NUTRITIONAL AND BIOCHEMICAL EVALUATION OF *MOMORDICA BALSAMINA* LEAF POWDER AT DIFFERENT HARVESTING STAGES

BY

MAROPENG SUSAN CHOSHI

A MINI-DISSERTATION SUBMITTED FOR THE DEGREE OF MASTER OF SCIENCE IN HORTICULTURE IN THE DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURE AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR : DR M.Y. MAILA

CO-SUPERVISOR : PROF D. SIVAKUMAR

NOVEMBER, 2022

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DECLARATION

I, Maropeng Susan Choshi, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Horticulture has not been submitted previously by me or anybody for a degree at this or any other university. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

Candidate: Maropeng Susan Choshi	________________ Signature	________________ Date

DEDICATION

I dedicate this to my supportive parents and siblings.

ACKNOWLEDGEMENTS

Greater thanks are the Almighty God for his love, faithfulness, favour, wisdom and guidance throughout the course of this programme. Isaiah 40:31 “But those who trust in the LORD will find new strength. They will soar high on wings like eagles. They will run and not grow weary. They will walk and not faint” is a scripture that gave me courage during challenging times of my journey. I express my sincerest gratitude to my supervisory team Doctor M.Y. Maila and Professor D. Sivakumar for the opportunity, guidance, support, insightful comments, productive criticism and useful suggestions during the research. Thank you to the team from the Green Biotechnology Research Centre of Excellence (GBRCE) and Limpopo Agro-Food Technology Station (LATS) and the Post-harvest Technology (phytochemistry) Laboratory at the Tshwane University of Technology, for the technical support. Acknowledging also the National Research Foundation (NRF) and AgriSETA for the financial support.

My most heartfelt appreciation goes out to my beloved family and friends who morally supported me in all ways and kept me motivated, my research colleagues and all people who have contributed.

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ABSTRACT

Momordica balsamina Linn., is one of the African leafy vegetables (ALVs) that holds a great deal of nutraceutical and pharmaceutical properties, attributed to the presence of micro-nutrients and secondary metabolites. These compounds form an important part of the human diet to reduce malnutrition and offer a wide range of remarkable preventive and therapeutic properties. Generally, the levels of nutritive and non-nutritive substances in ALVs are influenced by the plant's developmental stages and hence the stages in which the edible plant parts are harvested. A greenhouse study was undertaken, (1) to determine the effect of harvesting at different growth stages on the nutritional quality of *M. balsamina* and (2) to evaluate the effect of harvesting at different growth stages on the biochemical composition of *M.* balsamina leafy vegetable. Seeds of *M. balsamina* were planted in seedling trays and then when the seedlings were bearing three (3) leaves, they were transplanted into 20-cm-diameter plastic bags, containing a mixture of steam pasteurised loam soil and Hygromix at 3:1 v/v. Six harvesting stages, namely: vegetative, bud development, flower initiation, fruit set, fruit ripening, and physiological maturity, represented treatments, and were replicated ten (10) times. The experiment was laid out in a randomised complete block design (RCBD). At the vegetative stage, harvesting of *M. balsamina* leaves commenced and was continued at each at each growth stage, with plant variables being measured and recorded. The harvested leaves were freeze-dried and ground into fine powder for nutritive and biochemical analysis. Data were subjected to analysis of variance (ANOVA) using Statistics 10.0 software. In the first objective, data on the plant variables, namely, stem diameter (SD), vine length (VL), number of leaves (NL), leaf area (LA), chlorophyll content (CC), and leaf colour [brightness (L*), redness (a*), greenness (b*) and hue (h)], were measured and recorded, whereas data on the selected nutrients, namely, Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), and Phosphorus (P) were determined and recorded. The growth stages had highly significant ($P \leq 0.00$) effect on the measured plant variables, contributing 92, 87, 85 and 81% to the total treatment variation (TTV) in VL, NS, NL and LA, respectively. No significant ($P \geq 0.5$) effect was observed in SD. Similarly, treatments had a highly significant effect in CC and leaf colour, contributing 28, 33, 68, 71 and 63% to TTV in CC, brightness (L*), redness (a*), greenness (b*) and hue (h), respectively. Treatments highly increased VL (42-234%), NS (10-970%), NL (70-140%) and LA (53-86%) in *M. balsamina* leafy vegetable at all growth stages. Chlorophyll content increased by 10-37% at all growth stages, but experienced a decline (30%) at maturity stage. With regard to the leaf colour, the brightness of the leaves increased by 1% at the bud development stage, then followed by a decline of 1-11% at all the other growth stages. In contrast, the redness and the greenness of *M. balsamina* leaves were reduced at early developmental stages by 14-22% and 10-18%, respectively, but when the plants started setting fruits, the redness and the greenness of the leaves increased by 16-27% and 16-73%, respectively, up until physiological maturity. Harvesting of *M. balsamina* leaves at different growth stages again significantly influenced the nutritional quality of the leaf powder, contributing 46, 93, 68, 93, 95, and 96% to the TTV in K, Ca, Mg, Zn, Fe and P, respectively. The accumulation of K (56-95%), P (5-12%) and Zn (10-12%) in *M. balsamina* leaves were found to be high at early growth stages, but were reduced from fruit maturity stage up to physiological maturity by 84-91%, 154-173% and 81-229%, respectively. Notably, Fe was increased by 5% only, when the leaves were harvested at the bud development stage, but was reduced (6-508%) in all the other growth stages. Contrarily, Ca and Mg, were increased by 41-12033% and 101-2129%, respectively, from early vegetative stage throughout to physiological maturity. In the succeeding objective, the biochemical compounds, namely, total phenolic content (TPC), ascorbic acid (AA), and radical scavenging activity (DPPH) were determined. A highly significant ($P \leq 0.01$) effect was observed on the biochemical quality of *M. balsamina,* contributing 96, 98, and 97% to TTV in TPC, AA and DPPH, respectively. Relative to the vegetative stage, treatments increased TPC by 17% at the physiological maturity stage. However, in the rest of the tested growth stages, treatments decreased TPC by 25-40%. Harvesting at different growth stages also decreased AA concentration decreased by 32-58% at all the growth stages, when compared to the standard control. In contrast, the same treatments increased DPPH by 23-42% from the bud development, throughout to physiological maturity stage. In conclusion, harvesting at different growth stages influenced the nutritional and biochemical quality of *M. balsamina*. The nutritional and biochemical components evaluated where all adequately available at the vegetative and bud development stages.

Keywords: African leafy vegetables, antioxidant, Curcubitaceae, growth stages, health benefits, indigenous knowledge, leaf powder

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

In most African countries, especially South Africa (SA), the major burden of sicknesses is from infectious diseases associated with under development, poverty, poor nutrition, chronic diseases owing to a western type of diet and lifestyle, human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS), as well as the current Corona virus disease epidemic. Insufficient nutritional knowledge and awareness in many African diet often characterised by sufficient quantity and poor-quality nutrition has contributed to the present-day food choices (Steyn *et al*., 2006). Generally, leafy vegetables, amongst other vegetable groups, contain potential nutritional value. African leafy vegetable (ALV) species are regarded as providers of low-cost quality nutrition with lots of health benefits for many households in urban and rural areas (Chweya and Eyzagurre, 2007). However, a lot of ALV crops remain under-utilised in most parts of the country (SA) despite their nutritional and health benefits, on account of the idea that they are generally considered as a "poor man's crop'' mostly associated with poverty.

Momordica balsamina Linn., in the family Cucurbitaceae is an ALV commonly referred to as 'African pumpkin' or 'balsam apple', a perennial tendril bearing vine, native to the tropical regions of Africa (Shah *et al.,* 2014). The leafy vegetable holds a wide range of pharmaceutical properties and nutraceutical values. The leaves, fruit, seeds and the bark of the plant contain a satisfying amount of various antioxidant, anti-bacterial, antiviral, and hypoglycemic substances, and properties, including flavonoids, glycosides, steroids, glycoside, terpenes and saponins. The therapeutic agent 'Momordin' has been firmly established to be capable of inhibiting the growth of HIV/AIDS and other popular viruses (Thakur *et al*., 2009). The antioxidant quality of *M. balsamina* had been the most prominent in quality. Generally, antioxidants are substances that inhibit the process of oxidation in living organisms, which is potentially damaging, resulting in inflammation and diseases. Antioxidant categories include antioxidant vitamins (vitamin A, vitamin C and vitamin E), phytochemical antioxidants (phenolic acids including, flavonoids and carotenoids) and antioxidant minerals (Iron, Zinc, and Manganese (Aziz *et al*., 2019).

The vegetative parts of *M. balsamina* are known to be a salient source of nutrients, having seventeen (17) amino acids and substantial mineral composition such as Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Sodium (Na), Phosphorus (P), Zinc (Zn) and Manganese (Mn) (Hussan and Umar, 2006). The leaves of *M. balsamina* are also reported to contain higher protein, fibre, fat, and ash content values than those reported for the exotic vegetables, except for sodium (Thakur *et al*., 2009). *Momordica balsamina* leaves are recommended as protein supplement for cereal-based diets in poor rural communities, as they have been proven to contain more protein content compared to other popularly consumed leafy vegetables such as cabbage (*Brassica oleracea*, lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*) (Flyman and Afolayan, 2008). Simultaneously, its high mineral content, especially potassium, can be utilised to manage hypertension and other cardiovascular conditions. The satisfying levels of iron, manganese, and zinc could help manage micronutrient deficiencies (Thakur *et al*., 2009). More health beneficial opportunities concerning *M. balsamina* are being noticed, advances are tried, and further research is developed.

