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NUTRITIONAL, PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF THE RED AND WHITE *MALAKWANG (HIBISCUS* SPECIES*)* LEAVES

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ABSTRACT

Malakwang (Hibiscus species*)* is a common vegetable in Uganda diets. This study established the nutritional, phytochemical composition, and antioxidant activities of the red and white *malakwang* leaves. *Malakwang* leaves were harvested six weeks after germination. Nutrients and phytochemicals were quantified and qualified using the methods of Association of Official Analytical Chemists (AOAC) and High-Performance Liquid Chromatography (HPLC). The antioxidant activity was determined using the 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) scavenging method. The quantity of crude fat was significantly higher ($p<0.05$) in the white *malakwang* leaf samples (6.9 %) than the red (3.3%). The red had significantly higher ($p<0.05$) quantities of calcium (1753.6 mg), magnesium (1814.4 mg), iron (233.4 mg), vitamin A (RE) (952.7 µg) and vitamin E (22.8) per 100 g when compared to the 1522.2 mg, 1297.9 mg, 179.7 mg, 857.6 µg, and 16.8 µg, respectively for the white *malakwang* leaves. The white *malakwang* leaf samples had higher ($p<0.05$) quantities of flavonoids (25.7 %) and alkaloids (5.5) than the 20.7 % and 2.1 %, respectively for the red *malakwang* leaves. The red *malakwang* leaf extract had more mucilage and coumarin contents than the white *malakwang* leaves. The white and red leaf extracts scavenged 61.3% and 52.7% of DPPH radicals, respectively, which are similar to the 62.2% scavenged by vitamin C. *Malakwang* leaves could significantly contribute to dietary intake of crude fibre, essential oil, calcium, magnesium, zinc, vitamin A (RE), C and E and phytochemicals hence suggesting a correlation with the cultural health claims for improving breast milk production, appetite, immunity and healing of sores and wounds. Further studies on the evaluation of nutrients and phytochemicals in *malakwang* leaves are recommended to approve the results of this study. Experimental trials in humans could be performed to evidence the effect of *malakwang* leaves on breast milk production and healing of sores and wounds. Also, the determination of essential fatty acids and the effect of preparation and post-harvest methods are recommended for further studies.

Key words: Nutritional, antioxidant activities, health benefits, indigenous, *malakwang* (*Hibiscus)* species

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INTRODUCTION

Malakwang (Hibiscus species) is an indigenous leafy vegetable widely consumed as a delicacy in the cultural diets in Uganda [1]. The two varieties commonly distributed in the regions are red (red venation) and white (green venation). *Malakwang* leaves have sour and bitter tastes, which make them popular in diets. Customarily, there are health claims, including stimulation of breast milk production, appetite, and an increase in serum ferritin levels associated with consuming and using *malakwang* leaves [1].

Nutritional assessment of *Hibiscus* species leaves revealed that the vegetables contain substantial amounts of vitamin A (RE), C, B-complex, E, iron, calcium and zinc [1-3]. Importantly, Mohammed [4] noted that minerals and vitamins cannot be synthesised by animals, hence they must be supplied by plants. Therefore, the inclusion of leafy vegetables in the diets of humans is imperative. *Malakwang* leaves added to protein foods like meat, beans and peas provide complemented valuable sources of vitamins, fibres, antioxidants, and minerals such as iron and calcium, which promote human health. Combining different food stuffs in a dish is frequently practiced in Uganda and is especially essential in the rural areas where they contribute substantially to the protein and mineral contents, which are usually in short supply in daily plant-based diets [4]. The available fibre can contribute significantly to the organoleptic properties such as the taste, flavour, texture and colour of diets [1].

Malakwang seems to show essential health benefits like improving iron bioavailability, digestion, absorption and antioxidant activities [4, 5]. These could be scientifically attributable to the contents of nutrients like vitamin C, E and zinc, as well as phytochemicals like alkaloids, tannins, flavonoids and saponins [1, 5, 6]. Phytochemicals are also associated with a broad spectrum of health-promoting effect namely antifungal, antivirus and anticarcinogenic properties [6, 7, 8].

Despite the widespread consumption rate and the health benefits attributed to *malakwang* vegetables [1], there are gaps and inconsistences in quantitative data on the nutrient and phytochemical composition of the red and white varieties. Such information is necessary for guiding effective diet planning, management and evaluation of health benefit claims. This study evaluated the differences in nutrient and phytochemical composition as well as antioxidant activity of the red and white *malakwang* leaves.

