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Research Article

Antioxidant Activity, Total Phenolic Content, and Nutrient Composition of Chayote Shoot (*Sechium edule*, Jacq. Swartz) from Kundasang, Sabah

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ABSTRACT

The chayote (*Sechium edule*) plant is believed to have significant nutritional importance due to its medicinal functions. It has been widely cultivated in Kundasang, Sabah for vegetable consumption. This study was carried out to determine the health benefits of this vegetable, especially the upper 3-foot of the shoot portion, in terms of antioxidant activity, total phenolic content and nutrient composition. It was divided equally into three parts, each a foot long and classified as: upper tier, middle tier and lower tier and amongst them, which tier gives rise to the best health benefits or will the traditional consumption of the whole 3-foot shoot be better for overall health well-being. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and Folin Ciocalteu test were used to determine antioxidant activity and Total Phenolic Content (TPC), respectively. The results showed that the upper tier of shoot parts had the highest value for antioxidant activity, with the lowest of IC_{50} value (245.12 ± 9.24 µg/ml). The lower part of the plant also shows the highest value for TPC with a value of 355.66 ± 5.84 mg/g GAE. Minerals, Ca, Mg, P, Mo, Fe and Al were the highest value in the middle tier portion. The proximate analyses showed that the upper tier of the shoot has the highest fat, crude protein, and carbohydrate contents. Crude ash has the highest value in the middle tier part. On the other hand, the moisture content and the crude fiber were high at the lower tier of the shoot portion. The upper tier of the shoot is recommended to be consumed for health benefits because it is high in antioxidant activity and proximate content and also rich in minerals. However, the traditional practice is to consume the whole 3-foot long of this vegetable is very welcome.

Keywords: Antioxidants, Chayote, Minerals, Proximate Analyses, Total Phenolic Content

Introduction

Sechium edule (Jacq.) Swartz (Cucurbitaceae), also known as chayote, is an herbaceous plant originally native to Central America [1], a versatile vegetable with edible leaves, fruits, shoots, stems, and roots [2]. This plant is widely cultivated worldwide in Mexico, Africa, Brazil, China, Malaysia, India, and Thailand [3]. Chayote is called cidrayote, chayote, chiote, Cho-Cho, choko, chow-chow, christophene, custard, hayatouri, huisquil, squash mango, mirliton, sayote, vegetable pear, and xuxu depending on some areas where it is planted [2, 4]. Chayote plants are herbaceous plants, monoecious, strong, and energetic creepers. It grows from a single, thick root, which produces roots rooted. Its stems are angular-corrugated and thickened to the base and woody, while

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there are many thin and firm branches at the top. The stem of this plant can reach 30 to 50 feet (9 to 15 m) in one-year [5]. Leaves have grooved petioles with a length of 8-15 cm. The size of the chayote leaf grows from the shoots to the bottom of the plant. Like almost all Cucurbitaceae, chayote plants develop tendrils for support [6]. Although adult green fruit is mainly eaten, other parts of the plant are also found to be eaten in young sprout and roots sections that can provide essential nutrient sources [7]. Researchers report that pharmacological studies confirm that leaves, fruits, and chayote seeds exhibit diuretic, antihypertensive, cardiovascular, and anti-inflammatory properties [6]. This plant is also reported to contain antibacterial [6–10] and antiepileptic activity [11]. Referring to the anti-cardiovascular properties of chayote, previous studies have found that flavonoids in shoots reduce serum lipid and cholesterol content and prevent atherosclerosis [9]. In addition, the intake of leaves and fruits of chayote plants has been found to dissolve kidney stones and other renal diseases and as a cure for lung disease due to its diuretic properties [12]. This crop is grown in many countries like China, India, and Madagascar [13]. The utilization of chayote as functional food has been previously suggested because of the content of flavonoids and their antioxidant activity [14]. The use of leaves and chayote seeds in a daily diet can lower blood pressure and dissolve the calcification of the urine [15].

This study is to evaluate which part of the chayote shoots (3-foot long, which is divided equally into three equal sections): the top tier or the middle tier, or the lower tier has the superior content of antioxidants, total phenolic compounds, and nutrients that will contribute to the maximum impact on health well-being and indeed the choice for consumption. If proven potent, this vegetable will be able to replace synthetic antioxidants in the food processing industry to some extent, which has been documented to have an adverse effect on humans [16]. A few synthetic antioxidants were approved in the food industry due to their toxicity and carcinogenicity properties [17]. This study examined chayote shoots from different extraction solvents. Natural antioxidants are said to be much safer than synthetic ones, especially in the food and medical industry. Therefore, the phytochemical analysis and antioxidant profile of chayote can provide an important tool for improving the value of the crop as a functional food [18].