1.1.1 Description of the research problem

Momordica balsamina, holds a great deal of nutritional and medicinal properties, attributed to the presence of micro-nutrients and secondary metabolites (Thakur *et al.,* 2009) and it is highly utilised by elderly folks as a health supplement in their diet. Harvesting of this nutritive ALV plant starts from early vegetative growth stages and continuous up to physiological maturity, depending on the availability of the crop. However, it is currently unknown as to when and at what growth stage(s) does the plant's nutritive content(s) occurs in sufficient amount, necessary for meeting the required nutritive and pharmaceutical value when harvested. The plant's age at harvest is known to affect its nutritional composition (Lee and Kader, 2000) and the concentration of many oxidative of leafy vegetables (Adegbaju *et al.,* 2020), where some substances are found in abundance, insufficient or in excess at some growth stages than the others.

Medicinal plants play a major role in many rural communities over the world in the treatment and prevention of disease and the promotion of general health (Ngarivhume *et al*., 2015). Many medicinal herbs are therapeutic at one dose and toxic or ineffective at another (Khumalo, 2020). This is a result of the variations of concentrations of nutritional and phytochemical substances. This has persisted as a challenge in the realm of alternative medicine as there's very little scientific and medical evaluation to assess their efficacy (Khumalo, 2020).

1.1.2 Impact of the research problem

Variability in the composition of the plants due to the physiological growth stage of the plant may influences the composition of plant extracts from the same species. The variations of nutritional and biochemical composition cause the concentration of active ingredients in concoctions of medicinal plants to differ widely (Ngarivhume *et al*., 2015). This wide variation in the amount of the active ingredient ingested creates the possibility of subminimum doses (Ngarivhume *et al*., 2015). The absence of standardised dosages and the lack of scientific knowledge on toxicity and composition of active ingredients causes low acceptability and adoption of African traditional medicine into national health systems.

1.1.3 Possible causes of research problem

The concentration and level of nutrient elements and phytochemicals might vary depending on crop variety, phenological stage and environmental conditions (Modi and Mabhaudhi, 2006). Nutritional variation is a result of bioavailability of soil nutrients and the nutrient uptake ability that depends on the nutrient's demand for growth, determined by the role the nutrients play in plant growth (Makokha *et al.,* 2019), whereas phytochemicals accumulate with the age of the plant in order to protect the reproductive phase of the crop for the next generation of the plant. On the other hand, harsh environmental conditions during plant growth can influence the accumulation of phytochemicals within a plant (Bhat and Karim, 2009).

Momordica balsamina is an ALV that is consumed by local people as a nutritional vegetable known to prevent various ailments, while many traditional healers in local communities use the crop as an ingredient for remedies administered to patients with various health complications (Chauke *et al*., 2015). Traditional healers' knowledge of the medicinal uses of plants such as *M. balsamina* and the preparation of traditional concoctions was passed down from their parents and grandparents, which has ultimately led to the promotion of incorporating such potent herbs as a vegetable in daily diets. This has resulted in the sequence of knowledge and active use of medicinal plants to be mostly culturally based (Chauke *et al*., 2015).

1.1.4 Possible solutions of the research problem

Investigating the variations of nutrients and biochemicals in *M. balsamina* can contribute to improving its quality, effectiveness and safety for consumption as both a nutritional leafy vegetable and a medicinal plant. This will present knowledge to local community consumers, health shops and traditional healers who often collect *M. balsamina* that is found freely growing, near fences and in the wild, on the appropriate and most beneficial stage for consumption and healing purposes. Scientific validation of African medicinal plants may eventually lead to more widespread use of traditional medicines in affordable health care systems, as in India and China, provided that thorough toxicological investigations, clinical studies and randomized controlled trials are conducted. African traditional knowledge and medicine thus have the potential to play a large role in primary healthcare, particularly in poor and isolated rural areas (Ngarivhume *et al*., 2015), as well as in the proper informed harvesting stages of the plant at different developmental stages.

1.1.5 General focus of the study

The study focused on the evaluation of the nutritional and biochemical composition of *M. balsamina* harvested at different growth stages in order to determine the occurrence and accumulation of various nutrients and biochemical compounds at specific growth stages whereby consumption of the ALV may be safe and effective for consumers, as well as pharmaceutical uses.

1.2 Problem statement

Although *M. balsamina* is utilised for pharmaceutical and nutraceutical purposes in most rural communities of SA, where a strong belief in its safety and effectiveness that is mostly culturally based still exist (De Wet *et al*., 2016), for centuries, like any other indigenous African plant, it had been informally utilised without proper scientific evidence (Awuchi, 2019). Also, minimal research has been conducted on *M. balsamina* as a possible cash crop and other important ALVs to address food and health security. Incidentally, *M. balsamina* contains functional nutritional and biochemical properties in its fruit, leaves, and roots. Nutritionally, the leaves and green fruit are cooked and eaten as a relish and medicinally, the infusions of this plant are used for intestinal and abdominal ailments (Hutchings *et al.,* 1996).

Again, most ALVs are harvested from an early growth stage to physiological maturity without empirical evidence of the crop's useful nutritional and biochemical properties in every growth stage. The harvestable stage is among the important factors that influence vegetable crops' nutritional and biochemical quality (Adebiyi and Oluwalan, 2019). Generally, the levels of nutritive and anti-nutritive substances in ALVs are influenced by the stages of plant development and hence the stages in which they are harvested (Maseko, 2018). In order to achieve advances and further develop the *M. balsamina* crop for nutraceutical and pharmaceutical purposes, one should consider that the plant part and the stage of harvest be investigated to contribute to the optimal dosage in terms of safety and efficiency (Bortolotti *et al*., 2019). As a result, it is of utmost importance to evaluate the nutritional and biochemical qualities of *M. balsamina* in relation to harvesting stages for proper documentation.

1.3 Rationale

Nutrition is important for the proper standing of life for every human being. Researchers, governments, and different organizations are deeply involved with the overall population's nutritionary standing, particularly children and pregnant mothers in developed and developing countries (Seena *et al.,* 2005). To alleviate nutritional impoverishment, true efforts should be targeted on exploiting under-exploited indigenous plants as nutrient supplement sources (Ogle *et al.,* 2001). The nutritional and biochemical properties of *M. balsamina* play a significant role in fighting against malnutrition and ensuring food security in most African countries, including SA (Chipurura, 2010). For most ALVs, there is an ideal stage of plant growth found most favourable for human consumption in terms of palatability and flavour, with levels of nutrients and biochemical substances varying with the plant's developmental stages (Maseko, 2018). Flyman and Afolayan (2008) suggested that maturity phases of *M. balsamina* leaves partakes a substantial impact on the plant leaves mineral levels, wherein their study earlier stages of the plant presented good quality. Therefore, since *M. balsamina* leaves seem to be rich in health-beneficial components, in this study, the researcher intends to evaluate the nutritional and biochemical composition of *M. balsamina* leaf powder at different harvestable stages.

1.4 Purpose of the study

1.4.1 Aim

The study aims to establish the nutritional and biochemical composition of *M. balsamina* dried leaf powder for nutraceutical purposes based on the harvesting strategy of the plant.

1.4.2 Objectives

1.4.2.1 To determine whether the nutritional composition of *M. balsamina* leaf powder will be influenced by harvesting at different growth stages of the plant.

1.4.2.2 To evaluate whether the biochemical composition of *M. balsamina* will be affected by harvesting at different stages of the plant.

1.4.3 Hypotheses

2.3.1 Harvesting at different growth stages of *M. balsamina* will not have an influence on the nutritional composition of its leaf powder.

2.3.2 Harvesting at different growth stages of *M. balsamina* will not have an influence on the biochemical composition of its leaf powder.

1.5 Reliability, validity and objectivity

The reliability of data was based on statistical analysis of data at the probability level of 5%, objectivity was achieved by ensuring that the findings were discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was reduced through minimising the experimental error by increasing the number of replications on the experiments conducted. The treatments were also randomised within the selected experimental design (Leedy and Ormrod, 2005).

1.7 Scientific contributions

Momordica balsamina has proven to be a very potent African herb. The outcomes of this research and future development of the product will contribute significantly to the nutraceutical and pharmaceutical industry and other end-users. The World Health Organization (WHO) recognises the benefits of alternative medicine, especially of traditional origin. However, it concerns the safety of the medicine and therefore encourages thorough research for scientific backing. This research could contribute information on the correct and safe harvesting stage of *M. balsamina*, for safe and efficient prescriptions of traditional medicinal treatments, daily consumption, and commercial production. The leaf powder product if developed, will be beneficial for nutrition, wellness programs and culinary use.

1.8 Structure of the mini-dissertation

The research problem of the study was introduced in Chapter 1, the work done, and the work not done on the problem statement being reviewed in Chapter 2. The research related to the objectives was addressed in Chapter 3 and Chapter 4, whereas the summary, significance of the findings, recommendations with respect to future research and with the conclusions that were intended to provide a take home message regarding the current study are in Chapter 5. The citation and references followed the Harvard style of author-alphabet as approved by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

African leafy vegetables (ALVs) refer to the collective of traditional leafy vegetables that form part of the culinary repertoire of contemporary African communities, while leafy vegetables refer to plant species of which the leafy parts, which may include young succulent shoots and leaves, flowers as well as young fruit, are used as a vegetable (Van Jaarsveld, 2014). The intake of traditional vegetables in Sub-Saharan Africa (SSA) has been advocated for by the World Health Organization's (WHO) global initiative program due to their non-nutrient bioactive compounds that possess health-promoting and protective properties. As a result, the use of traditional vegetables to combat specific nutrient deficiencies and sustain safe food has been researched intensively in South Africa (SA) (Sivakumar *et al.,* 2018).

