

BINDURA UNIVERSITY OF SCIENCE AND EDUCATION



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DEPARTMENT OF MATHEMATICS AND SCIENCE

**VITAMIN C AND PROTEIN CONTENT OF SELECTED INDIGENOUS
LEAFY VEGETABLES CONSUMED BY RESIDENTS IN MHONDORO
GRASSLANDS**

BY

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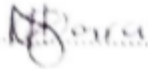
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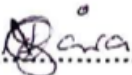
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DEDICATION

This work is especially dedicated to my family.

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Sincere gratitude and appreciation go to my supervisor Prof. P. Dzomba for his unwavering support, supervision, feedback and patience during this study. I would be amiss if I fail to acknowledge all laboratory personnel in the Chemistry Department for helping out during laboratory work. Lastly, I would like to thank my family for believing in me. Above all, I thank God for leading me all the way.

ABSTRACT

The encroachment of exotic vegetables is responsible for the decline in consumption and utilization of indigenous leafy since a lot of people nowadays have limited or no knowledge regarding the potential benefits of indigenous African leafy vegetables though history has it that they have been part of traditional diets in indigenous societies. The current work compared protein and vitamin C profiles of four indigenous leafy vegetables namely *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa*. The highest protein content was observed in *A. thunbergia*, followed by pumpkin leaves, *V. unguiculata* and least was *B. Pilosa*. The largest value of the amount of vitamin C content was observed in *Cucurbita* leaves (197.90 ± 1.71 mg/100 g) and the least was 167.55 ± 2.91 mg/100 g in *A. thunbergia*. *B. Pilosa* and *V. unguiculata* exhibited 182.44 ± 1.96 mg/100 g and 177.07 ± 1.09 mg/100 g respectively. The findings of this study substantiate the nutritive properties of the vegetables as well as their recognition as potent sources of protein and vitamin C that are readily available, economical and within the reach of many who have limited access to animal protein and packaged vitamin C supplements.

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PROJECT TITLE: To determine protein and vitamin C content in local vegetables in Murambwa grasslands community.

CHAPTER 1:

1.1 INTRODUCTION

This chapter sought to discuss on the background of the study, aim of the study ,objectives ,statement of the problem ,significance of the study, research questions ,limitations and delimitations of the study, hypothesis and definition of key terms

1.2 Background to the study

Indigenous leafy vegetables generally thrive naturally as native to a given area in the back of yards, dumping sites as well as in open lands or they colonized the region in the past and evolved there over time (Gupta et al., 2017). These vegetables significantly boost food security and enhance the quality of diets particularly among rural dwellers in the third world. (Mavengahama, 2014). A number of studies support that indigenous leafy vegetables have come in handy in plugging nutrition gaps through provision of wholesome, affordable and nutritious food alternatives (Ranum et al., 2014; Meldrum et al., 2018) owing to their elevated content of essential nutrients (Bua et al., 2017) including minerals (zinc, calcium, magnesium, iron and potassium), provitamin A, vitamin C and fibre (Nyadanu &Lowor, 2015; Schreinemachers et al., 2018). Additionally, these vegetables have very low content of carbohydrates and fats. Despite all the health benefits, consumption of indigenous leafy vegetables had witnessed a significant decrease as people turned to exotic ones (carrots, lettuce, tomatoes) that are not that economical to cultivate (Maseko et al., 2018). Some of the commonly consumed indigenous vegetables in Zimbabwe are nyevhe (*Cleome gynandra*), mowa (*Amaranthus thunbergia*), muboora (*Cucurbita* species), muchacha (*Cucumis anguria*), munyemba (*Vigna unguiculata*), mutsine (*Bidenspilosa*) among others (Gido et al., 2017).

These vegetables have medicinal functions because of their phytochemistry (Neugart et al., 2017). They also offer greater nutritional value compared to exotic variants thereby ensuring adequate supply of the nutrients required by the human body. Indigenous leafy vegetables are immune boosters because they contain large amounts of vitamins (Maseko et al., 2018). Vitamin A is an antioxidant that is vital in scavenging free radicals that cause diseases related to oxidative stress. There is little information documented pertaining to the nutritional value of the indigenous leafy vegetables because they are underutilized (Neugart et al., 2017). Vitamin C helps in the absorption of iron besides maintaining healthy teeth and gums. Folic acid is linked to the reduction of possibilities of birth defects and vitamin K prevents osteoporosis and inflammation (Gido et al., 2017). These vegetables are a vital source of dietary minerals such as iron, zinc, calcium, magnesium, sodium, phosphorus and potassium. Minerals are stable to cooking methods that are used in preparing the vegetables compared to vitamins. Minerals are important in nutrient metabolism and prevent degenerative ailments (Li et al., 2018; Chivenge et al., 2015).

