular Malaysia), Lemba (Moluccas), Capeba or pariparoba (Brazil), Bumbu, Domboo, Tombo and Ucheng-ucheng (Java) (Tawan et al., 2002; Mensah et al., 2013; da Silva et al., 2014). The importance of P. umbellatum as a medicinal plant has been described in various studies. The extract from its leaves and other parts of the plant have been used for the treatment of infectious and inflammatory diseases (Roersch, 2010), snake venom (Núñez et al., 2005) and as an anti-cancer remedy (Iwamoto et al., 2015). The plants also been used by witchdoctors in Cameroon (Agbor *et al.*, 2005; Roersch *et al.*, 2010), as a fragrance in Ecuador (Pohle & Reinhardt, 2004; Roersch *et al.*, 2010), and as fish bait in Ghana (Roersch *et al.*, 2010).

In many countries, the leaves of *P. umbellatum* is consumed as a leafy vegetable and condiment as was reported by Mensah *et al.* (2008). In Malaysia, *P. umbellatum* is also used as a leafy vegetable by locals especially by the Kenyah ethnic group residing in Sungai Asap, Belaga, Sarawak. The plant can occasionally be found growing on the forest floor within the Belaga area. It is a perennial or woody herb that commonly grows up to 1.0-2.5 m tall (Roersch, 2010). It flourishes in shady, moist habitats with moderate light penetrating through the forest gaps. The local people collect this plant from the nearby forests along their longhouses and sell it at the nearest market. The leaves are prepared as one of the ingredients for freshwater fish soup dishes and are also eaten as a relish together with their staple diet. The

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locals believe that the plant contain high nutritional values which are good for health. However, previous studies conducted have mainly focused on the medicinal uses of this plant rather than its nutritional attributes. Additionally, there is far less scientific data available on morphological descriptions and agronomic requirements of this species. Therefore, the present study was conducted for the purpose of describing the morphology of *P. umbellatum* as well as its nutrient and anti-nutrient contents.

MATERIALS AND METHODS

Sample Collection

The samples of *P. umbellatum* were collected at Sg. Asap secondary forest, Belaga, Sarawak. The selected samples were then placed in an ice chest for transportation to the Agronomy Laboratory, Universiti Putra Malaysia Kampus Bintulu, Sarawak.

Morphological Study

In the laboratory, the morphological characteristics of *P. umbellatum* at leaf, inflorescence and berry parts were observed and measured. The study involved the assessment of the qualitative and quantitative traits of the plant as described in Table 1. The qualitative traits were measured at the study site using a metric ruler and vernier caliper. Whereas micro measurements such as trichome length on leaf surface was observed under Keyence Digital Microscope VHX-600.

Table 1. The qualitative and quantitative measurements of *P. umbellatum*.

Plant Parts	Qualitative parameter	Quantitative parameter
Leaf	Shape, arrangement, trichome	Leaf dimension and trichome lengths
Inflorescence	Spike color, peduncle color	Spike and peducle dimensions
Berry	Berry shape, berry and pedicle color	Berry diameter and pedicle dimensions

Nutrient and Anti-nutrient Contents

Sample preparation

The samples of *P. umbellatum* were collected after measurements of qualitative traits were taken. The samples were then placed in the ice chest for transportation to the Agronomy Laboratory, Universiti Putra Malaysia Bintulu Sarawak Campus. In the laboratory, the edible parts of the samples comprising the leaves and petiole were then separated and selected for nutrient analysis. The leaves of *P. umbellatum* were washed under running tap water to remove any dirt and contaminant and were then subsequently rinsed with distilled water. The moist leaves were left to evaporate at room temperature. The leaves then were then oven dried in 60°C for 24 hours following the methods of Abuye *et al.* (2003) and ground into a dried powdered sample using a heavy duty blender. The powdered sample was stored in airtight containers until further analysis.