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MATERIALS AND METHODS

Experimental Design

The red and the white *malakwang* plants were grown at Makerere University Agricultural Research Institute Farm, Kabanyolo. The four sub-plots were planted in pairs of the red and white variety with a two-week interval per pair at a spacing of 15 x 30 cm and thinned to one crop per point. Insects were controlled by spraying the vegetation surrounding the garden.

Sample Collection and Preparation

Malakwang leaves were harvested at six weeks after germination from every first line of the three stratified lines, at about 7.00 am. The harvests were packed in the pale green polythene bags labeled R_1, R_2 and R_3 or W_1, W_2 and W_3 according to the stratified lines and transferred to the laboratory. The leaves were cleaned immediately by cutting off the soiled stem, washed under running water then air dried on the cleaned laboratory benches. The edible part of the leaves was picked and packed in 500 g perforated polythene packs and stored at 5 ºC. The fresh leaves were used within two days. The samples for proximate, vitamins A and E, mineral and phytochemical analysis were prepared by drying them in an "Air oven" DFTNL / C / MRC OVEN / 003, MRC Model DFO – 150, serial / No AI 18112901 - 1 at 60 ^{O}C for 12 hours, cooled in a desiccator and milled into a fine powder to produce a homogeneous analytical laboratory sample using a grinder (Vitamix Moulinex Auto Clean, England). The samples were sealed in small air-tight packs, labeled, and stored at 1^{O}C for subsequent analysis.

Preparation of the red and white *malakwang* aqueous leaf extracts

Aqueous leaf extract samples were prepared using the procedures described by Kate and Lucky [9], with modifications. Two different samples (100 g) each were dried in the shade for five days at 26 ºC and the constant weights (27 g) recorded. Each sample was macerated with a crucible and pestle to powder with uniform consistency and carefully transferred into the extraction bottle. Distilled water (270) ml at 50 ºC, was added into each extraction bottle gradually and shaken well. The solvent volume used was at a ratio of 10 ml per g sample. The bottles were tightly covered and kept in a dry dark place for 24 hours. The samples were carefully filtered using a layer of fine muslin cloth and concentrated in a high vacuum freeze dryer (Edwards High Vacuum BOC Ltd. Crawley, Sussex, England). The red and white *malakwang* extracts were weighed and packed in special freeze-dried air-tight packs, sealed and stored at 1 ^{O}C and used to determine the phytochemical composition and antioxidant activities.

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Chemical Analyses

Nutritional, Phytochemical Composition, and Antioxidant Activity of Red and White *Malakwang* Leaves

All analyses were done in triplicate. Moisture vitamins B and C contents were determined on fresh *malakwang* samples and the results expressed on a fresh weight basis. The rest of the analyses were conducted on dry *malakwang* leaves and the results expressed on a dry matter basis.

Proximate composition

Moisture

Moisture content was determined using the hot-air oven (Gallenkamp UK) at 90 ºC for 16 hours following the standard procedures of the AOAC [10]. The percentage moisture content of fresh *malakwang* leaves was calculated using the formula: [Moisture (%) = (B – C) x 100/A].

Dry matter percentage was calculated from moisture measurements using the formula: [A – (B – C) x 100/A].

Crude protein was determined using Kjeldahl method according to the AOAC [10] number 978.04 based on the total nitrogen content. Percentage crude protein was equal to (titre reading x 0.02 x 14 x 6.25 x df x 100/1000 x sample weight) where df was the dilution factor.

Total sugar was determined using the phenol-sulphuric acid method described by Nielsen [11]. The concentration of total sugar (g/100g) was calculated using the formula; absorbance – (intercept on vertical axis) x D x Vo x Vi/sample weight (g) x Slope x V x 10.

Crude fat was determined using the Soxhlet extraction method described by Nielsen [11] and the content was calculated by the equation; total fat (%) = $\underline{w_1 - w_2 \times 100 / W_{o.}}$;

Dietary fibre of food matrix was determined using the method described by Kirk and Sawyer [15] and was calculated in % as W1 – W2 /Ws x 100.

Mineral composition was determined using the wet ashing method of the AOAC [10]. Quantification of the mineral elements was performed using an Atomic Absorption Spectrophotometer (AAS). The blank was first measured, then the sample solutions were read off at different wavelengths for the different minerals, 520 nm for calcium, magnesium and iron and 213.9 nm for zinc against the blanks. The concentration of the mineral in the blank solutions and *malakwang* leaf sample was calculated using the formula: mineral (mg) = (a-b) x v x f x 100/w x 1000.