Material and Methods

Sample Preparation

The plants were derived from the highland area of Cinta Mata Farm in Kampung Mesilau Kundasang, Ranau Sabah. The 3 feet long (0.93 m) chayote plant measuring from the tip of the shoot to the lower part of the plant was analysed for this study (Figure 1). A total of 100 randomly composite samples were harvested from the open field, measuring 100 m x 200 m in size. The samples were wrapped up in brown paper and returned to UMS laboratory for further analysis. The plant parts were weighed and washed using distilled water to remove dust, if any. Subsequently, the plant was dried for a while in the open air of the laboratory space.

The 100 pieces of the 3 feet (0.93 m) plant stock were then cut into three (3) sections: top tier shoot, middle tier shoot and lower tier, each a foot long (0.31 m). Each composite section of the plant stock has a total of 100 samples. Each composite section is then chopped into small pieces (1-2 cm in size) to facilitate and accelerate the drying process, by increasing the surface area. The samples were dried using the dryer cabinet for 72 hours at 40-50°C and blended using an electronic blender into powder form. Subsequently, samples were stored in a vacuum backpack and stored at room temperature until extraction to determine the antioxidant assay and total phenolic content (TPC). In addition, the sample will be used to determine the nutrient content and proximate analyses

Figure 1. Chayote Shoot with tendrils, leaves, and stem together [18].

Sample Extraction

Determination of antioxidant and TPC was performed by taking 50 g of each sample blended in powder form and mixed with 1L ethanol (80%)

at room temperature (25 °C) for 48 hours. Then, the mixture is filtered using the Whatman No.1 filter paper, and the solvent is removed under vacuum at 40°C using a rotating evaporator. After that, the extracts are stored in a vacuum packing bag. The extraction must be carried out in a dark environment, and the extracted extracts are stored and refrigerated (-20 °C).

Determination of Antioxidant

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method for measuring the activity of free radicals in plants is used with some modifications [19]. DPPH testing is a widely accepted method for assessing the antioxidant activity of natural compounds. Measurements for the decline in DPPH absorption are the methods for determining anti-radical antioxidant in DPPH dissipation at 517 nm. The activity of free radicals was calculated as a percentage of the DPPH color changes using the following formula:

$$D(\%) = \frac{Ab - As}{Ab100} \dots\dots\dots (1)$$

where, as is the absorption of the solution when the sample extract is added to a certain level, and Ab is the absorption of the DPPH solution. Extraction concentrations provide 50% inhibition (IC_{50}) calculated from the percentage of inhibition against extract concentration.

Preparation of Buffered Water and Bligh-Dyer

An approximately 300 ml of double distilled water was transferred into a separating funnel and 2.04 g potassium dihydrogen phosphate (KH_2PO_4) was added to create a 0.05 M solution. The pH was adjusted to pH 7.2 by the addition of sodium hydroxide (NaOH) pellets and the mixture was extracted with 3 x 50 ml dichloromethane (DCM). The Bligh-Dyer solvent mixture was made up using buffered water: chloroform: methanol with a ratio of 4:5:10, respectively.

Determination of Total Phenolic Content (TPC)

The total phenolic content is calculated using the Folin-Ciocalteu method [20]. A 1 mg sample was dissolved first with 1 ml of methanol and then mixed with 5 ml of Folin-Ciocalteu reagent (which was dissolved ten times with water). After 2-5 minutes, the following sample was mixed with a 7.5% sodium carbonate (Na_2CO_3) solution of 5 ml into the test tube. The mix was left for 20 minutes in the dark at 25°C completing the reaction before measuring absorbance using UV-Vis Spectrophotometer at A765nm. Gallic acid was used as standard compared with the standard acid curve, i.e., Galic Acid Equivalents (mg/g GAE). A 1 mg dissolved acid in distilled water was used to provide standard curves. The values expressed in mg/g extract of garlic equivalent (GAE) acid. The blank reagent used is 1 ml of methanol, 5 ml of Folin-Ciocalteu reagent, and 5 ml of sodium carbonate (Na_2CO_3) (7.5%). The total phenolic content was calculated based on the following equation:

$$C(GAE) = c \times \frac{V}{M} \dots\dots\dots (2)$$

C = total phenolic content, mg/g plant extract, in GAE;
c = galic acid concentration from calibration, mg/mL;
V = volume extract, mL;
m = weight of plant extract, mg