Proximate composition

The moisture, ash, crude protein, crude fiber, crude fat, total carbohydrate and energy of *P. umbellatum* leaves were determined in triplicate according to the Association of Official Analytical Chemists (AOAC, 1990) methods. The percentage of crude protein content was estimated by multiplying of nitrogen content by a 6.25 factor (978.04, AOAC, 1990). The crude fiber content was extracted with 1.25% sulphuric acid (H_2SO_4) and 1.25% sodium hydroxide (NaOH) and then quantified using the 2010 Fibertec System Foss Tecator, Sweden (930.10, AOAC, 1990). The crude fat content was extracted using a 2055 Soxtec Avanti Manual System, Sweden according to method 930.09 (AOAC, 1990). The carbohydrate content was determined through difference of 100% DW sample with the total of crude protein, crude fiber and ash (AOAC, 1990).

Mineral content

The ash obtained from the ash content analysis was used for mineral digestion. A few drops of distilled water were added into porcelain crucibles followed by 2 mL of concentrated hydrochloric acid (HCl). Next, 10 mL of 20% HNO₃ was added and the samples was left to evaporate on a hotplate. The sample was then filtered using Whatman filter paper No. 2 into 100 mL volumetric flasks (method 922.02, AOAC, 1990). The concentration of K, Ca, Mg, Na, Fe, Zn, Cu and Mn were determined through the use of an Atomic Absorption Spectrophotometer (AA800 Perkin-Elmer, Germany) whereas P content was measured using the blue development method according to Murphy and Riley (1992) and quantified using a UV-Vis Spectrophotometer (Lambda 25 Perkin-Elmer, Germany).

Antioxidant content

TPC content was determined using the Folin Ciocalteu's method by Singleton *et al.* (1999). 1 g of the oven dried grounded samples was weighed and soaked in 10 mL of methanol 70% (w/v) and centrifuged at 1000 rpm for 10 minutes. 1 mL of the aliquots were pipetted into individual test tubes. Standard gallic acid in concentrations of 10, 20, 40, 60, 80 and 100 µg/mL was used as standards and 0.5 mL of Folin Ciocalteu's reagent was added. The tubes were incubated for 2 hours at room temperature for color development. The absorbance was measured at 750 nm by using a UV-VIS Spectrophotometer (Lambda 25 Perkin Elmer, Germany). The TPC content in the samples calculated by equation from the calibration curve and expressed in gallic acid equivalent (mg GAE/100 g) of dry mass.

The TFC content was determined using the method described by Kamtekar *et al.* (2014). 1 mL of the aliquots and 1 mL of blank as well as standard quercetin at 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL were placed into the test tubes followed by the addition of 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. The mixture was left to rest for 5 minutes before 0.3 mL of aluminium chloride solution was added. This mixture was allowed to stand for 6 minutes. Finally, 2 mL of 1 M sodium hydroxide was added and distilled water was added to make up the volume of 10ml. The resultant mixture was mixed thoroughly until a yellowish orange color developed. The TFC content was measured at 510 nm of absorbance using a UV-VIS Spectrophotometer (Lambda 25 Perkin Elmer, Germany). The TFC was expressed as mg of quercetin equivalents per 100 g quercetin equivalents (mg QE/100 g) on a dry mass basis.

Vitamin C content was determined by extracting 3 g of the ground samples using 3 mL of 10% metaphosphoric acid which was diluted using deionized water up to the volume of a 50 mL volumetric flask. The pH of the filtrate was adjusted to 5.0-5.25 using 4 M NaOH or 10% metaphosphoric acid and then 10 mg dithiothreitol was added. The mixture was incubated in the dark at room temperature for 1 hour. The mixture was then filtered and re-filtered through a 0.45 μ m membrane filter prior to injection for HPLC analysis. Vitamin C determination was performed using an HPLC system using a Zorbax 5 μ m ODS column (250 x 4.6 mm) with an analytical guard column C-130B (2 x 20 mm). The mobile phase was 0.5% KH₂PO₄ at a flow rate of 0.8 mL/min. Vitamin C was monitored at 245 nm. The results were expressed as mg of ascorbic acid per 100 g fresh weight (mgAA/100 g).