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Vitamin A (RE) was determined as β-carotene content using the standard procedures described by Kirk and Sawyer [15]. A factor of '12' was used to convert carotenoid to vitamin A using the formula: total vitamin A (µg/g) = A x vol x 10^4 x df /constant x swt/12.

Ascorbic acid content of the red and white *malakwang (Hibiscus* spp.*)* leaf was determined by the redox titration method described by Nielsen [11] using 2, 6-dichloroindophenol (DCIP) indicator. The value of ascorbic acid was calculated using the formula:

Vitamin C (mg/100g) = (*A-B) x C/W* x 100.

High Performance Liquid Chromatographic method described by Perveen *et al.* [12] and Gasior *et al.* [13] was used to determine vitamins B_1, B_2, B_3, B_6, B_9 and E. The HPLC system used was the Shimadzu 10AVP Elmer NCL 900 network chromatography interface, and a data processing unit com-paq. HPLC discovery C18, 25 cm x 4.6 mm, 5 m column (Supelco, Sigma – Aldrich) was used for the separation of B-vitamins.

Vitamin B

Two grams (2.0 g) of *Malakwang* leaf powder was refluxed for 15 min using 25 ml of 0.1 N HCL extraction solvent in a 250 ml round bottom flask. A filtrate of the extract was diluted to 100 ml in volumetric flask and centrifuged at 1400 rpm for 5 min. The supernatant (2 ml) was filtered through a 0.45 µm membrane filter and 20 µl of the filtrate was injected into the HPLC system. The content of each B – vitamin (x) was calculated using the equation from the calibration curves of B-vitamins standards.

Folic acid

Ten grams (10 g) of *malakwang* leaf powder was boiled in a water bath at 100 °C for 10 min using 30 ml 0.1 M phosphate buffer containing 1 % ascorbic acid. The extract was diluted to 50 ml with phosphate buffer and centrifuged for 10 min at 5000 g. Twenty-five microliters (25 µl) was injected into the HPLC equipment with an autosampler at 4 °C and a flow rate of 0.8 ml per min. Mobile phase ultrapure Water + 0.1 % formic acid (v/v and acetonitrile were used) and column Licrospher ^{R} RP – 18 Column 250 x 4.6 mm, 5 µm (RF – 10AXl Shimadzu Inc., Kyoto Japan) at a temperature of 30 °C, attached to fluorimetric detector.

Extraction of vitamin E

Two grams (2 g) of *malakwang* leaf powder was sonicated for 15 minutes in 70 ml of mobile phase to solubilize the compounds. The extract was diluted to 100 ml in volumetric flask with mobile phase, passed through a 0.45 µm membrane filter and 20 µl of the filtrate injected into the HPLC system. The mobile phase

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acetonitrile:methanol in the ratio of 75:25 at a flow rate 1.0 ml/minute was used and vitamin E was detected at wavelength of 220 nm.

Quantitative determination of phytochemicals from malakwang leaves

The determination of total alkaloids, flavonoids and saponins measures in *malakwang* powdered leaves was done using the method described by Harborne [14].

Alkaloids

Malakwang leaf powdered sample (5 g) was mixed with 200 ml of 10 % acetic acid in ethanol in a 250 ml beaker and covered for four hours at 28 °C. The mixture was filtered and concentrated in a water bath at 60 °C to a quarter of the original volume. The extract was precipitated with concentrated ammonium hydroxide and collected in a weighted filter paper, washed with dilute ammonium hydroxide (1 %), and dried in the oven at 80 °C. The alkaloid content of *malakwang* leaf was calculated using the formula;

$$\text{Alkaloid (\%)} = w_1 - w_2/w \times 100$$

Total flavonoids

Malakwang leaf powdered sample (5 g) was boiled in 50 ml of 2 M HCl solution for 30 minutes under reflux, allowed to cool, and filtered through Whatman number 42, filter paper. The sample solution (15 ml) was treated with an equal volume of ethyl acetate drop wise. The precipitated flavonoids was recovered in a weighed filter paper and calculated using the formula:

$$\text{Flavonoid (\%)} = (w_1 - w_2)/w \times 100$$

Saponins

The powdered sample (20 g) of *malakwang* leaves was treated with 100 ml of 20 % aqueous ethanol in a conical flask heated over a water bath at 55 °C for four hours with continuous stirring, filtered and the residue was re-extracted with another 200 ml 20 % ethanol. The combined extract was reduced to 40 ml over a water bath at 60 °C and centrifuged with 20 ml of diethyl ether in a 250 ml preparatory funnel. The purification process was repeated, then 60 ml of n-butanol was added and the combined n-butanol extract washed twice with 10 ml of 5 % aqueous sodium chloride, concentrated in a water bath at 90 °C and then dried in the oven to constant weight. The content of saponin in *malakwang* leaf sample was calculated using the formula:

$$\text{Saponins (\%)} = w1-w2/w \times 100$$

Tannins content was determined as described by Kirk and Sawyer [15]. The respective absorbance was measured in a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) at 760 nm. Total tannin content was

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determined from a calibration curve made with standard tannic acid (Sigma Chemical Co (St. Louis, MO, USA) using the formula;

Tannins (mg /100g) = C x extract value / Aliquot x sample wt x 100

Qualitative determination of phytochemicals from malakwang leaf extracts

The analytical method used to detect steroids and anthocyanin from *malakwang* extract was described by Harborne [14]. Keller-Kilani test was used to identify cardiac glycosides [16]. Fehling's test was used to identify the presence of reducing compound [16]. For the extraction of anthracenoside aglycones (emodols), coumarins, and anthocyanins, a soxhlet apparatus was used. The appearance of fluorescence under UV light indicated the presence of coumarins [17, 18]. Ruthenium red test was conducted to indicate the presence of mucilages [19]. The appearance of stains of red or pink color indicated the presence of mucilages.

Determination of Antioxidant activity of malakwang leaf extracts

The stable 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) was used to determine the radical scavenging activity potential of *malakwang* leaf extract following the principle of donating hydrogen to the free radical molecules rendering them harmless or neutralised [20]. *Malakwang* leaf extracts changed the DPPH (purple colour) in terms of radical scavenging power to diphenyl-1-picrylHydrazine (non – radical, yellow colour) and the scavenging power was measured in an ultraviolet spectrophotometer at 517 nm. Vitamin C was used as the antioxidant standard at the same concentration as the extracts. The radical scavenging activity of *malakwang* leaf extracts was calculated following the formula Inhibition % = {(Ab – Aa)/Ab} x 100. Where Ab = absorption of blank sample and Aa = absorption of the extract.

Data Analyses

Data generated in this study was subjected to analysis of variance (ANOVA) and transformed into mean values. The significance level was set at p=0.05. Statistical Package for Social Sciences (SPSS) version 24 was used to manage the data. The quantitative data of the red and white leaves and extracts are presented using percentages, whereas the qualitative measures used symbols; '+' to mean weakly present; '++' moderately present; '+++' strongly present, and '–' absent.

RESULTS AND DISCUSSION

Proximate and mineral composition

Table 1 shows the proximate and mineral composition of the red and white *malakwang* leaves. They indicate that the two different varieties of *malakwang* leaves differ significantly in their crude fat content, with the white leaves bearing significantly a higher ($p<0.05$) value than the red variety. The difference could be attributed to the genomics of the plants. The moderate fat measure detected in the

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white *malakwang* leaves appears to be consistent with the glossy appearance of the healthy leaves of the white variety [21]. Onunogbu [22] reported the ability of some plants to absorb and accumulate oil in the aerial parts of the leaves where essential oil constitutes from 0.2 - 0.5 % and contains mainly fatty acids, polyacetyenes and phenol acids imbedded in the internal secretory system [23]. The study of the essential oil found in the leaf mesophyll is important for the understanding of plant defense strategies, biosynthesis of the secondary metabolites, particularly terpenes and the investigation of metabolic engineering of plants that are more resistant to pathogens and insects and are an effective herbivore deterrent [23]. The secondary metabolites indirectly prevent the formation of free radicals [23]. The white *malakwang* type could possess the potential of accumulating fat. Nevertheless, the measure of crude fat in the white leaf was higher than the 1.5 %, 0.3 g, and 0.4 g reported by Ereifej [2], Edo [24], and USDA [25] for *Rumex acetocella*, *Hibiscus sabdariffa Linn*, and pumpkin *leaves*, respectively but lower than the 9.60 and 8.40 % reported for *Abelmoschus manihot* and *abelmoschus esculentus* leaves by Raimi [26].