For centuries, indigenous and traditional vegetables have been recognised to hold several agronomic advantages over their exotic counterparts, including superior adaptation to local environmental conditions and limited requirements for expensive external inputs, such as irrigation and agrochemicals. However, with ample research done on the cultivation practices of these crops, limited information is available on production systems, including optimum plant and harvest times, spacing, propagation, and harvest methodology (Uusiku *et al*., 2010).

For several decades, ALVs have been consumed in African diets (Fransisca and Eyzaguirre, 2007). Mooketsi, (2019) presented the status of consumption of ALVs in various African countries in which they are prominent. Cameroon has a wide range of African vegetables, including ALVs that are found in lowlands and highlands. Often, they are not available for consumption as crop production practices and pricing structures are a limitation. In Uganda, the agroclimatic conditions are suitable for a wide range of ALVs. They are mostly consumed seasonally, while only abundant in rainy seasons (Mooketsi, 2019). In Nigeria, it has been established that consumption of ALVs is high as wild vegetables are available throughout all seasons, including dry seasons. The majority of ALVs in Nigeria are harvested from the wild and play an economic role (Mooketsi, 2019). In Kenya, consumption is said to be limited because of the fear that ALVs can be toxic, despite their nutritional and agronomic advantages (Mooketsi, 2019). It is believed that in Ethiopia, rural people are endowed with knowledge concerning the use of ALVs, some of which are ensured to be consumed even during times of drought (Mooketsi, 2019).

In SA, there is a decline in consumption of ALVs partly because of low availability and poor perception. Low availability is because production continues to be on a small scale and they are considered wild species hence have never been domesticated nor formally commercialised. The rare presence of ALVs in retail stores has greatly contributed to their reduced consumption. This is due to decreased availability and their low status among some SA community (Maseko *et al*., 2017). Despite official statistics indicating a low consumption level of vegetables in Sub-Saharan Africa, it appears that African leafy vegetables are usually consumed with the staple food in various forms. (Kamga *et al*., 2013).

2.2 Work done on the problem statement

2.2.1 The existence of African leafy vegetables

For millennia, ALVs have been sourced and collected as weeds, predominantly in the wild and disturbed places (Jansen Van-Rensburg *et al*., 2007). Currently, most ALVs have been sourced from agricultural fields, near homes, disturbed places, homesteads, and some in livestock kraals (Olouch *et al*., 2009). A lot of ALVs are often not actively planted and not only are they collected from the wild, but they are also nurtured and maintained in agricultural lands and homesteads once they are discovered. The statement was supported by High and Shackleton (2000), reporting that over one-third of total edible plants in homestead plots of common areas in South Africa, such as Bushbuckridge (Mpumalanga province of SA), were from species that are freely growing. However, the ratio of collected ALVs to cultivated ones may vary from place to place.

According to Vorster (2009), the cultivation of a few selected species is based and ranked according to different criteria. The criteria could be divided into a taste or a food security preference. Included in the taste preference rankings were taste and ease of access for harvesting. The food security criteria used included aspects like how many products the plant supplied (leaves/fruit/seed), how long it is available in the new form, and the ability to store for an extended period. African leafy vegetable production has its advantages because of its uniqueness, such as short production cycles, the requirement of a few purchased inputs, thrives in poor soil, are resistant to pests and diseases, and are pretty acceptable to local tastes. In addition, ALVs are well suited to the small plots and limited resources of rural families and produce high yields with substantial nutritional value (Mwaura *et al*., 2013).

2.2.2 Domestication of various important edible African leafy vegetables

According to Bettinger *et al.* (2012), wild plant domestication brings into the cultivation of a wild plant, involving continuum steps of gathering, tendering, and cultivation to domestication. In Sub-Saharan Africa, many plants are freely growing in the wild or as weeds in areas of continuous cultivation. Several of these plants of interest have been domesticated through semi-cultivation or cultivation because when domesticated, they require few inputs and tend to grow and produce in areas where the cultivation of conventional vegetables is challenging (Van Rensburg *et al.,* 2004). The domestication of traditional leafy vegetables in West Africa has been commonly attributed to enabling easy access, having reliable vegetable sources throughout the years, and the potential extinction of ALVs from wild habitats due to current human activities of influence (Dansi *et al*., 2012).

Dansi *et al.* (2008) extensively outlined the different steps that exist in the plant domestication process, which include species are entirely wild and collected only when needed and wild species are maintained in the fields when found during land preparation (clearance, burning, and weeding) due to its proven utility and everyday need, its scarcity around habitations, and the difficulties for getting it on time, in quality, and in quantity. The author further alluded that these preserved plants are subject to regular observations for the understanding of their reproductive biology, whereby farmers start paying more attention to the preserved plants (weeding, protection against herbivorous) for their survival and average growth. A sort of ownership on the plants starts, with the reproductive biology of the species being known, and multiplication and cultivation of the species in the home gardens or in selected parts of cultivated fields are undertaken by farmers or (date of planting, sowing, or planting density, pest, and diseases management, etc.) in order to master mass production of the species in the future. The ownership of the plant is more rigorous, since the species is cultivated and harvested using traditional practices, in order to improve the quality of the product, farmers adopt specific criteria to select plants that better satisfy people's needs. The best cultivars/plants (good grain/fruit quality, resistance/tolerance to diseases and pests) are known, and a technical package is adopted for their development and mass production. At this stage, access to market is considered, and some species benefit from traditional postharvest technologies (method for processing, cooking or conservation, etc.) to meet consumers' needs and finally selection initiatives continue with cooking qualities, protection against pests and diseases in cultivation and storage. Income generation is more clearly taken care of; market demands (quantity and quality) are also taken into account, and species; varieties that meet consumers' preferences are selected (Dansi *et al.* (2008).

Van Zonneveld *et al.* (2021) summarised domestication steps from various literature into three categories: wild, semi-domesticated, and domesticated. Broadly, the term "wild" is applied when a species is predominantly harvested from natural stands; "semi-domesticated" when a species is both widely harvested from natural stands and is insignificant "cultivation"; and "domesticated" when a species is principally cultivated.

Spider plant *(Cleome gynandra* L.) is semi-domesticated in East Africa. It is mainly cultivated by subsistence farmers. It is semi-domesticated in that farmers collect seeds from volunteer plants and propagate them for home consumption and, in some cases, for sale in local markets (Chweya and Eyzaguirre, 2007). In eastern and southern Africa, the species is cultivated and sold in urban markets and supermarkets (Schippers, 2002) but also grows as a weed. In SA, the species is still regarded as wild (Sowunmi, 2015). In some rural parts, such as Limpopo province, *C. gynandra* is collected from the wild, cultivated and sold (Faber *et al*., 2010). in Zimbabwe, it grows as a weed. It is not formally cultivated as a commercial crop but for years it has been a semi-domesticated volunteer crop in farmers" fields near and around most (Masuka and Mazarura 2012). In West Africa, the species are spontaneous and widely collected and cultivated by local communities (Houdegbe *et al.,* 2018).

Most of the *Amaranthus* (Pig weed) species are harvested in the wild to be consumed as a food source. However, only a few are cultivated, and are among the leafy vegetable types most commonly sold at markets in tropical Africa (Achigan-Dako *et al.,* 2014). In SA, Amaranth is cultivated, but it mostly freely germinates as a volunteer crop after the first rains. It is harvested from the wild and domesticated through the regeneration of fallen seeds from the previous year's plants (DAFF, 2010). When practicing selective weeding, ALV species such as Amaranth are treated as crops and are allowed to grow without being disturbed. Of all the weeds that regarded as leafy vegetables in SA, Amaranth is part of the group of species that have the potential to be developed as crops. (Van Rensburg *et al*., 2007).

Balsam pear (Momordica charantia) originates from Africa, expanded and domesticated in South-east Asia. Origin of *M. charantia* in Africa and natural dispersal to Asia was inferred in molecular phylogenetic work that included all 60 species of Momordica, most of which are endemic in Africa. *Momordica charantia* grows in the wild in Africa, and the initial domestication was dated to 6,000 years ago, followed by the separation of further cultivars 800 years ago (Renner, 2020). The regions of eastern India and southern China have been suggested as possible centres of domestication (Walters and Decker-Walters, 1988).

Blackjack *(Bidens pilosa*) in West Africa is reported to still be harvested from the wild (level 0 of domestication) by the majority, while only 22.22% of the same population reported that *B. Pilosa* is being cultivated (level 4 of domestication) (Sanoussi *et al*., 2015). Wild jute *(Corchorus olitoriusis)* found near rural farms in some South African provinces (KwaZulu-Natal, Eastern Cape, Limpopo, and Mpumalanga). The vegetable is commonly used in traditional medicine to treat a cold, tumour, pyrexia, and persistent bladder infection is said to be one crop that has been domesticated through regeneration of the seeds fallen from the previous year's plants. (Dansi *et al.,* 2012). It is classified to be in the early stages of domestication, which suggests that there is an awareness of the food value and a sense of ownership of the crop (Nyadanu *et al.,* 2017). The domestication of *C. olitorius* remains challenged by pests and diseases presently observed among subsistence growers In West Africa, the species has been reported to be domesticated for several decades, and it is most popular among the western population of Nigeria. It is, however, still mainly collected from the wild in southern Africa (Mavengahama and Lewu, 2012).