Antinutrient contents

The oxalate content was determined by the methods described by Day and Underwood (1986). Approximately 1 g of the ovendried grounded sample was weighed and placed in a 100 mL conical flask. Next, 75 mL of 3M H2SO4 was added into the conical flask and stirred using a magnetic stirrer for one hour. The mixture was then filtered into a plastic vial and 25 mL of the collected filtrate was taken. The filtrate was titrated while hot against 0.05 M of potassium permanganate (KMnO4) until the mixture turned a persistent faint pink color (for at least 30 seconds). The volume of the KMnO4 titrated was recorded and calculated using the assumption that 1 mL of KMnO4 is equivalent to 2.2 mg oxalate.

Phytic acid was determined using the procedures of Lucas and Markakas (1975). About 2 g of the oven-dried grounded samples was weighed and put into a 250 mL conical flask. Next, 100 mL of 2% concentrated HCl was poured into the conical flask and left for three hours. The mixture was filtered using the Whatman filter paper No. 2 and 50 mL of the collected filtrate was moved to 250 mL beakers. Then, 107 mL of distilled water was added into each of the beakers followed by 10 mL of 0.3% ammonium thiocyanate solution as an indicator. The titration was carried out against the standard iron chloride solution which contained 0.00195 g iron per mL. The titration volume was recorded and used to calculate the phytic acid content using the formula: % Phytic acid = (titre value x 0.00195) x 1.19 x 100.

Statistical Analysis

The quantitative morphological characteristics of *P. umbellatum* and its nutritional content were analyzed using a one way analysis of variance (ANOVA) at $p \le 0.05$ using the Statistical Software Program, SAS Version 9.4.

RESULTS AND DISCUSSION

Morphological Characteristics

The leaves of *Piper umbellatum* grown in Malaysia are of ovate shape for the entire margin. The leaves form an alternate pattern between each other on the plant. The adaxial and abaxial surface has a non-glandular trichome also at the petiole (Figure 1 and Figure 2). It has big leaves with the mean dimension at 25.71

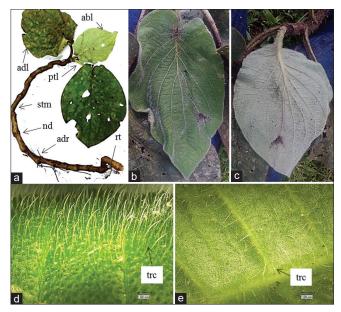


Figure 1: Vegetative parts of *P. umbellatum* (a) Woody shrub of *P. umbellatum* showing adaxial or upper leaf (adl), abaxial or lower leaf (abl), petiole (ptl), stem (stm), node (nd), adventitious root (adr) and root (rt), (b) leaf adaxial surface, (c) leaf abaxial surface, (d) microscopic image showing the presence of trichome (trc) on adaxial surface, and (e) microscopic image showing the presence of trichome (trc) on abaxial surface.

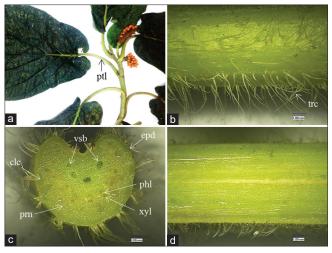


Figure 2: Vegetative parts of *P. umbellatum*: Petiole (a) *P.umbellatum* showing petiole (ptl), (b) abundance of non-grandular trichomes on the petiole, (c) cross section of the petiole consists of epidermis (epd), collenchyma (clc), parenchyma (prn), phloem (phl), xylem (xyl) and vascular bundles (vsb), and (d) longitudinal section of the petiole.

Saupi et al.

x 2.24 cm whereas the mean of the trichome length averages 1.58 mm (Table 2). The leaves possessed similar characteristics to those grown in the Dominican Republic (Roersch, 2010). The trichome growth exhibited various functional adaptations for environmental stress, increased water retention capability and mechanical defense against herbivores (Medeiros & Boligon, 2007; Kenzo *et al.*, 2008).

The inflorescence is axillary or leaf opposed spikes, the orientation is erect and usually in false umbels with the pedicels bearing the bracts (Figure 3). The inflorescence consists of an oblongoid spike which is creamy white to yellowish orange in color. The spike measures approximately 6.21×0.12 cm (length x width). It is attached to a light green color peduncle which develops to about 3.19×0.52 cm (length x width). The peduncle and spike length measured in the current study was within the range of those reported in other studies (Roersch *et al.*, 2010). Flowering occurs throughout the year, depending on water availability (Nwauzoma *et al.*, 2013).