The dietary fibre results of the red and the white *malakwang* leaves reported in this study were comparable (Table 1). It shows that the two varieties could have equal fibre growth capacity. Moreover, they were grown on the same fallowed soil during the rainy season justifying the amount of dietary fibre they exhibited. The result suggests that *malakwang* leaves could be used as a source of dietary fibre as it would need 200 g of the leaves to achieve the RDA (Recommended Dietary Allowance) of 28, 29 and 30-38 g of dietary fibre for expecting, lactating mothers and adults, respectively [27]. Dietary fibre is essential for forming soft faecal matter and assisting its smooth passage out of the body and for excretion of waste toxins from the intestine. Consequently, consumption of 100 g of cooked *malakwang* leaves daily could have the potential to offer sufficient fibre protection from conditions like constipation, haemorrhoids, colon cancer, irritable bowel syndrome, hiatus hernia, appendicitis, diabetes, obesity, coronary heart diseases, gallstones, and rectal fissures [28]. Nevertheless, the fibre results reported in this study are comparable to the 17.55, and 19.29% reported by Raimi [26], and USDA [25] in *Abelmoshcus esculentus* and *Hibiscus Cannabinus* leaves but higher than the 10 % reported by USDA [25] in *Hibiscus sabdariffa Lin* leaves and lower than the 22.6 and 25.44 % reported in *Rumex acetocella* and *Abelmoshcus monihot esculentus* by Ereifej [2] and Raimi [26], respectively. The variations in dietary fibre results of *Hibiscus* leaves compared to that of *malakwang* leaves could be due to the genetic makeup, and environmental conditions namely: soil fertility, water uptake, sun light, weed control and maturity level [27].

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The total sugar content reported for the red and white *malakwang* leaves were similar (Table 1). The amount of sugar reported in this study could supplement the carbohydrates content in the diet. Carbohydrates are the main source of energy for all metabolic activities in the human body and in particular the only source of energy for the human brain. Therefore, they are indispensable in the diet. The sugar contents are comparable to the 11.31 and 12.3% reported by USDA [25] and Edo [24] in *Hibiscus sabdariffa Lin* leaves but lower than the 41.25, 32.65, and 27.3 % reported by Raimi [26], and Ereifej [2] in *Abelmoshcus esculentus, Abelmoschus Manihot esculentus,* and *Rumex acetocella*, respectively (Table 1).

Similarly, crude protein results were the same for the red and white *malakwang* leaves (Table 1). The small amount of protein informed in *malakwang* leaves can supplement its value in vegetarian diet. Higher amounts of protein (21.16, 21.9, and 8.65 %) were reported in *Abelmoshcus esculentus* and *Rumex Acetocella* leaves by Raimi [26], and Ereifej [2], respectively. The 3.5 % and 3.15 g reported by Singh [3] and USDA [25] in *Hibiscus sabdariffa Lin* and pumkin leaves support the amounts found in *malakwang* leaves. The differences in crude protein results of this study from *Hibiscus* species studies could be due to the variations in genetic and environment factors like soil fertility, climatic changes, availability of solar energy for photosynthesis and ecotype.

Remarkably, the calcium, iron, and magnesium results reported in this study were significantly higher in the red leaf variety than the white (Table 1). The factors contributing to these variations could be due to genetic makeup of the different varieties with a stronger ability in the red to increase the rate of uptake of minerals from the soil and the rate of photosynthesis [29]. The high levels of calcium, magnesium, and iron in the leaves could also mean that the elements are widely distributed in the plants [30]. It suggests that calcium, magnesium, and iron in *malakwang* leaves (Table 1) could be used as a dietary source as it would need about 100 g of *malakwang* leaves to achieve the RDA of 1000 – 1200 g of calcium; 50 g leaves to achieve the RDA of 310 – 420 mg magnesium, and 10 – 15 mg iron [31]. Calcium is one of the key nutrients required for breast milk production and contributes to numerous basic health functions like absorption of nutrients, making and maintaining the skeletal structure (99 %), blood clotting, muscle contraction, maintaining a regular heartbeat, nerve function, and neuron-transmission. Iron improves blood volume to transport blood platelets, alkaloids, saponins, and nutrients required for destroying micro-organisms and repair of tissues. Nonetheless the reported measures of calcium in this study are higher than the 240 and 39 mg/100 g reported by Singh [3] for *Hibiscus sabdariffa* and pumpkin leaves, respectively. Also, the 5.0 and 2.22 mg/100 g iron contents reported by Singh [3], and USDA [25] for *Hibiscus sabdariffa and* pumpkin leaves are lower than the

amount reported in this study, respectively. Similarly, the 51 and 39 mg/100 g magnesium reported by Mahadevan and Kamboj [32] and USDA [25] for *Hibiscus sabdariffa* and pumpkin leaves are also lower compared to that of *malakwang* leaves.