Determination of Mineral Content

Analysis of calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (P), selenium (S), copper (Cu), zinc (Zn), molybdenum (Mo), iron (Fe) and aluminium (Al) were performed [21]. A 1 g of the prepared sample was inserted into a crucible and made ashes at 500 °C for two hours in the furnace and then left cool. Ten, drops of water were added, followed by 3 - 4 mL of nitric acid. The residual nitric acid was left to evaporate on a Bunsen burner at temperatures of about 100 - 120 °C. The crucible was then put into the furnace to continue ashing at 500 °C for an hour. The crucible was left cool in the desiccator. The resulting ash was dissolved into 10 mL of hydrochloric acid and then transferred to a volumetric flask and diluted with distilled water. Then, the solution was filtered using Whatman No. 1. The macro and micro-mineral elements were determined by using Mass-Spectrophotometry (ICP-MS).

Proximate Analyses

The nutrient composition of the sample was determined using AOAC standard [21]. The moisture content was measured by drying in the oven for 5 h at 105°C. A 5 g of sample was weighed in the crucible and dried in the oven. The moisture content was calculated using the following equation,

$$\text{Moisture content} = \frac{B - C}{B - A} \times 100 \ldots\ldots\ldots (3)$$

where, A is the weight of crucible (g), B is the weight of sample with crucible before (g), and C is the weight of sample with crucible after drying. Kjedahl method was used for the determination of protein content. The Soxhlet method is used to obtain fat content using the Soxhlet (FOSS Soxtex™ 2050) instrument. The crude fiber content in the sample was determined by the FiberBag system via Fibertherm (Gerhardt Fiberthern, Germany). The system produces carbohydrates, proteins and fats that can be dissolved from the sample to obtain coarse fiber content. The ash content was determined using ashing method (Mufle Furnace, Carbolite ELF11 / 141201, United Kingdom) at high temperature of about 550 °C. Carbohydrate content was determined by summing all the percentages of crude protein, crude fat, water content, crude ash, and crude fiber content. The sum of values is then deducted with a value of 100% and the minus results obtained are the value of the carbohydrate contents. All experiments were conducted in three replications. A 300 ml of double distilled water was transferred into a separating funnel and 2.04 g potassium dihydrogen phosphate (KH_2PO_4) was added to create a 0.05 M solution. The pH was adjusted to pH 7.2 by the addition of sodium hydroxide (NaOH) pellets and the mixture was extracted with 3 × 50 ml dichloromethane (DCM).

Statistical Analysis

The results of this study have been reported in means ± standard deviation. The data were analyzed using SPSS version 26.0 and used one-way variance analysis (ANOVA) with a significant difference ($P < 0.05$). Post-hoc HSD tests ($P<0.05$) were used to accomplish the analysis.

Results and Discussion

Antioxidant activity of Chayote shoot

In Table 1, the antioxidant activity of the chayote shoot is 245.12 ±9.24 µg/ml for the top tier of the shoot (1st foot), whilst the middle tier (2nd foot length) is 255.92 ± 10.79 µg/ml and the lower tier (3rd foot length) is 278.03 ± 7.62 µg/ml based on IC_{50} value. The lowest value is the indicator of the highest antioxidant activity, which is the top

Table 1. The IC_{50} value is based on the percentage of antioxidant scavenging activity in the chayote shoot of 3-foot long divided equally into three (3) equal sections of top tier shoot, middle tier shoot, and lower tier shoot, expressed in µg/ml.

Chayote Plant Parts	Chayote Plant Parts		
	Top Tier Shoot	**Middle Tier Shoot**	**Lower Tier Shoot**
IC_{50} (µg/ml)	245.12 ±9.24	255.92 ± 10.79	278.03 ± 7.62