The berry of *P. umbellatum* is a fleshy drupe or berry form, which is 4.22 mm in mean diameter (Figure 4). The berry attaches to a light green color pedicel which measures 0.69 x 0.10 cm (length x width). The characteristics are similar to those described in a study of *P. umbellatum* from Nigeria. The berry is commonly used for propagation and the color observed was a bright orange and red color. The striking color is to enable the plant to attract the attention of fruit-eating mammals as well as avians

Table 2. Quantitative and quantitative characteristics of *P. umbellatum*

Plant part	Qualitative traits		
Leaf	Shape	Ovate	
	Arrangement	Alternate	
	Margin	Entire	
	Trichome type	Non-grandular	
Inflorescence	Spike shape	Oblongoid	
	Spike color	Yellowish orange	
	Peduncle color	Light green	
Berry	Berry shape	Drupe	
	Berry color	Orange and red	
	Pedicle color	Light green	
	Quantitative traits		
Stem	Stem diameter (mm)	21.73 ± 2.98	
	Internode lenth (cm)	5.53 ± 0.34	
	Plant height (cm)	53.47 ± 21.35	
Leaf	Blade length (cm)	25.71 ± 1.70	
	Blade width (cm)	22.24 ± 0.14	
	Petiole length (cm)	14.87 ± 1.24	
	Petiole diameter (mm)	8.83 ± 0.57	
	Trichome length (mm)	1.58 ± 0.03	
Inflorescence	Number of spike per plant	3.00 ± 1.53	
	Spike length (cm)	6.21 ± 0.76	
	Spike width (cm)	1.02 ± 0.13	
	Peduncle length (cm)	3.19 ± 0.54	
	Peduncle diameter (cm)	0.52 ± 0.07	
Berry	Number of berries per spike	85.75 ± 19.45	
	Berry diameter (mm)	4.22 ± 0.12	
	Pedicel length (cm)	0.69 ± 0.03	
	Pedicel width (cm)	0.10 ± 0.03	

Data of quantitative traits were presented as mean \pm standard error of 9 samples

(Nwauzoma *et al.*, 2013). However, the berry of *P. umbellatum* found in Nigeria was reported to be of a light brownish color.

Nutrient and Anti-nutrient Contents

Proximate composition

The results for the proximate composition of *P. umbellatum* showed that the moisture content of the edible parts was relatively high in comparison to ash, crude protein, crude fiber, crude fat and the total carbohydrate content (Table 3). The trend of the proximate analysis recorded in the study was observed in the following order: Moisture > carbohydrate >

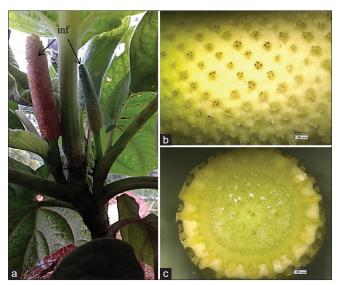


Figure 3: Reproductive parts of *P. umbellatum*: Inflorescence (a) Woody shrub of *P. umbellatum* showing inflorescence (inf), (b) flower spike showing individual, perianthless flowers with bracts, and (c) cross section of the spike.



Figure 4: Reproductive parts of *P. umbellatum*: Fruits (a) Orange berries, (b) yellow berries, (c) red berries, and (d) shoots beginning to grow from ripened berries.

crude fiber > ash > crude protein > crude fat. However, the proximate analysis performed only for the edible parts of the plant.

The moisture content of the fresh weight sample was found to be 63.27% and comparable with other selected Malaysian leafy vegetables as reported by Hoe and Siong (1999). However, the result obtained in the current study was approximately one times lower than *P. umbellatum* recorded by Mensah *et al.* (2013) in Nigeria. This may be influenced by several factors such as sample maturity and agro-climatic conditions (Gupta *et al.*, 2005). In addition, agro-ecological areas in Nigeria are heavily influenced by the airmass originating from the South Atlantic Ocean which increase the humidity in the country especially during the rainy season (Eludoyin & Adelekan, 2012). Due to the presence of high moisture content, appropriate handling and processing methods are necessary to increase the shelf life of *P. umbellatum* and reduce the rate of deterioration (Ooi *et al.*, 2012).