Above all, indigenous leafy vegetables are an economical and affordable alternative protein source with up to 25 g/100g protein content (Food and Agriculture Organization of the United Nations (FAO), (2018). Therefore, they are vital in feeding the economically-challenged rural and urban folks due to the fact that they are cheap compared to animal sources of protein (poultry, meat and fish) (Chivenge et al., 2015). Owing to population and environmental issues, there is need for more sustainable protein source alternatives. The current work is a comparative study of protein and vitamin C profiles of four indigenous leafy vegetables namely *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa*.

1.3 Aim of the study

This study aims at investigating the protein and vitamin C content of four indigenous leafy vegetables: *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa*.

1.4 Objectives

The main objectives of this study are:

- To determine the content of vitamin C in four indigenous leafy vegetables namely *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* using spectrophotometry.
- To determine the quantifiable proteins in the vegetables using the Kjeldahl method.

- To compare the protein and vitamin C content of the four vegetables under study.

1.5 Statement of the problem

People residing in the Mhondoro grasslands greatly depend on indigenous leafy vegetables (*A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa*) as an important part of their diet. There is scarce scientific information pertaining to the protein and vitamin C content of these vegetables. This knowledge gap spurs the understanding of the value of these vegetables nutritionally and the potential health benefits derived from their consumption. Lack of all-embracing information on the vitamin C and protein content of *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* consumed by the Mhondoro Grasslands community has restricted the development of strategies to enhance the overall status of nutrition and dietary intake of the people.

1.6 Significance of the study

This work is going to give important revelations regarding the protein and vitamin C content of *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* consumed by the Mhondoro Grasslands population. This in turn will provide a basis for understanding the nutritional value of the four vegetables and how they partake in fulfilling the dietary needs of that population. The findings of the study will significantly contribute to promoting food systems that are sustainable and have a semblance of the local culture of the people thereby promoting traditional cuisines.

1.7 Limitations of the study

The selected sample of indigenous leafy vegetables is small hence the findings of the study would fall short in representing all indigenous leafy vegetables consumed across Zimbabwe, hence generalization of the findings is not possible. Resources and time constraints have limited the study to Mhondoro grasslands only and the full diversity of the indigenous leafy vegetables is not fully captured herein.

1.8 Delimitations of the study

The findings of the study may not directly apply to communities not specified herein. Primarily this study focuses on protein and vitamin C content of the vegetables under study without considering other bioactive phytochemicals. Findings of the study are short term since they are based on samples collected in the farming season only.

1.9 Research questions

- What is the vitamin C and protein content of *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* consumed by Mhondoro Grasslands local population?
- How do the vitamin C and protein content indigenous leafy vegetables under study compare with each other?

1.10 Hypotheses

H₀: The protein and vitamin C content of *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* consumed by residents in Mhondoro Grasslands have no significant difference.

H₁: The protein and vitamin C content of *A. thunbergia*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* consumed by residents in Mhondoro Grasslands have significant differences.

CHAPTER 2: LITERATURE REVIEW

2.1 Indigenous African leafy vegetables

There are concerns about the decrease in the consumption and utilization of indigenous leafy vegetables in food applications in spite of them being a vital component of human diet since time immemorial (Musotsi et al., 2018). The encroachment of exotic vegetables is responsible for this decline since a lot of people nowadays have limited or no knowledge regarding the potential benefits of indigenous African leafy vegetables though history has it that they have been part of traditional diets in indigenous societies. Replacement of these vegetables poses a significant danger of them being extinct in Africa as the is paucity of seeds and information related to their performance and required inputs (Chivenge et al., 2015). Despite a notable decline in cultivation of traditional vegetables the world over, they offer diverse genetics and have the potential to enhance nutritional and food security (Berkelaa, 2022).

Food intake is to a greater extent influenced by such factors as meal culture concept, socio-economic, gender and cultural factors. With reference to these factors, consumption of indigenous African leafy vegetables is greatly affected by expertise in preparation of food, available resources, beliefs and gender relations (Borelli et al., 2020). As a matter of fact, there is rapid loss in knowledge of preparation of traditional vegetables as they are regarded as old-fashioned amongst the young generation who regard farming as detested profession (Mabhaudhi et al., 2015). Moreover, the imminent loss of biodiversity of these African traditional vegetables is further aggravated by lack of information related to their use, cultivation, harvesting, cooking, nutritional content and methods of preserving them (Labadarios et al., 2022). Research, commercial farming and development have been oblivious to these vegetables rendering them uncompetitive hence their sad retreat from most local diets globally (Labadarios et al., 2022). This has negatively impacted on their aptitude as important food ingredients.