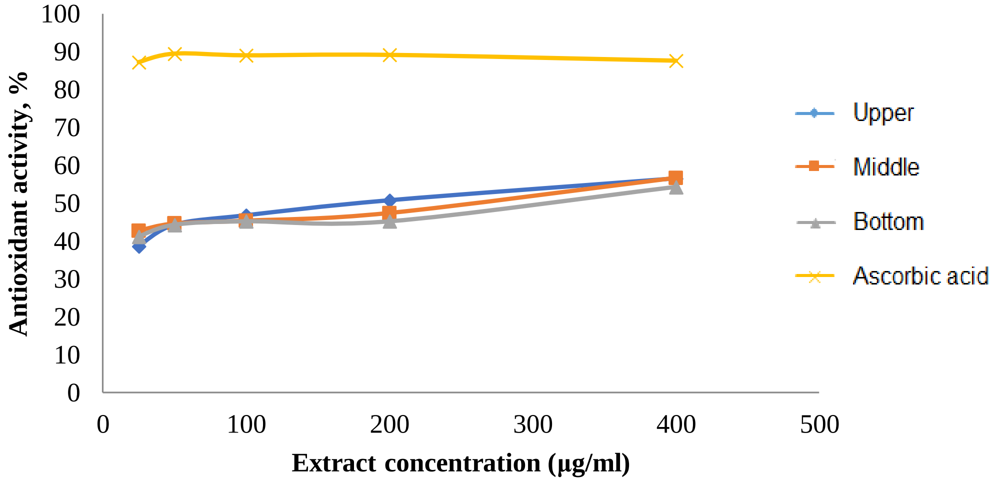

Figure 2. Percentage of radical scavenging of DPPH in 400µg / ml extracts from chayote shoot of 3-foot long divided equally in three (3) equal section of top, middle and lower tier shoot.

Table 2. The phenolic content in the chayote shoots when the 3-foot long is divided equally into three (3) equal sections, top, middle and lower tier shoot.

Chayote Plant Parts	**Chayote Plant Parts**		
	Top Tier Shoot	**Middle Tier Shoot**	**Lower Tier Shoot**
Total Phenolic Content (mg/g GAE)	355.66±5.84[a]	350.38 ±6.65[a]	310.73 ±6.68[b]

Values with different alphabetical letters in the row indicate significant differences at ($P<0.05$), with n=3.

tier of the shoot, followed by the mid-tier and the lowest antioxidant is the lower tier of the stem. No significant relationship was noted between the shoot and middle tier, but both differ significantly from the lower tier at $P<0.05$. However, the upper tier has the superior value over the rest even though a non-significant difference was noted in the middle tier (Table 1).

The percentage of scavenging activity against the extractant concentration is the highest in the upper tier shoot, followed by the middle and lower tiers (Figure 2). The results were lower than those previously reported [14]. The chayote fruits were found to contain slightly higher antioxidant values of 700 µg/ml [13]. The higher amounts of antioxidants values recorded in the shoots compared to the middle tier and the lower tier could be due to the significant amount of various polyphenol compounds, vitamin C, Vitamin A and phenolic acids [22, 23].

The highest total phenolic content (TPC) was in the top tier shoot (355.66 ± 5.84 mg/g GAE), followed by mid-tier (350.38 ± 6.65 mg/g GAE) and the least in the lower tier section (310.73 ±6.68 mg/g GAE) (Table 2), with no significant difference noted between the first two, even though the upper shoot has a higher value than the middle shoot. However, the upper and the middle shots differed significantly from the lower shoot section ($P<0.05$). This TPC result corresponds with the antioxidant activities of the top shoot, middle shoot, and lower shoot (Table 1), where the highest scavenging activity is seen in the upper shoot, followed by the mid and the lowest value in the lower shoot.

The cause of the variation of TPC may also be attributed to factors such as maturity stage, geographic location, climatic environment, harvesting and handling of plants, and storage methods used [24]. Other reported fruit contain a range of TPC values of 60-260 mg/g GAE [27], a value much lower than the content of the Chayote‘s leaves (Table 2). This suggests that consuming chayote leaves is more beneficial than consuming fruits. Consuming both is very much welcome, where TPC is an important source of antioxidants, richly found in fruits & vegetables [25].

Mineral contents

It is interesting to note that mineral concentration values such as Ca, Mg and Fe are significantly higher in the fruit compared to the leaves and stems [26]. In a study conducted based on different plant locations (terrestrial factors), plant age and plant species have been found to influence the plant mineral concentrations [27]. In addition, other causes of variation may also be attributed to factors such as maturity stage, geographic location, climatic environment, harvesting and handling of plants and storage methods used [24]. However, other studies found that Ca, Fe and P minerals are present in both plant leaf and stem [28, 29]. The variation in the minerals content in the leaves and stems can be attributed to terrestrial factors and fertilizers applications [30].