2.2.3 Cultivation attempts of African leafy vegetables

In Sub-Saharan Africa, ALVs are actively sown and are considered as cash crops (Enoch *et al*., 2020). Generally, seeds for cultivation purposes are collected from the previous season's crop of the African indigenous vegetables of preference, including ALVs, whereby seeds are stored for later use. In most cases, to ensure an abundance of the crop, during rainy seasons, stored seeds are scattered along the outskirts of agricultural land or against fences for climbing species such as *M. balsamina.* However, lack of proper quality seeds has remained a constraint in hindrance to the commercial production of ALVs and the success of the crop (Olouch *et al.,* 2009). It has been observed that several species have low seed germination rates as a result of poor seed quality, seed dormancy, and improper seed processing (Abukutsa-Onyango, 2005). For instance, the poor germination of *S. scabrum* has been attributed to improper seed extraction, resulting in inadequate removal of sugars and germination inhibitors.

Sun-drying of *C. gynandra* seeds was reported to improve the overall germination compared with shade-dried seeds (K'opondo *et al.,* 2005) and, along with *C. olitorius* have been reported to show poor germination and dormancy problem (Schippers, 2002). Seeds ought to be treated before sowing. Seed production at the farm level is very often of poor quality. This follows poor/ non-positive seed selection, as species isolation distance for the main crop, resulting in cross genetically mixed succeeding plants resulting from unprevented cross-pollination (Diouf *et al*., 2007). Seeds require to be dried for a few days (four to five), sealed in packages, and stored (Abukutsa-Onyango, 2005). Different vegetable seeds' stability differs even under similar conditions, where some need to be stored longer than others. Such as in the instance of freshly harvested seeds of *C. gynandra*, which require six months to one year to ensure the success of breaking physiological dormancy.

2.2.4 Commercialisation and marketisation of African leafy vegetables

Commercialisation in agriculture refers to the production of crops for sale in the market rather than for family consumption, whereas marketisation of agricultural products refers to the surplus of the produced crop after consumption (Mahaliyanaarachchi and Bandara, 2006). Commercialisation depends on the utilisation frequency, which drives the marketisation and scale of food production. Studies have attempted to quantify the frequency of utilisation of ALVs in SA. Van Rensburg *et al.* (2007) have reported the variable utilisation of indigenous leafy vegetables favouring exotic vegetables. This was attributed to the limited availability as they are not cultivated but mostly gathered from cultivated fields or the wild (Maseko *et al.,* 2017). Low levels of utilisation are owing to the western influence that has modified present food consumption patterns (Hassleman and Sizane, 2006) and general loss of indigenous knowledge, supporting that the present generation lacks sufficient knowledge of wild species collection from the wild (Mavengahama *et al*., 2013).

The general awareness of the nutraceutical and pharmaceutical value has led to an upsurge in demand for ALVs (Mwangi and Mumbi, 2006). In Nairobi, the consumption of African vegetables increased from 131 to 600 tonnes in recent years. Unfortunately, the supply of these vegetables has not kept pace with the increasing demand (Wafula *et al*., 2016). Production, marketisation, and utilisation of ALVs are interrelated and seem to influence the intensity of each other. Their expansion in production, marketing, and consumption could be attributed to increasing consumer awareness about their health and nutritional benefits (Schippers, 2002). High marketing returns have motivated the commercialisation of ALVs by small-scale farmers, who produce and supply the crop either individually or collectively in groups (Gido *et al.,* 2017).

Despite the rich content of nutrients they possess, the consumption of ALVs had shown a decline due to the introduction of exotic vegetables such as cabbages (*Brassica oleracea*), spinach (*Spinacia oleracea*), and lettuce (*Lactuca sativa)*. Several factors have resulted in the popularity of ALVs: poor seed distribution, postharvest handling, and marketing. On the other hand, the diversification in agro-bio has been shown to improve and contribute to livelihoods and food security, and African indigenous vegetables are a good candidate because of their agronomical advantages (Aleni, 2018).

2.2.5 Nutritional value of African leafy vegetables

The utilisation of plants for the provision of essential nutrients aimed at improving and maintaining human health has always seen a rapid increase through much of human history due to existing information, and cultural practices passed down to generations. Indigenous leafy vegetables play a significant role in improving the nutrition and health conditions of humans owing to their nutrient-dense properties in comparison to familiar vegetables (Van *et al*., 2004). Vegetable plants are generally good sources of fibre, water, vitamins, minerals, amino acids, and carbohydrates. African leafy vegetables (Figure 2.1) grown naturally in the sub-Sahara African environment have received considerable attention in the past decade as an inexpensive source of traditional and essential nutrients such as proteins, fats, oil, minerals, and vitamins present in varying quantities among species and families for the maintenance of the human health (Oboh *et al.,* 2017; Otang-Mbeng and Mashabela, 2020). More so, these ALVs are highly nutritive and form an important part of the human diet to reduce malnutrition, especially in rural communities. Information on the nutritional value of leafy vegetables can increase their consumption to help reduce nutrient deficiency diseases (Fabbri and Crosby, 2016).

Figure 2.1**:** Images of six African leafy vegetables

2.2.6 Nutritional value comparison of several important African leafy vegetables

For decades, *M. balsamina* had been consumed as a vegetable food in various countries such as SA, Sudan, and Cameroon (CABI, 2020). The young fruits, shoots, and leaves contain high quantities of essential minerals and antioxidants when prepared and consumed as leafy vegetables (CABI, 2020). In rural communities, the consumption of *M. balsamina* is being encouraged to supplement protein and they are cooked alone or mixed with groundnuts (*Arachis hypogaea*) (Behera *et al*., 2011; Thakur *et al.,* 2009). The leaves are an important source of essential nutrients, including potassium, magnesium, phosphorus, calcium, vitamins A and C, iron, amino acids, manganese, zinc, and sodium (Table 2.1). Its high potassium content is beneficial for managing cardiovascular diseases such as high blood pressure (Thakur *et al.,* 2009).

Cleome gynandra is rich in protein, manganese, sodium, calcium, iron, vitamins A and C (Table 2.1). In most African countries, the leaves are boiled and used for stew and side dishes. Moreover, the fresh leaves are consumed in combination with crushed meals and the dried leaves are pulverised and added to lactating or weaning foods. The vegetable is beneficial to pregnant and breastfeeding women (Van de and Venter, 2006). In specific communities, the leaves are boiled and soaked in sour milk for about 72 hours and consumed to improve vision and provide energy. *Cleome gynandra* leafy vegetable consumption among all age groups is often limited by its inherent bitter taste attributed to the existence of the chemical compound, proanthocyanidins, commonly known as condensed tannins, located in the vegetative parts of the plant (Ramphele *et al*., 2020). Previous work on *C. gynandra* showed high beta-carotene content and low fatty acids (Mibei, 2013). Also, *C. gynandra* and Amaranth *spp.* are low in fiber, protein, and fat (Matenge *et al.,* 2017), comparable to studies reported by Uusiku *et al*. (2010). A further study conducted by Sowunmi (2015) reported high amounts of potassium, sodium, calcium, zinc, and iron for *C. gynandra*.

Amaranth leafy vegetables are commonly found in most rural areas in SA where the tender shoots and leaves are eaten raw or boiled, used in soups in combination with other vegetables considering its high calcium, phosphorus, iron, and moisture content (88.98% - 90.35%) (Njeme *et al*., 2014; Olumakaiye, 2011)

. Previous studies reported a high dry matter protein content (17.5 to 38.3%), folate, riboflavin, and vitamin C in *Amaranthus spp.* (Table 2.1) (Mnkeni *et al.,* 2007; Njeme *et al.,* 2014).

The leaves of *B. pilosa* are known to have vitamin E, beta-carotene, fibre, and antioxidants (Table 2.1). According to the United Nations Food and Agriculture Organization, Bartolome *et al.* (2013) reported the nutritional value of *B. pilosa* with a high amount of calcium, moisture, and carotene. Together with different leafy vegetables, this vegetable is consumed in some rural areas in SA (Njeme *et al.,* 2014). The plant has been studied to possess blood pressure-reducing properties attributed to its fibre, potassium, magnesium, calcium, vitamin C, zinc, iron, and copper contents (Gavhi, 2019), compared to *C. gynandra* and *M. balsamina.*

Corchorus olitorius is a leafy vegetable consumed as food, especially in tropical and subtropical regions in Africa and Asia, as well as SA. It contains protein, fibre, vitamins, calcium, sodium, phosphorus, iron, and potassium (Choudhary *et al*., 2013). An earlier study by Legwaila *et al.* (2011) reported that *C. olitorius*, *Amaranthus ssp*, and *C. gynandra* contained significant amounts of protein, calcium, beta-carotene, and vitamin C when compared to conventional vegetables like *S. oleracea* and *Brassica oleracea*. A previous study revealed that the *C. olitorius* plant contained a higher amount of magnesium when compared to conventional vegetables such as *S. oleracea* and *B. oleraceae*. However, there was no significant difference in wild jute and spinach protein content even though the protein and fibre content of wild jute was higher than those found in cabbage (Ndlovu and Afolayan, 2008). The vegetable is also known to contain a high amount of thiamine (B_1), riboflavin (B_2), vitamin E, calcium, iron, fibre, and carotenoids (Musa and Ogbadoyi, 2012; Schippers, 2002). Moreover, a nutritional analysis conducted on six species of Corchorus, found wild jute to possess high amounts of crude protein, potassium, and beta-carotene except iron compared to others (Choudhary *et al.,* 2013) (Table 2.1-Table 2.2).