African indigenous leafy vegetables have evolved over time to become resistant to adverse environmental conditions such as drought because they have been neglected by both farmers and academics and because of this they are a guarantee to food security since they are drought-resistant (Dinu et al., 2016). In South Africa, intake of indigenous leafy vegetables differs from place to place and people consume them because they believe the vegetables have the ability to reduce risks posed by some illnesses (Lipton & Saghai, 2017). Lately, these vegetables are being

acknowledged as future crops owing to their meritorious medicinal properties, therapeutic functions and climatic resilience (Borelli et al., 2020). They are nutrition-rich, economical and available locally. Therefore, they can stand as smart foods of the future (Chivenge et al., 2015). Inarguably, indigenous African leafy vegetables remain largely untapped and they are paramount to achievement of zero hunger (Lipton & Saghai, 2017). The current widespread economic crisis coupled with high cost of food and fuel together with increase in interest rates have mounted pressure on the ordinary consumers who are struggling to meet their basic needs resulting in catastrophic food insecurity (Mabhaudhi et al., 2015). Research has shown that traditional vegetables provide a plethora of medical benefits among them prevention of the onset of some cancers, including lung, skin, uterine, cervical and gastrointestinal cancers; macular degeneration, cataracts and other degenerative conditions linked to oxidation and free radical damage (Borelli et al., 2020). Additionally, the vegetables promote healthy immune systems and minimize the risk linked to blood pressure and cardiovascular diseases (Lipton & Saghai, 2017). Beyond any reasonable doubt, African indigenous leafy vegetables not only form condiments in traditional cuisines but are a pivotal part of nutrition (Borelli et al., 2020). and are potent traditional medicine that can provide a source of income for the rural folks (Chivenge et al., 2015; Mokganya & Tshikhawe, 2018; Vu & Nguyen, 2017).

2.2 Cowpeas, (*munyemba* or *Vigna unguiculata*)

The plant is referred to as the poor man's meat because it is a noteworthy protein, mineral and vitamin source for the economically-challenged rural people with restricted access to animal protein (Rasheed et al., 2020). The plant is resilient to drought, adapting to a wide range of climate conditions and types of soil. It is commonly grown in the tropics and sub-tropics of the African continent, Southeast Asia, the United States and Latin America (D'Alessandro & Zolla, 2012). Cowpeas have gained industrial recognition in food formulations due to their functional benefits coupled with its nutritional prowess (Tovar et al., 2014). Cowpea is endowed with towering protein-content which is within the reach of many hence the lobbying for its consumption. Additionally, it is complementary an alternative source of protein for vegan people besides expanding food options contributing to dietary variety. (Clerens et al. 2012; D'Alessandro & Zolla 2012; Rasheed et al. 2020).

The content of protein in cowpea is significant and is responsible for a large chunk of its dry mass. Generally, the protein content of cowpea regardless of the type falls between 16 and 31 %

(Clerens et al. 2012; Tovar et al., 2014). In another study, the protein content was found to be 23.56 to 26.14 % (Rasheed et al., 2020). A study done on inter-cropped and solitary cowpea South African plots revealed that its protein content was between 23.7 and 26.4% The major protein abundant in cowpea are albumins, globulins, and glutelin while prolamins are available in smaller quantities (Musotsi et al., 2018; Labadarios et al., 2022). The amino acid profile for cowpea whole grain was shown to be high in lysine, arginine and leucine. Due to this fact, cowpea safely meets the essential amino acid requirements of a cereal crop-derived human food to a greater extent (Mabhaudhi et al., 2015). Addition of cowpea to tannin and non-tannin staple diets or foods made of sorghum greatly elevated the protein content of a number of traditional African dishes (Dinu et al., 2016). The leaves, decorticated seeds, whole grain and aerial parts of cowpea contain substantial amounts of indispensable amino acids as lysine, leucine, tryptophan and arginine in comparison to cereals hence their capability to meet the essential amino acid requirements of the human diet appreciably. One draw-back of cowpea is lack of sulphur-containing amino acids such as methionine and cysteine hence the need to be complemented with other cereals, meat, vegetables and dairy products so as to accomplish a balanced diet (Musotsi et al., 2018; Dinu et al., 2016).