Macronutrient

Macronutrients are required in large quantities by plants and micronutrients are needed in smaller amounts [31]. The lack of any of these elements can inhibit growth and reduce crop yields [32-34]. In Table 3, the top tier shoot recorded the highest amounts of P (239.19 ± 3.77 µg/g), moderate in the middle tier section (206.84 ± 5.12 µg/g) and lowest in the lower tier of the shoot 145.09 ± 0.31 µg/g) and all differ significantly. P is considered as a highly mobile nutrient [26, 35] and is biologically active at the plant growing tips [36]. It is interesting to note that Ca is the highest in the lower part of the shoot (135.59 ± 0.98 µg/g) and moderate in the middle tier section of the shoot (131.38 ± 0.68 µg/g) and lowest in the upper tier shoot (105.79 ± 0.34 µg/g). This is expected since Ca is the least mobile of all nutrients [37, 38] and will

Table 4. Proximate concentrations (%) of chayote shoot, of 3-foot long divided equally in three (3) equal sections of top, middle and lower tier shoot.

Proximate analyses (%)	**Chayote Plant Parts**		
	Top Tier Shoot	**Middle Tier Shoot**	**Lower Tier Shoot**
Moisture	89.48 ± 0.06^a	90.29 ± 0.25^a	90.55 ± 0.63^a
Fat	0.21 ± 0.02^c	0.19 ± 0.01^{bc}	0.14 ± 0.02^a
Crude protein	3.33 ± 0.29^a	3.15 ± 0.20^a	3.01 ± 0.24^a
Crude fibre	1.35 ± 0.08^a	1.87 ± 0.04^{bc}	2.04 ± 0.14^c
Ash	1.27 ± 0.02^a	1.17 ± 0.05^a	1.15 ± 0.12^a
Carbohydrate	4.36 ± 0.24^b	3.20 ± 0.19^a	3.12 ± 0.13^a

Values are expressed as the mean ± standard error of the mean (SE); with n = 3; Letters represent significant difference at $p < 0.05$ using ANOVA and Tukey's HSD test.

Values are expressed as the mean ± standard error of the mean (SE); with n = 3; Letters represent significant differences at $p < 0.05$ using ANOVA and Tukey's HSD test.

accumulate in the mature tissue. In comparison with other vegetables, the results of the study show that the chayote plant contains a high value of Ca concentration compared to other vegetable species such as green peppers (*Capsicum annuum*) and broccoli (*Brassica oleracea* L. var. Italica), with 8.7 µg/g and 100 µg/g, respectively. However, spinach (*Spinacia oleracea*) contains 4-fold higher of Ca concentration at 600 µg/g [39]. Therefore, for macronutrients, P is the highest in all the three plant parts with an average of 197.04±3.07 µg/g followed by Ca at 124.25± 1.35 µg/g and Mg 66.07±3.60 µg/g, and the least being Na with a value of 4.03±0.56 µg/g. Antagonistic factors may contribute to the low Mg concentration in the leaves of Chayote, where high Ca concentration in the soil may suppress the Mg uptake [40]. On the other hand, minerals such as Ca and P are found to exceed the concentration values [28, 41].

Micronutrient

Microminerals in Table 3, such as Cu, Zn, Mo, Fe, and Al are not significant ($p > 0.05$) amongst all three plant tiers. However, Cu is the highest found in the upper tier shoot area (0.37 ± 0.04 µg/g) compared to other tier zones. Cu is required in small amounts of 0.05-0.5 µg/g in the growing process between 3-10 µg/g in plant tissue and acts as an enzyme activator and intensifies flavour and colour in vegetables [42]. Cu also participates in numerous physiological functions, for example, as an essential cofactor for many metalloproteins [5]. Zn is almost equal amounts with no significant difference amongst them. This nutrient is important for chlorophyll and auxin formations and the conversion of starch to sugar [43]. According to Ji et al. [44] translocation mechanisms of minerals may contribute to the high supply factor of Zn towards the lower tier of the shoot. Iron is the highest number of micronutrients found in the leaves of vegetable chayote, dominating most in the mid-tier of the shoot (3.56 ± 0.71 µg/g), followed by the top tier shoot section (2.78 ± 0.4 µg/g) and the lowest in the lower tier of the shoot section with 2.41 ± 0.8 µg/g with no significant difference amongst them. Iron plays a critical role in the plant metabolic processes in DNA synthesis, respiration, and photosynthesis and many metabolic pathways are activated by Fe and a prosthetic group constituent of many enzymes [45]. Iron can be toxic if it is on high levels due to the tendency to form hydroxyl radicals that can damage the cell's mass [46]. The concentration of other elements, such as the presence of low Mg can also affect the concentration of Fe in the leaves due to their negative syngenetic effect [40]. The pH of the growing medium is critical in the availability of Fe for plant uptake.