Ash content is used to indicate the presence of mineral nutrients in a plant sample. The ash content recorded in the present study was 8.62% which is similar to other leafy vegetables such as *Basella alba* (8.23%) and *Morus alba* (8.91%) as found in prior studies done by Saha *et al.* (2015) and Iqbal *et al.* (2012) respectively. However, this value was found to be approximately three times lower than *Peperomia pellucida* (31.2%) which is consumed in Malaysia (Ooi *et al.*, 2012). The high ash percentage found in the *P. umbellatum* indicates a high mineral nutrient content.

The crude protein obtained during the current study was found to be 1.14% which is lower in comparison to other studies. Crude protein in *P. umbellatum* as recorded by Mensah *et al.* (2013) was 3.9%, which is roughly three times higher than the readings from the current study. A comparison with *P. pellucida* also showed that the vegetable had nine times the amount of protein as opposed to *P. umbellatum* (Ooi *et al.*, 2012). This indicates that *P. umbellatum* is not a complete source of dietary protein, hence its consumption with other protein rich sources such as meats is highly recommended.

However, in terms of crude fiber the results obtained from this study showed *P. umbellatum* to have a high content composition of 19.32%. This value was similar with those obtained from a previous study carried out by Mensah *et al.* (2013) who found a value of 20.20% for crude fiber. The results

Table 3. The nutrient and antinutrient composition of *P. umbellatum*.

Proximate	Concentration (%)		
Moisture*	63.27 ± 0.78		
Ash	8.62 ± 0.11		
Crude protein	1.14 ± 0.07		
Crude fiber	19.32 ± 0.75		
Crude fat	0.86 ± 0.05		
Total carbohydrate	33.33 ± 1.37		

Data represented in means \pm standard error (SE) of dry weight. Values noted with asterisk (*) are represented as fresh weight

showed that *P. umbellatum* is a good source of dietary fiber. Fiber is considered an important nutrient for health due to its potential to reduce the occurrence of colon disease, cancer, diabetes, heart disease and also to help improve digestion (Alam *et al.*, 2016).

The crude fat content obtained from the current study was only 0.86%, it was the lowest percentage out of all the other compositions. The result fell within the range of various leafy vegetables consumed in India (0.19-4.19%) (Saha *et al.*, 2015). This showed that *P. umbellatum* is a low fat food and thus its consumption may prove beneficial to reduce the incidence of obesity and cardiovascular diseases (Dewell & Ornish, 2007). Thus the consumption of *P. umbellatum* can be recommended as part of a dietary plan for reducing body weight.

The total carbohydrate content obtained in this study was 33.33% which was comparable to the results of prior studies regarding *P. umbellatum* (38.00%) and *P. pellucida* (46.58%) (Ooi *et al.*, 2012; Mensah *et al.*, 2013). The high carbohydrate content enables the plant to supply a significant amount of useful energy for one to carry out their daily activities (Yisa *et al.*, 2010; Saha *et al.*, 2015).

Mineral Content

The K content recorded in the study was the highest in comparison to other elements. The trend of the mineral elements observed in this study was in the following order: K > Ca > Mg > P > Fe > Mn > Na > Zn > Cu. P. umbellatum contains a significantly high K content with a concentration of 1280.20 mg/100g (Table 4). The values recorded were relatively low when compared to other Malaysian leafy vegetables such as P. pellucida (6977.00 mg/100 g) and Limnoscharis flava (4202.50 mg/100 g) (Saupi et al., 2009; Ooi et al., 2012). However, the values were comparable to other leafy vegetables such as Pangium edule (1157.25 mg/100g) and Gnetum gnemon (Ainul Asyira et al., 2016). The K content in P. umbellatum contributes approximately 36.58% of the 3500 mg/d Recommended Dietary Allowance (RDA) (WHO, 2012). A high intake of K in a daily diet could potentially reduce the risks of coronary heart disease as well as other chronic diseases (Weaver, 2013; Ainul Asyira et al., 2016). Potassium is a significant mineral for plants as it helps to govern enzyme activity, protein synthesis, stomatal activity, photosynthesis, in