A study has shown the leaves and fruits of *M. charantia* to be high in carbohydrates compared to the amount present in the seed (Bakare *et al*., 2010). Similarly, calcium was found to be the highest mineral present in the leaves with a low amount of vitamins A, C, E, B_9, and cobalamin. In contrast, a minimal amount of vitamin A, D, K, niacin (B_3), and pyridoxine (B_6) were present in the methanolic and petroleum ether extracts of the leaves. According to Ayeni *et al.* (2015), the leaves of *M. charantia* contain a low quantity of sodium (6.58mg/100g) and magnesium (5.88mg/100g) and a high amount of phosphorus (24.36mg/100g), potassium (32.84mg/100g), and calcium (22.36mg/100g) (Table 2.2).

2.2.7 Phytochemical value of African leafy vegetables

The presence of naturally occurring bioactive compounds known as phytochemicals in ALV is mainly responsible for plants' intrinsic medicinal properties. They are secondary metabolites in plants with no nutritive value but possess a wide range of remarkable preventive and therapeutic properties (Patra, 2012). The phytochemicals play a defensive role in plants against pathogens and herbivore-generated attacks, aiding them to strive in extreme conditions (Kennedy and Wightman, 2011). Also, they are useful in drug discovery for the treatment of several diseases, food fortification and bring to light the significance of the traditional system of treatment (Mojab *et al.,* 2003). Furthermore, Moyo *et al.* (2012) reported that the health benefits of consuming leafy vegetables owe to the presence of phytochemicals, including vitamins and proanthocyanins. These bioactive constituents from plants exert their health improving, disease-preventing, and therapeutic effects in isolated or combined forms with other phytoconstituents to bring about health benefits (Fawzi, 2013).

Interestingly, medicinal plants, owing to their phytochemicals, have gained relevance in the healthcare sector, with about 40% of medicines being produced from them (Babalola and Shode, 2013). The common phytochemicals with extensive pharmacological activities found in different parts of plants, as well as indigenous leafy vegetables at different concentrations and quality depending on the plant harvesting time, including flavonoids, alkaloids, lignans, terpenoids, phenolic acids, tannins, lignins, and saponins (Weldegerima, 2009). Previous studies have shown that tannins, flavonoids, and saponins possess antioxidant and anti-microbial activities, while alkaloids possess anti-hypertensive, anti-plasmodial, and anti-bacterial properties (Mallikharjuna *et al.,* 2007; Stephen *et al*., 2009). These phytochemicals present in indigenous leafy vegetables (Table 2.3), can inhibit protein digestion, and prevent cardiovascular diseases and cancers (Mibei, 2013).

Table 2.1: Nutritional proximate composition of six selected African leafy vegetables

Nutrients	*Balsam apple* *(M. balsamina)*	*Spider plant* *(C. gynandra)*	Pigweed (*Amaranthus ssp.*)	Wild jute *(C. olitorius)*	Blackjack (*B. pilosa*)	*Balsam pear* *(M. charantia)*
Ash	127.0±17 mg	3.0±0.04 %	1.90±0.06 %	5.58 %	2.2 g	15.42±2.08 mg
Fibre	37.2±7.9 mg	1.59±0.4 %	1.84±0.50 %	–	3.9 g	3.31±1.25 mg
Carbohydrate	32.5±0.8 mg	–	–	19.56 %	8.4 g	32.34±0.24 mg
Fat	53.7±8.6 mg	0.66±0.03 %	0.88±0.10 %	11.99 %	0.5 g	3.68±0.68 mg
Protein	287.7±1.8 mg	6.42±0.41 %	5.60±0.10 %	20.72 %	3.8 g	27.46±1.60 mg
Moisture	3.77±0.22 mg	88.8±0.10 %	84.1±0.10 %	83.06 %	85.1 %	17.97±1.00 mg

Table 2.2: Nutritional mineral composition of six selected African leafy vegetables

Nutrients	*Balsam apple* *(M. balsamina)*	*Spider plant* *(C. gynandra)*	Pigweed (Amaranthus *spp.)*	Wild jute *(C. olitorius)*	Blackjack (*B. pilosa*)	*Balsam pear* *(M. charantia)*
Calcium	2.22±0.50 mg	33-288 mg	480 mg	30.55 mg	1354 mg	20510±5.77 ppm
Phosphorus	3.24±0.01 mg	–	–	6.68 mg	504 mg	312.3 ± 44.8 mg
Iron	0.14±0.01 mg	2.9 mg	10 mg	19.53 mg	17 mg	98.00±0.23 mg
Zinc	0.39±0.01 mg	0.8 mg	3.46 mg	4.71 mg	22 mg	120.00±1.15 mg
Sodium	0.06±0.02 mg	–	–	54.56 mg	290 mg	2200.00±1.15 mg
Potassium	27.05±0.27 mg	–	1598 mg	6.68 mg	–	413.00±1.45 mg
Magnesium	3.82±0.06 mg	76 mg	470.9 mg	76.69 mg	658 mg	255.00±0.69 mg
References	Flyman and Afolayan, 2007; Jonathan *et al*., 2020	Matenge *et al.,* 2017 ; Njeme *et al*., 2014	Kachiguma and Matenge et al., 2017; OtangMbeng and Mashabela, 2020	Idris et al., 2009; OtangMbeng and Mashabela, 2020;	Njeme *et al*., 2014; Xuan and Khanh, 2016;	Bakare et al., 2010; Zhang *et al*., 2009

Table 2.3: Photochemical constituents of six African leafy vegetables

Phytochemicals	Balsam apple *(M. balsamina)*	Spider plant *(C. gynandra)*	Pigweed (*Amaranthus spp.*)	Wild jute *(C. olitorius)*	Blackjack (*B. pilosa*)	Balsam pear *(M. charantia)*
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	–	+	+	–	+
Terpenoids	–	+	–	–	+	+
Phenols	–	+	+	+	–	+
Saponins	+	±	+	+	+	+
References	Abubakar et al., 2018 ; Karumi *et al*., 2004.	Mibei *et al*., 2012	Ahmed et al., 2018 Martinez *et al*., 2013;	Mibei *et al*., 2012	Okoli *et al*., 2009	Daniel *et al*., 2014

Key: + Present, – absent, ± unclear.

Generally*, Momordica species* contain phytochemicals such as alkaloids, saponins, glycosides, anthraquinone, resins, flavonoids, steroids, carbohydrates, and terpenoids with disease prevention and therapeutic effectiveness (Nagarani *et al.,* 2014; Thakur *et al.,* 2009). Previous studies have suggested that the antioxidant properties of plants are due to the presence of phenolic compounds and flavonoids, which can bind to metals involved in the production of free radicals, mop off free radicals, and protect the antioxidant defense system of the body (Baba and Malik, 2014).

Like other *Momordica* species, *M. balsamina* possesses flavonoids, terpenoids, cardiac glycosides, saponins, and steroids. The methanolic extract of *M. balsamina* has been found to contain flavonoids, tannins, coumarins, phenols, terpenoids (Souda *et al.,* 2018). These phytochemicals are comparable to those reported for other species of *Momordica*. A report by Thakur *et al.* (2013) reported that the anti-microbial activity of *M. balsamina* was due to the presence of tannins and flavonoids. These phytochemicals exert their effects by disabling bacterial-bonding enzymes and cell envelop proteins by forming a complex with starches and proteins. A class of defense proteins known as 'balsamin' isolated from the methanolic extract of *M. balsamina* has been found to possess anti-microbial activity (Ajji *et al.,* 2016; Kaur *et al.,* 2012).

Additionally, eleven phenolic compounds with antioxidant properties have been isolated from *M. balsamina* (Olalere and Gan, 2020) (Table 2.4).

Table 2.4: Antioxidant phenolics extracted from *Momordica balsamina*

Compounds	Chemical formula	Adducts	References
Yakuchinone A	$C_{20}H_{24}O_3$	–H	Chun *et al*., 2009
Methyl-5-O caffeoylquinate	$C_{17}H_{20}O_9$	–H	Jayasinghe *et al.,* 2012
Agrimol E	$C_{33}H_{38}O_{12}$	–H	Yin *et al*., 2011
Decaffeoylacteoside	$C_{20}H_{30}O_{12}$	–H	Kim et al., 2009
Kukoamine A	$C_{28}H_{42}N_4O_6$	–H	Chahel *et al.,* 2019
Geraniin	$C_{41}H_{28}O_{27}$	+HCOO	Yeh *et al.,* 2019
1,2,3,6-Tetra-O-galloyl-*β*Dglucopyranoside	$C_{34}H_{28}O_{22}$	–H	Zhou *et al.,* 2019
Mallotinic acid	$C_{34}H_{26}O_{22}$	–H	Yang *et al*., 2015
Terchebin	$C_{41}H_{30}O_{27}$	–H	Richman *et al.,* 1996
Laevigatin A	$C_{34}H_{26}O_{23}$	+HCOO	Fecka *et al*., 2015
1-O-Galloylpedun-culagin	$C_{41}H_{28}O_{26}$	+HCOO	Tanimuru *et al.,*2005

Momordica charantia, due to its various phytochemical components, had been used in traditional medicine for primary healthcare in different parts of the world (Bortolotti *et al*., 2019). The plant contains essential bioactive compounds such as alkaloids, tannins, saponins, terpenoids, carotenoids, and flavonoids which are comparable to those found in *M. balsamina* and other leafy vegetables which possess pharmacological efficacy against several diseases such as diabetes mellitus, dementia, hypertension, Alzheimer's disease, and erectile dysfunction (Mukit *et al.,* 2018; Oboh *et al*., 2017). Ayeni *et al.* (2015), reported the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides in the plant's leaves, where the quantitative analysis showed alkaloids as the highest compound present. More so, a previous work that chemically characterised the extract of *M. charantia* leaves using an advanced standard technique such as high-performance liquid chromatography (HPLC) revealed the presence of phenolic compounds such as catechin, kaempferol, ellagic acid, gallic acid, rutin, quercetin, and isoquercitrin which exhibited antioxidant and cardioprotective properties (Saliu *et al.,* 2019). These plant-constituents, either in extracts or isolated forms, exert their pharmacological effects by inhibiting the activities of enzymes (such as arginase, phosphodiesterase-5, α-amylase, monoamine oxidase, α-glucosidase, butyrylcholinesterase), and scavenging free radicals (Oboh *et al.,* 2017). Prior studies on *M. charantia* have revealed the presence of bioactive compounds such as charantin (steroid), polypeptide-p, and vicine which may, however, exert their hypoglycemic effects by stimulating the secretion of insulin and modifying carbohydrate metabolism for the effective control of blood glucose level in the human body (Krawinkel and Keding, 2006; Kumar *et al.,* 2010). Further, studies have shown that tetracyclic triterpenoids such as lanostane, protostane, cucurbitane frequently used in the management of diabetes are present in *M. charantia* (Putta *et al.,* 2016).