The levels of zinc reported in the two varieties of *malakwang* leaves were the same (Table 1) but higher than the 3.2% reported by Mahadevan and Kamboj in *Hibiscus sabdariffa Lin.* This could be ascribed to the rate of growth due to soil fertility, water, level of maturity, and the genetic makeup of the plants [32]. Sodium was remarkably low in *malakwang* leaves. A low level of sodium is recommended for the control of high blood pressure offering a health benefit to humans.

The Vitamin composition of red and white *malakwang* leaves

Table 2 displays the vitamin composition of the two varieties of *malakwang* leaves. Generally, they differed significantly in their vitamins A, and E levels, with the red *malakwang* leaves bearing higher values than the white variety. Vitamin A (RE) is important for health of the immune system, and vision as well as reproduction, growth and development. Vitamin E also strengthens the immune system, prevents blood clotting and helps the body to use vitamin K. The notable amounts of vitamin A, riboflavin, and ascorbic acid reported in this study suggest that *malakwang* leaves could be used as a dietary source of these vitamins as it would require about 200 g of *malakwang* leaves a day to achieve the RDI (Recommended Dietary Intake) [31]. Vitamins A, C and E are antioxidants and as such, act as a general de–toxicants [33]. The epidemiological evidence suggests that vitamin A and carotenoids are important dietary factors for reducing the incidence of heart disease and might control cancer since they are important for healthy cell division and may reduce the risk of some types of cancer [34]. Vitamin C absorbs soluble iron by chelating or maintaining the iron in the reduced form and provides protection against oxidative stress - induced cellular damage by scavenging of reactive oxygen species. Also, it may help to control tumor progression, infections, and inflammation [35]. Vitamin E is necessary for the prevention of deficiency symptoms including peripheral neuropathy and hemolytic anemia. It is a powerful, lipid – soluble chain-breaking antioxidant [35]. Therefore, it is vital to supplement vitamins C and E contents of *malakwang* leaves during preparation by introducing fresh fruits and vegetable salads into the diet. The results of vitamin A (RE) are consistent with the 1000 µg/100 g vitamin A (RE) reported by Singh [3] for *Hibiscus sabdariffa* but higher than the 0.59 µg/100 g sample reported for *Abelmoshcus esculentus* by Rubaihayo [1]. The value of ascorbic acid reported in this study (Table 2) is higher than the 25 and 10.1 mg reported for *Abelmoshcus esculentus* and *Hibiscus Sabdariffa Lin* by Rubaihayo [1] and Mahadevan and Kamboj [32].

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The thiamine values reported in this study are similar to the 0.45 mg reported by Edo [24] for *Hibiscus sabdariffa Lin* leaves but higher than the 0.2 and 0.094 mg reported for *Hibiscus sabdariffa* and pumpkin leaves by Singh [3] and USDA [25] respectively. On the other hand, the niacin contents (Table 2) are lower than the 1.4 and 0.92 mg reported for *Hibiscus sabdariffa linn* and pumpkin leaves by Singh *et al.* [3] and USDA [25] respectively. The USDA [25] reported lower folic acid (0.0 µg/100 g) for pumpkin leaves. The variations in vitamin quantities of *malakwang* (*Hibiscus)* leaves compared to edible *Hibiscus* leafy vegetables could be credited to variations in genetic factors, soil fertility and moisture, availability of solar energy for photosynthesis, and ecotype factors [36].