Table 4. The minera	l content in	Р.	' umbellatum.
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Mineral	Concentration (mg/100 g)	
K	1280.20 ± 120.69	
Р	291.14 ± 21.30	
Ca	570.60 ± 21.57	
Mg	323.80 ± 18.31	
Na	4.00 ± 1.05	
Fe	16.34 ± 0.95	
Zn	3.16 ± 0.22	
Cu	2.18 ± 0.26	
Mn	12.10 ± 0.62	
Na/K	0.01 ± 0.00	
Ca/P	2.00 ± 0.14	

Data represented in means \pm standard error (SE) of the dry weight

Saupi et al.

addition to increasing crop production (Prajapati & Modi, 2012; Wang *et al.*, 2013).

The concentration of P in the P. umbellatum was estimated to be approximately 291.14 mg/100g. The amount is comparable to Justicia flava (292.00 mg/100g) and Emex australis (290.00 mg/100g), which are common leafy vegetables consumed in South Africa (Odhav et al., 2007). The consumption of 100 grams of P. umbellatum can provide 41.59% of the RDA which is recommended to be 700 mg/d for adults, lactating and pregnant women (Sawka, 2005). Phosphorus is required for the body to carry out cellular metabolism and skeletal mineralization and its deficiency may cause osteoporosis especially in women (Heaney, 2004). According to Karp et al. (2007), phosphorus is not a limiting nutrient as it is readily available in various vegetables and plant-based foods, however a high percentage of P which is present in plant based foods are bonded with phytate making it unavailable for body absorption.

High concentrations of Ca was also observed in the *P. umbellatum* at 570.60 mg/100g ,these results were similar to those reported by Ooi *et al.* (2012) and Saupi *et al.* (2009) in other leafy vegetables. The Ca obtained in the study can contribute 43.89% of the 1300 mg/d RDA. *P. umbellatum* has good potential as a source of dietary Ca and its intake may contribute to bone and teeth health, nerve regulation and muscle function (Ng *et al.* 2012). Ca deficiency may lead to rickets in children and osteomalacia in adults (Soetan *et al.*, 2009; Ainul Asyira *et al.*, 2016).

The Mg content recorded in the present study was found to be 323.80 mg/100g which is comparable to *L. flava* (228.10 mg/100g) which is commonly consumed by Malaysians (Saupi *et al.*, 2009). Magnesium is required in appropriate amounts to carry out many of the bodily functions such as glucose metabolism and insulin homeostasis (Song *et al.*, 2004; Barbagalo *et al.*, 2003). The consumption of *P. umbellatum* in one serving of 100 g is estimated to contribute about 77.10% of the 420 mg/d of the RDA for Magnesium (Otten *et al.*, 2006). Mg deficiency was reported to cause Type 2 diabetes mellitus (He *et al.*, 2006).

P. umbellatum was found to have a low content of Na with a concentration of just 4.00 mg/100g. This result was comparable to the Na content of Erechtites hieracifolia (5.30 mg/100g), a wild leafy vegetable commonly consumed in Indonesia (Srianta et al., 2012). However, this amount is 16 times lower than G. gnemon (65.00 mg/100g) (Rukayah, 2002). This shows that P. umbellatum contain low concentrations of Na, which is concurrent with various other studies showing that some varieties of leafy vegetables contain low amounts of Na (Gupta et al., 2005; Saha et al., 2015). The RDA for Na is recommended at 2300 mg/d for adults. From the results obtained P. umbellatum can contribute 0.17% of the required RDA for Na from a 100g serving. The addition of salt during the preparation of dishes can also increase the amount of Na consumed. The overconsumption of Na may cause high blood pressure, cardiovascular diseases and stroke (WHO, 2012).