Al is almost equal in all three tiers with no significant difference with each other ($P<0.05$). The Al in soils of the tropics, is in low pH values of less than 5.5 [47]. However, the Kundasang area is mostly metamorphic derived soils with soil pH of between 5.0 to 5.5 and are not expected to contain high Al concentrations as reflected in the foliar analyses [48]. Mo is almost uniform in all the three tiers of the shoots with no-significance difference to each other ($P<0.05$). This nutrient is required in a small amount for normal plant development and the range in most plant tissues is between 0.3-1.5 µg/g and those recorded in the growing medium range between 0.01-0.20 µg/g [43]. The mineral movement is said to occur from the older leaves to the younger parts of the leaf and therefore, the difference is not noticeable between the three tiers of shoots [36, 38].

Proximate analyses

The chemical composition of moisture, fat, crude protein, ash, and carbohydrates contents was analysed as shown in Table 4. The top tier of the shoot of chayote is the richest in all the investigated proximate components, with the middle tier showing the same content and the lowest being the lower tier, with the exception of crude fibre, exhibiting the opposite trend and moisture content as well. The moisture content exhibits a descending trend with 89.48 ± 0.06 % in the upper tier, the mid-tier with 90.29 ± 0.25 %, and the lower tier having 90.55 ± 0.63 % and all values were not-significant to each other, indicating the distribution of water is almost uniform throughout the 3-foot shoot of the chayote. Furthermore, the location in Kundasang is considered an elevated site with relatively high humidity [49].

Fat content is the highest in the upper tier (0.21 ± 0.02 %), followed by 0.19 ± 0.01 % at the mid-tier and 0.14 ± 0.02 % in the lower tier, with no significant difference. Different fat concentrations in many plants may be due to plant metabolism [41]. The crude protein content, with the top tier, has the higher value (3.33 ± 0.29%), followed by the mid-tier (3.15 ± 0.20 %) and lower tier (3.01 ± 0.24 %). All were non-significant to each another, indicating a uniformity in the protein content of the chayote shoot. Other studies reported higher protein content in the stem and not in the leaves of chayote [4]. The crude fibre was observed with the highest content in the lowest tier zone (2.04 ± 0.14%) but not in other parts of the zone. This result is very much anticipated since the lower zone is the most mature part of the shoot, followed by the mid-tier section, and the youngest shall be the tip of the shoot (Table 4). According to Vieira et al. [16] higher crude fiber content is found in the leaves than in the stem, which is contradicted in this current study. The different content of crude fibre may be attributed to the differences in plant species, plant maturity, seasonal variation, fertilizer, or analytical techniques [19]. In contrast, Cucurbita pepo plants also derived from the Cucurbitaceae family found in sub-Saharan Africa show lower levels of crude fibre content compared to the chayote plants [50].

The content of crude ash is higher in the upper tier of the shoot (1.27 ± 0.02 %), whereas the mid-tier (1.17 ± 0.05 %) and lower tier sections (1.15 ± 0.12 %) showed no significant difference. The previous study reported that chayote ash content in the leaf and stem was in the range of 0.90-1.20 % which was close to the current study [4].

The carbohydrate, mostly the sugar fractions, is superior in concentrations in the upper tier (4.36 ± 0.24 %), compared to the mid-tier section (3.20 ± 0.19 %) and the lower tier section (3.12 ± 0.13 %). The finding of the different patterns for carbohydrate content in different parts of the chayote shoot is supported by the work of Ismail et al. [51] on *Strobilanthes crispus*. According to Schiltz et al. [52], the concentration of carbohydrates increases throughout plant growth with the higher carbohydrate content at the higher level of plant development.

Conclusion

The best plant part to be consumed in the upper tier of the shoot portion, a foot from the tip down. This top portion of the plant part is rich in antioxidant and proximate contents compared to the middle and the lower tiers section. These have been seen from the TPC (mg/g GAE) and proximate readings as well. This makes the first foot portion of Chayote shoot the most prolific and highly nutritious part of the plant that highly rich in nutrients.

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