The leafy vegetable *C. gynandra* had been found to contain ferulic, vanillic, and chlorogenic acids, as reported by Matenge *et al*. (2017). Furthermore, a qualitative phytochemical analysis was conducted using four samples of *C. gynandra* (fresh, shadow, sun, boiled). All samples contained flavonoids, terpenoids, alkaloids, and amino acids whereas, saponin was only present in the fresh sample and was comparable to those found in *M. charantia* (Mibei, 2013).

Ahmed *et al*. (2013) conducted a qualitative study on a species of Amaranth (*Amaranthus Viridis*), revealing the presence of tannins, flavonoids, and cardiac glycosides in the leaves and seed extract, while a quantitative analysis revealed the presence of high amounts of saponins, tannins, alkaloids and cardiac glycosides in the leaves compared to the seed extract. Furthermore, Matenge *et al.* (2017) found *Amaranthus spp.* to be extremely high in phenolic content compared to *C. gynandra*. Other phytoconstituent found include ferulic acid, 3,4-dihydroxbenzoic, chlorogenic acid.

The extensive medicinal and nutraceutical properties of *B. pilosa* used in the treatment of more than forty diseases, including diabetes, had been attributed to its phytochemical constituents (Bartolome *et al*., 2013). It is widely used in SA to treat ulcers (mouth and stomach), ear infections, headaches, and renal disorders (Gavhi, 2019). About 201 bioactive compounds, including various flavonoids, aliphatics, aromatics, terpenoids, chalcones, okanin glycosides, phytosterols, fatty acids, phenolic acids, aurones, porphyrins, and phenylpropanoids, have been found and isolated from this plant (Silva *et al.,* 2011; Xuan and Khanh, 2016). These bioactive compounds in isolated forms, extracts, or in combination with other compounds have shown anti-microbial, antioxidant, anti-inflammatory, anti-diabetic, and anti-cancer properties. A review by Islam (2013), reported that the aqueous and hexane leaf extracts of *C. olitoriusis* possessed significant antioxidant activities due to high total polyphenols, ascorbic acid, total flavonoids, carotenoids, and phenol contents. It has also shown anti-tumor activity attributed to the presence of galactolipid (Islam, 2013).

2.2.8 *Momordica balsamina* L. as a leafy vegetable

2.2.8.1 Origin and distribution of *Momordica balsamina*

Momordica balsamina L. (Cucurbitaceae), also known as ‘balsam apple’ or ‘bitter melon’, is native to the tropics of Africa but is now invasive in Australia, Asia, Europe, and the central parts of America (MWNH, 2004; Tropical Plants Database, 2020d). It grows freely in southern African regions such as Botswana, Swaziland, Namibia, and South Africa. Duenas-Lopez, (2019) suggested that this crop is native to all parts of Africa, with the exception of Chad, and that it is a widespread crop mainly SA and

Eswatini (Table 2.5- Table 2.6). It grows in Limpopo, KwaZulu-Natal, the Eastern and Northern Cape provinces of SA.

Table 2.5: Distribution table of *Momordica balsamina* across Africa (Duenas-Lopez, 2019.)

Country/Region	Distribution	Origin	References
Angola	Present	Native	POWO (2020)
Benin	Present	Native	POWO (2020)
Botswana	Present	Native	Jeffrey (1978)
Burkina Faso	Present	Native	POWO (2020)
Cameroon	Present	Native	POWO (2020)
Chad	Present	Introduced	Brundu and Camarda (2013)
Eritrea	Present	Native	POWO (2020)
Eswatini	widespread	Native	POWO (2020)
Ethiopia	Present	Native	POWO (2020)
Mali	Present	Native	POWO (2020)
Mauritania	Present	Native	POWO (2020)
Mozambique	Present	Native	POWO (2020)
Namibia	Present	Native	POWO (2020)

Table 2.6: Distribution table of *Momordica balsamina* across Africa (Duenas-Lopez, 2019.)

Country/Region	Distribution	Origin	References
Niger	Present	Native	POWO (2020)
Nigeria	Present	Native	POWO (2020)
Senegal	Present	Native	POWO (2020)
Somalia	Present	Native	POWO (2020)
South Africa	Widespread	Native	Jeffrey (1978)
Sudan	Present	Native	POWO (2020)
Tanzania	Present	Native	POWO (2020)
Zambia	Present	Native	POWO (2020)
Zimbabwe	Present	Native	POWO (2020)

The leaves of *M. balsamina* have been a popular vegetable in the eastern parts of SA and the local tribes call it 'tshibavhe or lubavhe' (Venda), 'nkaka' (Tsonga), 'duwana', or 'mohodu' (Sotho and Xhosa), 'intshungwana' (Zulu), or 'laloentjie' (Afrikaans) (MWNH, 2004; Tropical Plants Database, 2020d).

In SA, the crop has been ranked amongst popular ALVs where the popularity of specific species is a function of many factors, including availability, ease of preparation, taste, consistency, and appearance (Oelofse and van Averbeke. 2012). Currently, it is rarely found due to extreme climatic conditions and the lack of rain, though it tries to survive during harsh conditions (Akaka - Arca del Gusto - Slow Food Foundation, 2021). It is a drought-tolerant species that is readily available during dry seasons. It is widely available in the vicinity of villages and has been classified as a ruderal, which is common in areas with disturbed soils and agricultural soils (Cunningham *et al.,* 2012). It is also readily and freely growing in disturbed soils of gardens, fields, kraals, and ploughed areas. *Momordica balsamina* grows in white, yellow, red, and grey sandy soil, loam, clay, alluvial, gravelly, and calcareous soil. It also thrives in full sun and semi-shade in grassland, savanna, woodland, forest margins, coastal dune forests, and riverbank vegetation (Welman, 2004). Peak availability is between the month of December to January, during the rainy season and has sometimes been reported to be available throughout the year (Shackleton *et al.,* 1998). In tropical Africa, *M. balsamina* grows in drier regions and it thrives in coastal bushland on the sand, woodland, wooded grassland, riverine fringes, riverbanks, and dry riverbeds (Duenas-Lopez, 2019).

2.2.8.2 Morphological description of *Momordica balsamina*

Welman, (2004) reported that *M. balsamina* is a perennial herbaceous climber, with a tuberous rootstock, several to many stems, and grows up to 2 m long and over, occasionally to 10 m. The tendrils are simple; leaves are waxy, the lower surface is paler than upper, deeply palmately 5-7-lobed, up to 12 cm long, margin toothed, and stalked. It develops bright green leaves; flowers are pale yellow, round, and somewhat warty and solitary. Male and female flowers are monoecious. It bears striking orange to red spindle-shaped fruit with 9 or 10 regular or irregular rows of cream or yellowish short blunt spines. When ripe, the fruits (25-60mm) burst apart automatically into three valves that curl back (also opens when the tip is touched), revealing numerous seeds covered with a brilliant scarlet, extremely sticky coating. The seeds are embedded into a sweet edible red fleshy pulp tasting like watermelon.

2.2.8.3 Nutraceutical use of *Momordica balsamina*

Momordica balsamina is a vegetable species frequently eaten as a relish, on average six to seven times a week in most rural villages (Shackleton, 1998). A relish is usually made from the vegetable incorporated in a more significant proportion or the one that has the predominating flavour (Mabogo, 2012). In SA, 'balsam apple' is cooked while green can also be added to other vegetables, such as *C. olitorius*. It is eaten with a traditional maize meal (porridge) or added to other vegetable dishes. The young fresh leaves and fruits of this vegetable are preferably picked, then boiled together in water with an addition of salt, tomatoes (optional), and grounded nuts for added flavours. They are then cooked from 15 to 30 minutes, depending on receipts and the thickness of leaves, and served with porridge, hot or cold (Nkaka - Arca del Gusto - Slow Food Foundation, 2021).

The leaves and young fruits are eaten together and are cooked as a leafy vegetable in Cameroon, Sudan, and other parts of southern Africa and contain high levels of minerals and ascorbic acid with high antioxidant activity. The cooked vegetable is also declared companion to porridge in these countries. The consumption of *M. balsamina* as a leafy vegetable has been widely encouraged in poor rural communities as a supplement for protein, for cereal-based diets (Thakur *et al*., 2009). For many years, it has been used as an edible food plant and widely as a bitter flavouring agent and for a wide range of medicinal and veterinary purposes in many countries (Bean, 2007) and as livestock fodder. Otherwise, the leaves and stems have been used to feed camels, goats, and sheep (Bosch, 2004). The leaves are boiled, after some time, the water is drained to eliminate some of the bitter taste. Portions of the harvest are immediately dried under sunlight (Shackleton *et al*., 1998), where the dried leaves can be used solely as spices and herbs and can be stored for a more extended period when dry.