The iron content of *P. umbellatum* was found to be approximately 16.34 mg/100g which is about seven times lower than *P. pellucida*

(119.30 mg/100g) (Ooi *et al.*, 2012). Whereas, these values were comparable to that of *Vernonia amygdalina* (16.43 mg/100g) as found by Gupta et al *et al.*, (2012). The RDA of Fe for adults was between the ranges of 8-18 mg/day, hence the Fe content of *P. umbellatum* was found to be within this range (Sawka, 2005). This constitutes about 90.78% of the RDA for Fe. Iron is a major component of haemoglobin, proteins, enzymes and myoglobin (Sawka, 2005). Therefore, a deficiency of this mineral can lead to health problems such as astherosclerosis, autoimmunity disease, Parkinson's disease, fibrosis as well as other related diseases (Brewer, 2007).

The zinc content in P. umbellatum was relatively low with a concentration of 3.16 mg/100g. The values recorded were comparable to L. flava (0.66 mg/100g) and this can contribute about 28.72% and 39.50% of the RDA in one 100g of serving for adult males and females respectively. In general, Zn is required for many bodily functions and metabolic activities such as cell and protein division. A deficiency of Zinc causes anemia and retardation of fetal brain cells in pregnant women, although such deficiency rarely occurs (Chasapis et al., 2012). Copper is a micro element which was detected in low concentrations in P. umbellatum during the current study. P. umbellatum was found to contain 2.18 mg/100g of Cu this was the lowest value recorded in comparison to the other elements detected. The Cu contain in the P. umbellatum is roughly two times higher than the RDA which recommends an intake of 0.9mg/d (Sawka, 2005). Nonetheless, the consumption of P. umbellatum although exceeding the RDA for Cu is not regarded as harmful or toxic as the content was still lower than 10 mg/d of the Tolerable Upper Intake Level (UL). Cu deficiency in the general population is rarely reported (Sawka, 2005).

P. umbellatum was recorded to contain 12.10 mg/100g concentrations of Mn. This amount was three time lower in comparison to the content of *Moringa oleifera* leaves (43.27 mg/100g) (Saha *et al.*, 2015). The findings suggest that *P. umbellatum* has a low Mn content in comparison to other vegetables. Manganese is required by the body to regulate and bind various enzymes in the body such as arginase, superoxide dismutase and pyruvate carboxylate (Crossgrove & Zheng, 2004). The occurrence of Mn deficiency is rare though Mn toxicity has been known to affect the central nervous system (Sawka, 2005).

A diet with Na/K ratio <1 is recommended and considered a good diet for reducing non-communicable diseases such as cardiovascular disease, stroke and hypertension (WHO, 2012; Oulai *et al.*, 2014). The result showed that the *P. umbellatum* possesses a Na/K ratio of 0.01 <1, thus the consumption of this plant is considered healthy. Prior studies conducted on various leafy vegetables also obtained a Na/K ratio which was <1 (Hassan & Umar, 2006; Ng *et al.*, 2012; Srianta *et al.*, 2015). The effect of these two elements in combination for combating high blood pressure and hypertension are larger than the effect of K or Na alone (Sacks *et al.*, 2001; Perez & Chang, 2014). The consumption of vegetables to lower cardiovascular disease risk have been reported by other researchers, thus *P. umbellatum* and other vegetables should be included a healthy daily diet (Mellendick *et al.*, 2018). A Ca/P ratio which is > 1 is recommended in a healthy diet whereas values <0.5 are considered to be insufficient (Adeyeye & Aye, 2005). The Ca/P ratio obtained in the study was 2.00 > 1, thus the consumption of this plant can be considered as a healthy diet. Prior studies on various leafy vegetables also obtained similar results in terms of the Ca/P ratio at > 1 (Gupta *et al.*, 2005; Ndlovu & Afolayan, 2008; Oulai *et al.*, 2014). Both Ca and P interact to form calcium phosphate which is required for the formation of hydroxylapatite, a mineral compound in bone osseous tissue (Lee *et al.*, 2014).

Antioxidants

The presence of plant secondary metabolites potential antioxidants has attracted much attention due to the ability of antioxidants to defend against oxidative stress from free radical activity (Hossain & Shah, 2015). Plant based foods especially vegetables have been touted as natural antioxidant as they tend to naturally contain high levels of phenolic compounds, flavonoids and ascorbic acid (Cartea *et al.*, 2011; Hossain & Shah, 2015). The contents of phenolic (TPC), flavonoids (TFC) and vitamin C obtained in the present study are presented in following order: TPC > TFC > vitamin C (Table 5).