Figure 1: *V. unguiculata* leaves

2.3 Pumpkin leaves (*muboora*, *Curcubita maxima*)

Pumpkins fall under the Cucurbitaceae family. They are classified into *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita mixta* and *Cucurbita moschata* basing on their texture, stem and shape. (Xanthopoulou et al., 2009). Pumpkin is the predominant species out of twelve that fall under the genus *Cucurbita*. Pumpkin production, inclusive of gourd and squash surpassed 27

million tons in 2018 alone worldwide (Nguyen et al., 2020). In 2019, production was approximately 23 million tons (FAO, 2019). Pumpkins exist in a variety of shapes, colors and sizes depending on climate as well as variety. The applications of pumpkin in the pharmaceutical, processing, agriculture and feed industry greatly increased in the recent past owing to the presence of nutritional and health protective polysaccharides of the fruit, protein and oil in the (Sojak & Glowacki, 2010).

Maheshwari et al., (2015) regard pumpkin seeds as a nutritional powerhouse with no side effects on human health even when consumed regularly. The protein content of pumpkin seed oil ranges between 25.4 % and 41.59 % (Ardabili et al., 2011). In a separate study, Achu et al., (2013) report that pumpkin seed oil obtained from pumpkins collected from various places in Cameroon contain a minimum of 28 % and a maximum of 40% protein and all of the Cucurbitaceae family seed oils are an excellent source of essential amino acids. Figure 1 illustrates some varieties of pumpkin fruits.



Figure 2: Some pumpkin fruit varieties

Srbinoska et al., (2012) compared the chemical composition of *Cucurbita pepo* and *Cucurbita maxima* harvested in Macedonia and proved that protein and total nitrogen content of *C. pepo*

was greater than that of *C. maxima*. In another study in Kenya, Karanja et al., (2013) proved that Cucurbitaceae family seeds were rich in oil, protein and fiber. SteinerAsiedu et al., (2014) researched on the nutrient composition and protein quality of four species of pumpkins. The protein content was 46.10 % to 69.10 % translating to very high protein levels in the varieties that were investigated. Kwiri et al., (2014) state that Zimbabwean pumpkin seeds have very high protein content together with other nutrients and minerals. Al-Anoos et al. (2015) reported 33.03 to 34.76 g/100 g protein in Chinese and Egyptian varieties of the *C. maxima*. According to Cakarevic et al., (2020) protein obtained from pumpkin oil cake can be used in the encapsulation of bioactive compounds. Fish oil encapsulated with pumpkin seeds oil exhibited improved oxidative stability because pumpkin oil added antioxidants (tocopherols, carotenoids and phytosterols) to the blend (Ogrodowska et al., 2020).

Protein hydrolysate obtained from pumpkin seed improved both non-enzymatic and enzymatic antioxidant compounds in plants. Moreover, in salt-stressed plant the protein hydrolysate rehabilitated minerals and boosted growth of the plants (Sitohy et al., 2020). With reference to the discussion about nutritional analyses of pumpkin, there is every reason to determine the protein content of its leaves as there is an increase in the demand for healthy foods lately. Figure 3 shows a leaf of *C. maxima*.



Figure 3: *C. maxima* leaves

2.4 Black jack (*mutsine*, *Bidens Pilosa*)

The plant belongs to the genus *Bidens* (Asteraceae) that has more than 240 different species. It is a perennial plant that is easy to grow. The plant is mostly found in tropical and subtropical regions of the world (Gido et al., 2017). The United Nations Food and Agricultural Organization regards *B. Pilosa* as an edible plant since the year 1975. Since then, the plant has found

applications traditionally as a food and medicine in the Oceania, Africa, Asia and America (Maseko et al., 2018). There are claims that the plant has more than 40 different bioactive compounds (Nyadanu &Lowor, 2015; Schreinemachers et al., 2018). It is known to treat more than 40 diseases including cancer, infectious diseases, asthma and managing wounds and inflammation (Ranum et al., 2014; Meldrum et al., 2018). A lot of research has been done with regards to the functions of phytochemicals of the plant but there is limited documentation of the protein content of the plant. Figure 2 gives a summary of the composition, medicinal uses, food application and the main bioactive compounds of *B. Pilosa*.

Figure 4: Summary of the uses and composition of *B. pilosa*

A picture of the plant *B. Pilosa* is shown in figure 5.