Diaz, (2016) argued that although *M. balsamina's* red seed covering or aril is reputed to be edible, the fruit or 'apple' and its peeled seeds are poisonous. If ingested raw or cooked, they can produce a delayed muscarinic toxidrome characterised by nausea, salivation, emesis, diarrhea, and less commonly, hypoglycemia. Although 32Momordin extracted from *M balsamina* is a toxalbumin, stereo-chemically similar to ricin, gastrointestinal toxicity after 'balsam apple' ingestions is short-lived and inconsequential. A study conducted by Jonathan *et al*. (2020) has shown that 'balsam apple' leaves are considered non-toxic and safe to consume. A toxicity profile was conducted and found necessary to determine the minimum and maximum non-lethal doses, which provide information on effective dose or overdose. The result showed that *M. balsamina* is a safe herbal vegetable.

Some vegetables serve as piquants or spices and are only required to be added only in small quantities. *Momordica balsamina* is considered a piquant and most piquants have an acrid or bitter taste, some are even poisonous (Mabogo, 2012). The bitter taste in *M. balsamina* is attributed to a high percentage of alkaloids. Alkaloids are anti-nutrients that are a class of naturally containing organic nitrogen-containing bases. They are essential nitrogenous compounds produced as metabolites that cause biological effects based on consumption. Some common alkaloids include morphine and nicotine (Sood *et al*., 2012). Alkaloids are considered to account for the bitterness in many traditional leafy vegetables. There are two groups of alkaloids, namely pyrrolizidine and quinolizidine (Essack *et al*., 2017). Antinutritive compounds such as saponins have also been reported to be the cause of bitterness (Sinha and Khare, 2017). In South Africa, *M. balsamina* has been majorly acknowledged for its medicinal properties. However, its consumption as a leafy vegetable has declined due to its extremely bitter taste (Oelofse and van Averbeke. 2012).

2.2.8.4 Pharmaceutical use of *Momordica balsamina*

For decades, medicinal plants have been used in traditional medicine for the management of various diseases such as diabetes mellitus, cancer, hypertension, rheumatoid arthritis, and cardiovascular diseases (Davids *et al.,* 2016; Ochwang'I *et al.,* 2014; Rahimi, 2015). They have been a significant natural source for the unearthing of potent novel pharmaceutical compounds used in the treatment of several illnesses, and such alternative medicine is still extensively practiced by the

African communities and around the world (Nanyingi *et al.,* 2008), including countries where conventional medicine is notably practiced. This has led to an increase in the demand for medicinal plant-based products.

In addition to their pharmacological efficacy, medicinal plants have become of significant commercial value throughout the world. They possess various preventive and restorative effects such as anti-hypertensive, anti-diabetic, anti-hyperlipidemia, anti-microbial, anti-cancer, and antioxidant activities (Sangeetha and Rajamani, 2019). Their use over centuries for preventing, diagnosing, and treating different ailments is an outcome of helpful indigenous knowledge, beliefs, and cultural practices (Rafe, 2017). Various studies indicate that SA is a habitat of over 30,000 plant species, and over 80 % of its and the world's population still rely on medicinal plants for the management of various ailments due to their availability, low cost, potential efficacy, low toxicity, and transient side effects when compared to synthesized medications (Mahomoodally, 2013; Nasri *et al.,* 2013; Okoye *et al.,* 2014; Street and Prinsloo, 2013). Remarkably, several medicinal plant-derived products and ethno-pharmaceuticals are now being utilised by the western populace (Motlhanka *et al.,* 2010).

Momordica species have been used for primary healthcare to manage different disease conditions in various communities in Africa, Asia, Australia, and India (Nagarani *et al.,* 2014; Thakur *et al.,* 2009). *Momordica balsamina* is utilised in African countries like SA, Namibia, Botswana, Swaziland, and Mozambique as medicine for treating malaria, diabetes mellitus, and its associated complications (Kgopa *et al*., 2020; Ramalhete *et al*., 2016). Researchers have reported biological activities such as antioxidant, anti-diabetic, and cardioprotective properties (AbdErahman *et al.,* 2018; Olalere *et al.,* 2019). It is also used in the management of skin infections, rheumatoid arthritis, and abnormal uterine bleeding (CABI, 2020). The leaves, seeds, fruits contain phytochemicals such as alkaloids, steroids, glycosides, terpenes, and saponins which give the plant a wide range of biological activities such as anti-inflammatory, anti-microbial, analgesic, anti-diabetic, antioxidant, anti-plasmodial, anti-HIV, anti-trypanosomal, anti-diarrhoeal, anti-viral, and hepatoprotective properties as illustrated in Figure 2 (Behera *et al*., 2011; Pofu and Mashela, 2013). In West Africa and Syria, the fruits and leaves of *M. balsamina* are used to treat an injury, while the aqueous extract has been reported to have menstrual pain-relieving properties in young women (Thakur *et al*., 2009).

Makuse and Mbhenyane (2012) reported the hypoglycemic and hypertensive properties, while Bharathi and John (2013) reported the milk-stimulating effect of *M. balsamina*. Hammer et al. (2017) reported the inhibitory activity of the aqueous extract of *M. balsamina* leaves on gastric ulcers. The whole plant is used to manage fever, ulcers, skin diseases, mental sickness, and relieve pains in the intercoastal space (Thakur *et al.,* 2009). The anti-hypertensive, cardioprotective, nephroprotective, and osteoprotective properties have been attributed to its high potassium content (Flyman and Afolayan, 2007). A study showed that people who consumed high amounts of potassium found in vegetables had healthy blood pressure levels than those who consumed large quantities of processed food, suggesting the therapeutic value of potassium content in *M. balsamina* (Perez and Chang, 2014). Another review reported the anti-diabetic activities of *M. balsamina* crude leaf, fruit, and seed extract through the stimulation of insulin production and sensitivity and the inhibition of intestinal glucose absorption (Wajid *et al.,* 2019). Ludidi *et al.* (2019) reported the cardioprotective and hypoglycemic potential of methanolic extract of *M. balsamina.* The methanolic extract of *M. balsamina* was found to possess antioxidant activities attributed to the presence of significant amounts of flavonoids and phenolic compounds, containing carboxyl and hydroxyl groups, capable of binding to metals such as copper and iron (Jonathan *et al.,* 2020) (Figure 2.2).

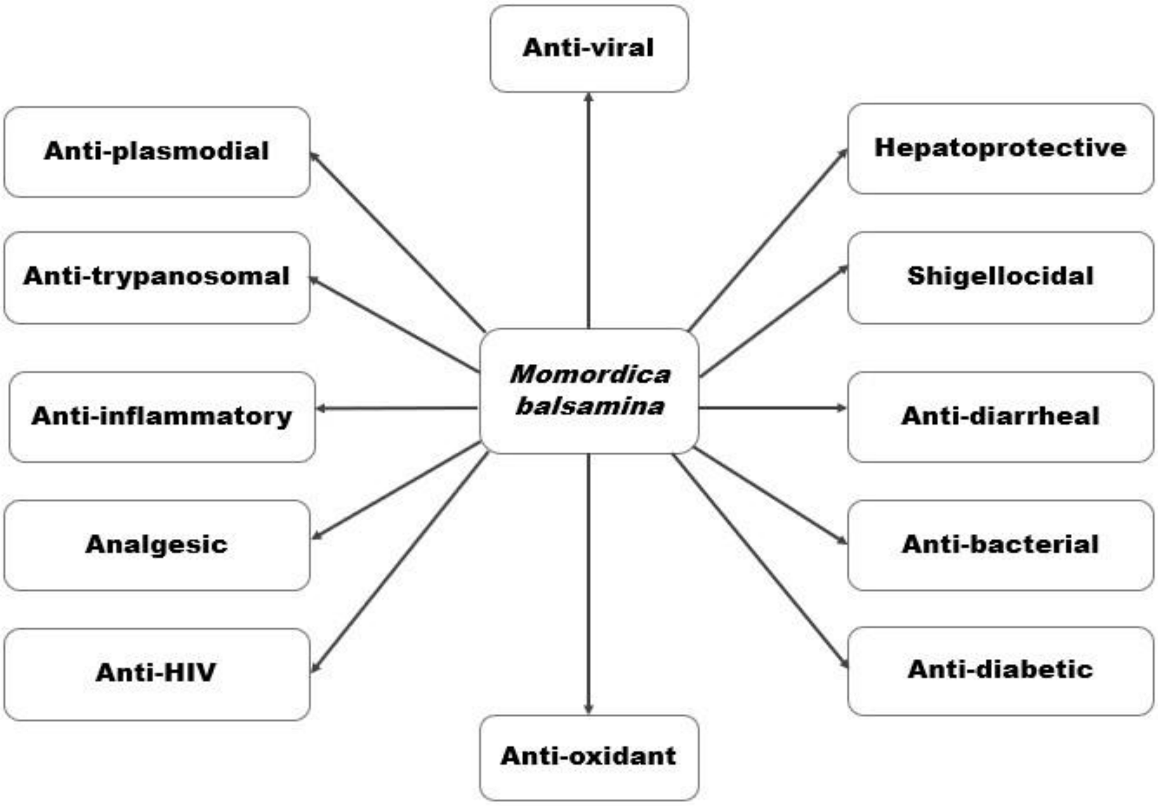

Figure 2.2**:** Pharmaceutical properties of *Momordica balsamina* (Thakur *et al.,* 2009)

It is important to note that several bioactive compounds with various medicinal properties have been identified, extracted, purified, and characterized from *M. balsamina.* Balsaminapentaol, balsaminol A and B, cucurbalsaminol A and B have been isolated from different plant parts. These bioactive compounds, especially the cucurbitane-type triterpenoids, have been reported to possess free radical scavenging and anti-diabetic properties (Nakamura *et al.,* 2006). Triterpenoids and balsaminols isolated from *M. balsamina* have been reported to possess anti-microbial and anti-malarial properties (Ramalhete *et al.,* 2016). Also, the fruit pulp of *M. balsamina* and momordin (I and II) have shown inhibitory effects on HIV after fourteen days of treatment and other viruses (Puri, 2010; Thakur *et al*., 2009).