The phytochemical composition of red and white *malakwang* leaves

Table 3 shows that the two *malakwang* leaf types differ significantly in their alkaloids and flavonoids levels, with the white leaves bearing significantly higher ($p<0{\cdot}05$) alkaloids and flavonoids levels than the red variety, probably due to genetic factors. Based on epidemiological study reports, the considerable contents of flavonoids and alkaloids in *malakwang* suggest that the leaves could possess active compounds that serve as a potential source of therapeutic health remedies. These health benefits include reducing the risk of various cancers as well as preventing menopausal symptoms [33], lowering the risk of coronary heart diseases, protecting the body from oxidative stress diseases, boosting immunity [37], and preventing common ailments like flu and cough. Alkaloids have also been known for having diuretic, antispasmodic, anti-inflammatory, and analgesic effects, including management of hypertension [33]. Tannins are known for the treatment of urinary tract infections [33]. Saponins reduce the uptake of glucose and cholesterol in the gut through intraluminal physicochemical interactions and may help in digestion [38]. *Malakwang* leaves could offer similar remedies and may account for its popularity in the traditional remedies namely slowing weight gain, managing hypertension and cleaning mouth sores and wounds [9]. Notably, this study reported higher quantities of flavonoids, alkaloids, tannins and saponins for the red and the white *malakwang* leaves than the 5.54, 3.11, 0.45, and 1.19 % reported by Arvind and Alka [6] for *Hibiscus sabdariffa* leaves. Also, Raimi *et al.* [26] reported lower (4.51 and 4.71 %) flavonoids in *Manihot esculentus* and *Abelmoschus esculentus* leaves, respectively. The contributing factors could be attributed to the ability of the different plant species to develop secondary metabolites due to genetic factors under favourable environmental factors namely soil fertility, optimal rate of photosynthesis in the leaves owing to good solar energy, soil moisture, and the time of harvest of the leaves [33].

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The Phytochemical constituent of aqueous extracts of *malakwang (Hibiscus* species) leaves

The extracts from the two leaves differ in their quantities of mucilages, coumarins and saponins, with the white bearing a higher quantity of saponins whereas the red leaf extracts had higher quantities of mucilages and coumarins (Table 4). The higher quantities of mucilages and coumarins in the red leaf extracts compared to the white could be due to the growth rate caused by high rate of photosynthesis controlled by genetic makeup of the two varieties. Mucilage is an essential substance in building stool and giving it a bulking-softening effect. Therapeutically, it could perform anti-inflammatory action, and reduction of bowel irritation by forming a protective film against toxin absorption from the bowel, controlling cough, supporting bronchial functions, and preventing urinary spasms [25]. Coumarins possess a range of different physiological activities including anti-cancer, antioxidant, anti-inflammation, anti-HIV, anti-coagulant, anti-bacterial analgesic and comparative immune modulation [37].

Antioxidant activities of *malakwang (Hibiscus* species) aqueous leaf extract

Table 5 displays the DPPH radical scavenging potential results of the red and white leaf extracts in 45 minutes. They coincide significantly, although the white leaf extract exhibited a higher scavenging percentage when compared to the red type. Notably, the percentage inhibition of the white leaf extract is also similar to that of vitamin C (Table 5). The results suggest that the white *malakwang* leaf variation could have the potential of effective antioxidants. This could be accredited to the presence of phytochemicals possessing antioxidant activities, namely flavonoids, alkaloids and tannins (Table 3), for example, polyphenols can neutralise free radicals [35] and act as metal chelation, thus preventing oxidation caused by highly reactive hydroxyl radicals [34]. Similarly, the existence of nutrients like vitamin A, C, E, fibre, and zinc (Table 1) that offer antioxidant activities, and which were found in fair quantities could have contributed to the scavenging strength of *malakwang* leaf extracts [7]. The results suggest that *malakwang* leaves could have antioxidant potential and may provide strong support to the ethnic health claims.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Malakwang leaves have a considerable level of nutrients and phytochemical compounds. The red leaves are significantly richer ($p<0.05$) in calcium, iron, magnesium and vitamins A and E. The white leaves are significantly richer in crude fat, flavonoids and alkaloids, which could exhibit a wide range of health functions and antioxidant activities. The antioxidant scavenging potential of the white leaf extract is comparable to vitamin C. The red and the white *malakwang* leaves could be prioritised for diet and pharmacological purposes, respectively. Further studies

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on the quantities of nutrients and phytochemical in *malakwang* leaves are recommended to support the results of this study. Experimental trial could be conducted to investigate the health claims of increasing breast milk production, the healing of wounds and improving blood volume. Further scientific studies could be conducted to investigate the essential fatty acids present in *malakwang* leaves in addition to the effects of preparation and post-harvest processing methods.

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Conflict of Interest

There was no conflict of interest.