Figure 5: *B. pilosa* plant

2.5 Mowa (*Amaranthus thunbergii*)

All amaranth belongs to the genus *Amaranthus* spp. in the family *Amaranthaceae* and sub-family *Amaranthoideae* (Topwal, 2019; Achigan-Dako et al., 2014). *Amaranthus* means a flower that is eternal or not wilting, unfading, or life everlasting (Rastogi, et al., 2013). The leaves and grain of amaranthus are rich sources of plant protein and amino acids (Reyad-ul-Ferdous et al., 2015). Protein originating from amaranth is considered healthier than that from the animal because plant

sources have restricted or no fat, hence free of cholesterol. The leaves of amaranth are known to reduce cholesterol which triggers cardiac health issues. There is a school of thought which proposes that consumption of *Amaranthus* leaves reduces appetite due to high levels of protein (Peter & Gandhi, 2017) which reduce blood insulin level thus controlling appetite.

Amaranthus protein is beneficial to gluten-intolerant individuals. The limiting proteins (lysine, threonine, methionine, and tryptophan) are abundant in *Amaranthus*. Lysine is present in the plant in significant amounts and it helps promotes hair growth, delays the onset of aging processes and also maintains the skin. Research has shown that maximal inclusion of amaranth leaves in one's diet reduces hair loss and early hair graying There are 8-9 g of protein in a one cup serving of amaranthus seeds. Amaranth protein is relatively rich in essential amino acids such as lysine and tryptophan, of which lysine is one of essential amino acids which other cereals lack (Sokolova et al., 2021; Ramdwar et al., 2017; Schnetzler & Breene, 2018; Njeme et al 2014). Figure shows the *A. thunbergia* plant.



Figure 6: *A. thunbergii*

In addition, green *Amaranthus* leaves are highly rich in vitamin C. Reports suggest that a cup of boiled and drained *Amaranthus* leaves provides 90% of daily dietary requirement of vitamin C (Lee et al., 2017; Sarker & Oba, 2018). The plant also possesses antioxidant properties due to the presence of phenolic compounds and flavonoids. These compounds are necessary in the biosynthesis of carnitine and collagen (Jiménez-Aguilar & Grusak, 2017). Additionally, they defend against diseases such as atherosclerosis, cancer, cataracts, neurodegeneration, emphysema, retinopathy, arthritis and cardiovascular diseases (Jimoh et al., 2017). Therefore, the

interest to explore and exploit plants has increased greatly because they are considered safe and economical alternative therapies (Sarker & Oba, 2018). Inarguably, amaranthus leaves as a component of regular is a potential nutraceutical therapy that requires exploration as a buffer against non-communicable diseases that have ravaged people's lives lately.

CHAPTER 3: METHODOLOGY

3.1 Materials and equipment

Kjeldahl apparatus, digester, UV-vis spectrophotometer (Genesys 10S), glassware, distilled water, water bath, 2,4-Dinitrophenylhydrazine, thiourea, ascorbic acid, sodium hydroxide, boric acid, hydrochloric acid, bromine water, sodium hydroxide, acetic acid, metaphosphoric acid, potassium sulphate, copper sulphate, bromocresol green, methyl red, sulphuric acid, ethanol.

3.2 Collection and preparation of Plant samples:

Tender leaves of *A. thunbergii*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* were randomly collected from fields and grasslands in Murambwa village, Mhondoro Ngezi district, Mashonaland West Province in Zimbabwe (18° 39' 58'' south, 30° 21' 17'' east). The samples were placed in khaki envelopes separately and transported to the laboratory immediately where wet mass of each sample was recorded before they were washed with running tap water and rinsed using distilled water thrice. The washed samples were cut into small pieces and allowed to dry in air under a shade. After completely drying the samples were pulverized into fine powders; their dry mass measured and they were stored in airtight containers.

3.3 Determination of protein (Kjeldahl method)

Preparation of reagents

- 40 % sodium hydroxide solution was prepared by dissolving 200 g of sodium hydroxide pellets in distilled water and making up the volume to 500 ml using distilled water
- 4 % boric acid solution was prepared by dissolving 20 g of boric acid in distilled water and making the volume up to 500 ml using distilled water.

- Bromocresol green/methyl red indicator solution was prepared by dissolving 100 mg of methyl red in 100 ml 95 % hydro-ethanol solution. The same mass of bromocresol green was weighed and treated exactly the same manner. Methyl red and bromocresol green were mixed in the ration 1: 5 by volume.
- The blank was prepared by mixing all the reagents except the sample.