The TPC content in *P. umbellatum* was found to be at concentrations of 510.63 mg GAE/100g which is about two times higher than *Gnetum gnemon* (253.45 mg GAE/100g) (Kongkachuichai *et al.*, 2015). *P. umbellatum* contains a high concentration of TPC which shows its potential as a medicinal plant. The application of *P. umbellatum* as a medicinal plant has been reported in many previous studies (Roersch, 2010, Núñez *et al.*, 2005). The phenolic compounds have the ability to act as an anti-carcinogenic compound by protecting body cells against oxidative damage (Silva *et al.*, 2007).

P. umbellatum is also rich in flavonoids with a concentration of 377.82 mg QE/ 100g. The results were comparable to Mussaendra afzelli (367.00 mg QE/100 g) and Artemisia arboresense (325.00 mg QE/100g) (Agbo et al., 2015; Djeridane et al., 2006). Flavonoids are the largest subgroup of polyphenols are important as anti-cancer, anti-coronary heart diseases, anti-Alzheimer's disease and anti-cardiovascular diseases (Jang et al., 2018; Bakhtiari et al., 2017; Raffa et al., 2017). Vitamin C which is also a strong antioxidant was recorded to be present abundantly in leafy vegetables. P. umbellatum was found to have a concentration of 140.40 mg/100 g of vitamin C which is about 5-16 times richer than various leafy vegetables as reported by Saha et al. (2015). P. umbellatum provides a good source of vitamin C which has the potential to reduce the risk of chronic diseases such as cancer (Kongkachuichai et al., 2015). Older people tend to consume leafy vegetables as a component of their daily diet due to the purported anti aging properties of food with high Vitamin C content (Samy et al., 2014).

Anti-nutrient content

Oxalate and phytate are the anti-nutritional factors which are commonly found in plant based foods. The result from this

Table 5. The phenolic, flavonoids and vitamin C content of *P. umbellatum*.

Concentration		
510.63 ± 10.12		
377.82 ± 22.04		
140.40 ± 2.80		

Data presented as mean \pm S.E

Table 6. The nutrient and antinutrient composition of *P. umbellatum*.

Antinutrient (mg/100g)	Concentration (mg/100 g)		
Oxalate	3.52 ± 0.54		
Phytate	411.67 ± 4.84		

Data represented in means \pm standard error (SE) of the dry weight.

study shows that the phytic acid contained in *P. umbellatum* was comparatively higher than the oxalate content. The oxalate content was found to be in concentrations of 3.52 mg/100g (Table 6). This was within the range recorded for other leafy vegetables consumed in India (1.72 – 9.42 mg/100g) (Saha *et al.*, 2015). Oxalate potentially binds to minerals such as K, Ca, Mg and Na, thus limiting the availability of these nutrients for body intake. The overconsumption of oxalate can cause the formation of calcium oxalate salt crystals which can precipitate in the kidney or urinary tract (Gemede & Ratta, 2014).

Phytate in *P. umbellatum* was present in high concentrations; the content was recorded at 411.67 mg/100g. The results obtained fell within the range recorded in other various leafy vegetables (103.16 – 924.70 mg/100g) as conducted by Saha *et al.* (2015). Phytic acid has a tendency to chelate to metal ions such as Ca, Mg, Zn, Cu and Fe to form insoluble complexes which are not readily available for body absorption, thus limiting the accessibility to these nutrients (Gemede & Ratta, 2014).

CONCLUSIONS

The consumption of *P. umbellatum* as a leafy vegetable can provide essential nutrients for body intake which is beneficial for consumers of this plant. The plant exhibits a high phytate content which could possibly reduce the availability of other nutrients. Soaking and boiling prior to consumption are the best methods suggested to reduce the anti-nutrient contents found in this plant. Taking into consideration the general nutritional value of this plant, it has suitable potential to be commercialized and introduced as a food crop. Thus, it is recommended that future studies be conducted to ascertain it's agronomical requirements.

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62

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