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Table 1: Proximate and mineral composition of the red and white *malakwang* leaves

Parameter	*Malakwang* leaf Variety	
	Red	White
Moisture (%)	80.6[a] (0.50)*	79.4[a] (0.5)
Dry matter (%)	19.4[a] (0.5)	20.7[a] (0.5)
Crude protein (%)	4.3[a] (0.0)	4.0[a] (0.0)
Sugar (mg/100 g)	13.2[a] (0.6)	12.5[a] (0.2)
Crude fat (%)	3.3[a] (0.0)	6.9[b] (0.1)
Dietary fibre (%)	13.3[a] (2.1)	16.7[a] (1.0)
Calcium (mg/100 g)	1753.6[a] (0.0)*	1522.2[b] (0.02)
Magnesium (mg/100 g)	1814.4[a] (0.0)	1297.9[b] (0.02)
Sodium (mg/100 g)	27.1[a] (0.2)	26.2[a] (0.4)
Iron (mg/100 g)	233.4[a] (13.8)	179.7[b] (6.9)
Zinc (mg/100 g)	5.6[a] (0.4)	6.5[a] (0.2)

SE* Standard error; values are means of three replicates. The mean values with different superscript letters in the same raw are statistically different at $p< 0.05$

Table 2: Vitamin composition of the red and white *malakwang* leaves

Parameter	*Malakwang* Leaf Variety	
	Red	White
Vit. A (RE) (µg/100 g)	952.7[a] (2.7)*	857.6[b] (7.8)
Thiamine (mg/100 g)	0.5[a] (0.0)	0.4[a] (0.0)
Riboflavin (mg/100 g)	2.9[a] (0.0)	2.2[a] (0.0)
Niacin (mg/100 g)	0.1[a] (0.1)	0.2[a] (0.0)
Pyridoxine (B_6) (mg/100 g)	5.6[a] (0.0)	5.1[a] (0.0)
Folic acid (B_9) (µg/100 g)	0.6[a] (0.0)	0.5[a] (0.0)
Ascorbic acid (mg/100 g)	420.4[a] (24.2)	369.2[b] (3.8)
Tocopherol (µg/100 g)	22.8[a] (0.0)	16.8[b] (0.0)

SE* standard error; values are means of three replicates. The mean values with different superscript letters in the same raw are statistically different at $p< 0.05$

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Table 3: Phytochemical composition of the two *malakwang* leaf varieties

Parameter	Unit	Variety	
		Red	White
Alkaloids	%	2.1[b]	5.5[a]
Flavonoids	%	20.5[b]	25.7[a]
Saponins	%	2.7[a]	3.5[a]
Tannins	%	2.1[a]	1.9[a]
Flavonosides	mg/g	0.2[a]	0.9[a]
Reducing compounds	mg/g	0.1[a]	0.1[a]
Anthracenocides	mg/g	0.1[a]	0.02[a]
Anthocyanosides	mg/g	1.1[a]	1.4[a]
Coumarins	+	+	+
Starch	+	+	+
Steroid glycosides	+	+	+

Key: values are means of three replicates. The mean values with different superscript letters in the same raw are statistically different at $p < 0.05$. Symbol '+' indicates the existence of a particular phytochemical in small amount

Table 4: Phytochemical constituents of aqueous extracts of the red and white *malakwang* leaves

	Phytochemical tested	White (Green venation)	Red (Red venation)
1	Mucilages	+	+++
2	Saponins	+++	++
3	Tannins	+++	+++
4	Alkaloids salts	+++	+++
5	Aglycones of anthracenosides	++	+++
6	Coumarins	+	+++
7	Cardiac glycosides	+++	++
8	Anthocyanins	-	-

Key: values are means of three replicates. The symbol '–' means absent; + weakly present; ++ moderately present; +++ strongly present precipitate

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Table 5: Antioxidant activities of *malakwang* aqueous leaf extracts reaction with DPPH compared to ascorbic acid

Concentration	Reaction Time (minute)	% radical (DPPH) scavenging capacity		
		Ascorbic acid	Red *malakwang* extract	White *malakwang* extract
Extract	0	2.71	2.63	2.71
	30	59.84	47.85	56.6
	45	62.22[a]	52.71[a]	61.32[a]
Dilution	0	1.46	3.07	3.02
	30	48.23	31.76	36.2
	45	59.91	37.89	42.05

Values are means of three replicates. The mean values with single letter superscript in the same raw are not statistically different at p <0.05

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