Digestion

Triplicate 1 g samples of *A. thunbergii*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* dry powder were weighed into Kjeldahl flasks. To each of the samples, 30 ml of concentrated sulphuric acid, 3.5 g potassium sulphate and 0.4 g copper sulphate were added. The mixtures were slowly heated at 400 °C in a fume-hood until the color changed to pearlescent blue. The solutions were allowed to cool and were then diluted to 100 ml using distilled water.

Distillation

10 ml of the digested solution were added to 10 ml of 40 % sodium hydroxide and fixed to the distillation apparatus. 10 ml of boric acid and 4 drops of indicator were added to the ammonia receiving flask. 30 ml of distillate for each sample were collected.

Titration

The collected distillates were titrated separately with 0.1 M hydrochloric acid and titre were recorded and percent nitrogen was determined as follows:

$$\% \text{ nitrogen} = \frac{(\text{ml titre sample} - B) \times \text{molarity HCl} \times \text{dilution factor} \times 14.007}{\text{mg sample} \times 10} \times 100$$

The conversion formula used to find percent protein in samples is:

$$\% \text{ protein} = \% \text{ nitrogen} \times \text{conversion factor}$$

3.4 Spectrophotometric determination of vitamin C content

3.4.1 Preparation of samples

2 g of wet, pulverized sample macerated for 12 hours with intermittent shaking in 50 ml of a solvent made of 5% metaphosphoric acid and 10% acetic acid by volume. The mixture was quantitatively transferred to a 100 ml volumetric flask and diluted to the mark with the same solvent. The mixture was filtered to obtain a clear filtrate which was analyzed for vitamin C content. The same treatment was done for all samples.

3.4.2 Estimation of vitamin C content

Bromine water was added to the filtrate in excess to obtain dehydroascorbic acid by oxidation of ascorbic acid in the samples. Thiourea was added dropwise to remove excess bromine and clear the solution. Calibration standards of concentrations 1.25; 2.5; 5; 10 and 20 ppm were prepared using ascorbic acid by serial dilution. 1 ml of 2,4-DNPH was added to all standards and samples as well as the blank. The mixtures were incubated in a thermostated water-bath at 37 °C for three hours after which they were cooled in an ice bath. 5 ml of 85 % sulphuric acid were added to each chamber and the absorbance was recorded at 521 nm.

3.5 Statistical analysis of data

Data collected for all parameters was analyzed using IBM SPSS Version 26. Difference among means was tested at $p \leq 0.05$.

CHAPTER 4: RESULTS

4.1 Protein content

The protein content of four plants: *A. thunbergii*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* is presented in table. The highest protein content was observed in *A. thunbergia*, followed by pumpkin leaves, *V. unguiculata* and least was *B. Pilosa*. Table 1 presents the findings of the study on the protein content of the plants under study. All values were significantly different at $p \leq 0.05$

Table 1: Protein content of indigenous leafy vegetables under study

Vegetable name	Mean % protein	SEM
<i>V. unguiculata</i>	12.34	0.76
pumpkin leaves	14.01	0.09
<i>A. thunbergii</i>	18.94	0.33
<i>B. pilosa</i>	8.16	0.25

The protein content results are presented as a graph in figure

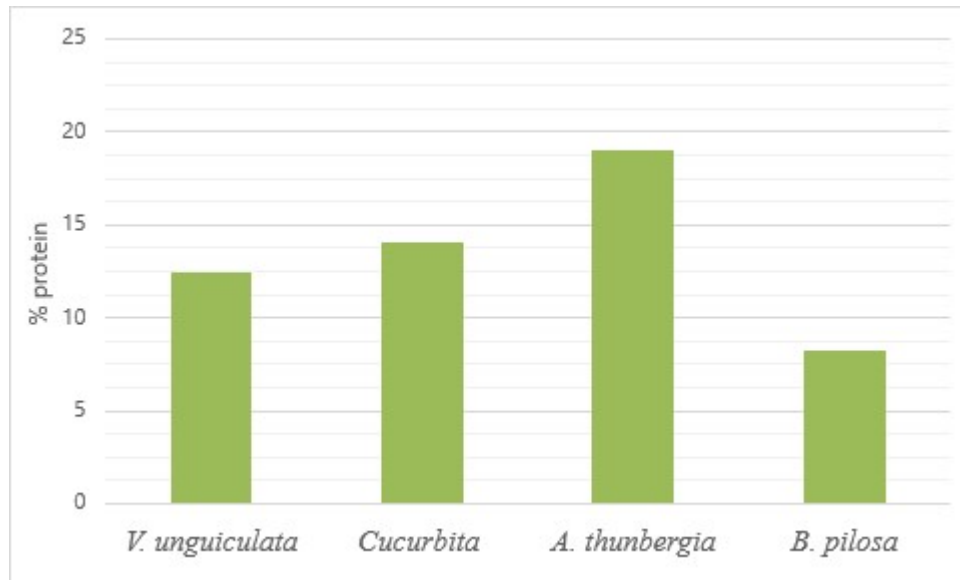


Figure 7: Comparison of protein content in *A. thunbergii*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa*

4.2 Vitamin C content

The findings of this study on content of vitamin C in 4 African indigenous vegetables are presented in table 2 and figure 9. Figure 8 is the calibration plot for standard ascorbic acid. The values presented were statistically different at $p \leq 0.05$. The largest value of the amount of vitamin C content was observed in *Cucurbita* leaves (197.90 ± 1.71 mg/100 g) and the least was 167.55 ± 2.91 mg/100 g in *A. thunbergia*. *B. Pilosa* and *V. unguiculata* exhibited 182.44 ± 1.96 mg/100 g and 177.07 ± 1.09 mg/100 g respectively.

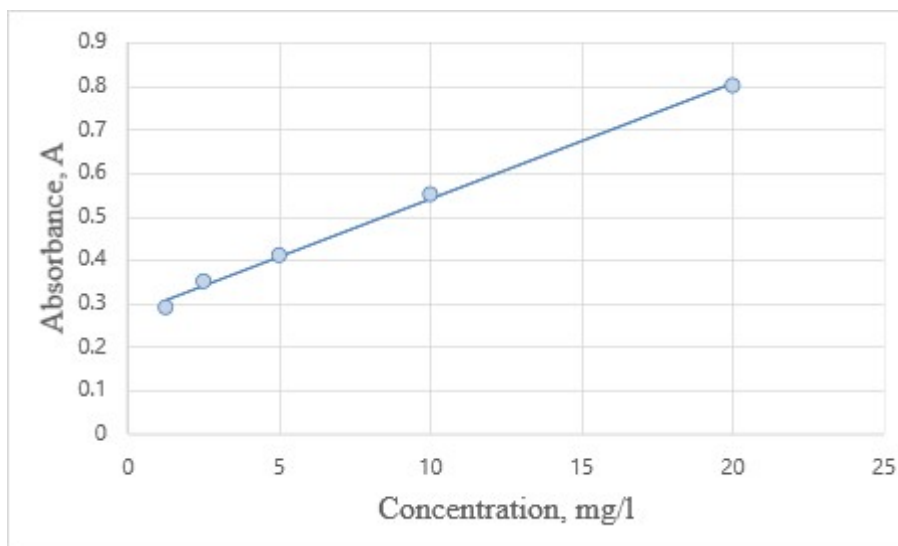


Figure 8: Calibration curve for determination of vitamin C

Table 2: Content of vitamin C in mg/100 g of sample

Vegetable name	Vitamin C content \pm SD, mg/100 g
<i>V. unguiculata</i>	182.44 \pm 1.96
<i>Cucurbita</i> leaves	197.90 \pm 1.71
<i>A. thunbergii</i>	167.55 \pm 2.91
<i>B. pilosa</i>	177.07 \pm 1.09

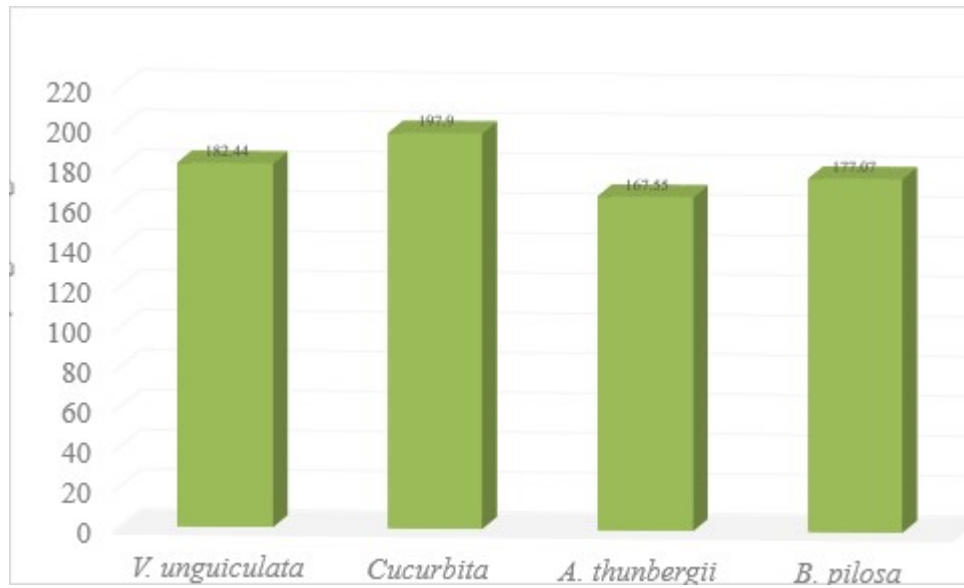


Figure 9: Graphical comparison of Vitamin C content of 4 leafy vegetables consumed in Mhondoro

CHAPTER 5: DISCUSSION CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this work, the protein and vitamin C content of four indigenous African leafy vegetable (*A. thunbergii*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa*) were determined using Kjeldahl method and spectrophotometry respectively. The calibration curve for the determination of vitamin C is shown in figure 8. The equation of the curve was $y=0.0266x+0.2738$ and its correlation coefficient was 0.9968. There were significant variations in vitamin C levels across the investigated cultivars at $p \leq 0.05$. The largest value of the amount of vitamin C content was observed in *Cucurbita* leaves (197.90 ± 1.71 mg/100 g) and the least was 167.55 ± 2.91 mg/100 g in *A. thunbergia*. *B. Pilosa* and *V. unguiculata* exhibited 182.44 ± 1.96 mg/100 g and 177.07 ± 1.09 mg/100 g respectively. The values observed herein were above those reported by Ayua et al., (2016). Vitamin C content was significantly higher in *Cucurbita* leaves.

These findings suggest that all the leafy vegetables investigated in the current study have the potential to provide substantial amounts of dietary vitamin C required by the body when incorporated in the diet of the people of Mhondoro. There is a school of thought arguing that the difference in nutrient content across cultivars is genetical (Marles, 2017). The samples were harvested in summer when they are abundant and have reached maturity stage. The implication is that despite harvesting at maturity stages, the vegetables need careful processing and cooking so that the vitamin consumed is maximized.

In addition, the protein content of 4 selected indigenous leafy vegetables was investigated. The results obtained exhibited significant differences in protein content of the vegetables at 0.05 confidence level. The highest protein content was observed in *A. thunbergia*, followed by pumpkin leaves, *V. unguiculata* and least was *B. Pilosa* (table 1; figure 7). The findings are vital in the face of protein deficiency in the sub-Saharan Africa owing to the exponential growth of human population against demand for animal protein. Choice of indigenous vegetable cultivars extends beyond all other factors to entail nutrient content as well. The consumption of these vegetables must be prioritized. Amaranth, according to the results herein has the highest protein content.

The results obtained in this study are indicative of the fact that African indigenous leafy vegetables can be utilized as a source of protein in human diets. According to the World Health

Organization (WHO) (2007), range of protein requirements in human diet is 0.66 and 0.69 g/kg. The study has shown *A. thunbergii*, *Cucurbita* species leaves, *V. unguiculata* and *B. Pilosa* contain substantial amounts of protein and vegetable protein is a viable alternative to animal protein in the daily diet chart.

5.3 Conclusion

This work evaluated the vitamin C and protein content of four indigenous leafy vegetables that are widely consumed as vegetables in Mhondoro. All the vegetables were found to have appreciable quantities of protein and vitamin C. The findings of this study substantiate the nutritive properties of the vegetables as well as their recognition as potent sources of protein and vitamin C that are readily available, economical and within the reach of many who have limited access to animal protein and packaged vitamin C supplements.

5.3 Recommendations

Indigenous African leafy vegetables such as *A. thunbergii*, *Cucurbita* species, *V. unguiculata* and *B. Pilosa* are promising crops that can be utilized to alleviate malnutrition in developing countries hence understanding their detailed nutritional profiles is imperative. The current study focused on vitamin C content and protein content of matured plants. A detailed evaluation of these plants' agronomic characteristics at various stages of maturation is recommended. Furthermore, protein isolates of these plants are alternatives for incorporation in cereal foods to promote nutritional value. Further research is required in qualitative properties of the plants as well as verification of safe levels of intake. Most importantly, there is need to evaluate environmental and socio-economic issues relating to cultivation, harvesting and processing of these vegetables

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