2.3 Work not yet done on the problem statement.

The nutritional value and biochemical quality of *M. balsamina* have been studied extensively. However, the available empirically based information on the effect of growth stages on these qualities is not comprehensive. To date, there has been an investigation on the effect of plant maturity on leaf mineral content. *Momordica* *balsamina* holds substantial qualities, it is thereof important to evaluate its nutritional and biochemical properties focusing on various growth stages .

CHAPTER 3
EFFECT OF DIFFERENT HARVESTING STAGES ON THE NUTRITIONAL COMPOSITION OF *MOMORDICA BALSAMINA* L. INDIGENOUS LEAFY VEGETABLE

3.1 Introduction

Essential mineral elements are arbitrarily divided into macronutrients (elements required in higher quantities) and micronutrients (elements required in smaller quantities (Asyria *et al*., 2016). *M. balsamina* leaves have proven to be an essential green leafy vegetable as a source of nutrients to supplement other significant sources. *The leaves of M. balsamina* serve as a good source of mineral elements, particularly microelements. They are an essential source of essential nutrients, including potassium (K), Magnesium (Mg), Phosphorus (P), calcium (Ca), vitamins A and C, iron, amino acids, manganese (Mn), zinc (Zn), and sodium (Na) (Thakur *et al.,* 2009). Comparing the leaves mineral contents with recommended daily consumption values, *Momordica balsamina* leaves could be a good supplement for some mineral elements, particularly K, Ca, Mg, Fe, Cu, and Mn (Hassan and Umar, 2006).

Different vegetable nutrient elements serve great importance and roles in human health. Inadequate intake of nutrients results in various deficiencies that manifest as diseases. Not only can adequate vegetable consumption protect humans from some chronic diseases such as diabetes, cancer, obesity, metabolic syndrome, and cardiovascular diseases, but it as well improves risk factors related to these diseases (Ülger *et al.,* 2018). Different nutrient elements contribute differently to human nutrition. According to Kirschmann (2007), Iron (Fe) is a component of many enzyme systems, such as haemoglobin, which functions in oxygen transportation and cellular respiration. Calcium (Ca) is an element necessary for the solid growth and maintenance of bones, which must be accompanied by Magnesium (Mg) and Phosphorus (P) to function effectively. Magnesium and Phosphorus are required for enzymes concerned with oxidative phosphorylation and utilisation of carbohydrates and proteins for growth and production of energy, respectively (Trumbo *et al.,* 2001). Zinc is vital in carbonic anhydrase and other highly purified enzymes essential for carbohydrate metabolism. At the same time, Potassium (K) is known to send oxygen to the brain (when united with P) and for the synthesis of muscle protein, stimulating kidneys to eliminate poisonous body wastes and regulate the heartbeat (Trumbo *et al.,* 2001). There are various and specific daily recommended intakes of nutrients, including those prominent in *M. balsamina,* that need to be considered. Recommended intakes of nutrients can be converted into recommendations for average intakes of food according to age, sex (gender), and physiological status.

Morphological characterizations have great potential to provide a visual indication of distinctive developmental stages. This can help farmers and users harvest at the appropriate time for specific qualities at the best concentrations. As plants grow, they develop a gradient towards reproductive maturity (ontogenetic aging) and, after a time, reach a threshold above which the newly developing shoots have the capacity to fruit and flower (du Perez, 2003). In contrast, those below the threshold are still juvenile. The transition from juvenility to maturity state is called Phase Change (Leaky, 2004). *Momordica balsamina* is classified as a Cucurbitaceae crop, a crop family of the most diverse climbing species. It is classified as a herbaceous vine. Vines are plants that cannot remain free standing to any appreciable height (Putz, 2001). They are generally long and slender. As a result, being long and slender and the environmental heterogeneity they experience are suggested to have inclined them towards showing visible developmental changes.

Plants encounter profound changes, and their degree has been designated as either homoblastic or heteroblastic. The majority of plants, including *M. balsamina*, undergo heteroblastic development, in which the juvenile to adult transition is gradual. This also includes the developmental changes in floral form in the reproductive phase. Heteroblastic development traits that occur include changes in leaf size and shape, leaf anatomy, stem thickness, reproductive status, and physiology, to mention a few (Lee and Richards, 2002). Changes from one stage to the next are controlled primarily by some internal regulatory mechanism that presumably assesses the developmental status of the plant and causes the appropriate phase shifts. Developmental progression from one stage to the next depends on internal control, but to some extent, sensitivity to external conditions has been superimposed on the internal control (Lee and Richards, 2002).

In this current study, understanding the nutritional quality at different harvesting stages will aid in the consumption of appropriate quantities of *M. balsamina* as a healthy crop for nutrition purposes. Flyman and Afolyan (2008) have suggested that the findings are relevant in communities where home gardens and wild vegetable gathering are promoted as strategies to increase the intake of micronutrient-rich foods.

3.2 Materials and methods

3.2.1 Experimental site

Momordica balsamina plants were raised under greenhouse conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province (23°53'10"S, 29°44'15 E), between late winter to late spring (August-November) in 2021. The site experiences average day and night temperatures of 28 and 21˚C, respectively, with maximum temperatures regulated with thermostatically activated fans, wet walls, and a net covering the greenhouse roof. The greenhouse tunnel is 30.15 m long and 8.10 m wide (Figure 3.1). For nutritional analysis, the experiment was conducted at Limpopo Agro-Food Technology Station (LATS) laboratory located at the University of Limpopo, Limpopo Province (23°53'10"S, 29°44'15 E).

Figure 3.1: The greenhouse tunnel under which *Momordica balsamina* indigenous leafy vegetable experiment was conducted.

3.2.2 Treatments and research design

Six harvesting stages, namely: vegetative, flower-bud development, flower initiation, fruit set, fruit ripening, and physiological maturity, served as treatments and replicated ten (10) times (n=60). The vegetative stage served as the standard control. The experiment was laid out in a randomised complete block design (RCBD).

3.2.3 Research procedures

The seeds of *M. balsamina* were planted and raised in a 200-hole seedling tray filled with Hygromix-T (Hygrotech, Pretoria North) under greenhouse conditions, where they were irrigated when the moisture content dropped below 50%. After 7 days, fully developed seedlings were hardened-off for a week outside the greenhouse prior to planting. The hardened seedlings were uniformly selected and were transplanted directly into 20-cm-diameter plastic bags, each containing growing media mixture of steam-pasteurised loam soil (300°C for an hour) and Hygromix-T (3:1 v/v). Each pot contained one seedling and all pots were arranged on greenhouse benches at intra- and inter-row spacing of 0.2 m. Irrigation was achieved by using 250 ml of tap water after every second day, depending on the moisture content. The plants were not fertilised, but regular pest and disease scouting/control were done following recommendations by DAFF (2014).

3.2.4 Data collection

3.2.4.1 Plant variables

Data collection was performed while the plants were still in their respective growing positions before destructive harvesting. The various developmental stages representing the treatments were determined when 50% of individual plants per row showed developmental changes to the next growth stage. The number of days to flowering was determined when 50% of the plants per row had flowered. Plant growth variables, including the chlorophyll content (CC), were collected on four randomly selected leaves per plant using a chlorophyll meter (MINOLTA, SPAD-502), stem diameter (SD) (mm) was measured using a Vernier caliper at about 5 cm from the medium surface, vine length (VL) (cm) was measured from the media surface to the tip of the flag leaf using a measuring tape. The number of leaves (NL) and shoots (NS) per plant were counted manually, the leaf area (LA) of three (3) randomly selected plant leaves was obtained using a grid method, and leaf colour [brightness (L*), redness (a*), greenness (b*) and hue (h)] was determined using a colorimeter.

3.4.2.2 Nutritional composition

a. Sample preparations

Momordica balsamina tender leaves were harvested at each developmental stage and stored at −80°C before freeze-drying. The samples were freeze-dried using a benchtop freeze dryer (VirTis Sp Scientific, Model #2kbtes-55 Gardiner, NY, USA) at – 47°C to – 53°C for seven (7) days. The freeze dryer pressure was maintained below 200 millitorrs. After 7 days, the freeze-dried samples were subjected to grinding in a Wiley-mill grinding machine (MF10 Basic, IKA WERKE, USA) to pass through a 1-mm-pore sieve (Makkar, 2000).

b. Procedures

Twelve (12) samples weighing 400 mg of dried *M. balsamina* leaf powder, were digested in an acid solution with a PerkinElmer Titan MPS vessel. Reagents of about 5.0 ml of nitric acid (HNO_3) and 3.0 ml of hydrogen peroxide (H_20_2) were added to the mixture and was carefully shaken. At least 10 minutes elapsed before closing the vessel and then heated with a specific temperature program (Table 3.1) in the microwave. A standard of 75 ml digestion vessel was used. The results expected a clear solution in mg/L. The selected essential mineral elements of potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), Iron (Fe), and phosphorus (P) in mg/L were determined and data was recorded.

Table 3.1: Nutritional analysis temperature program.

Step	Target Temp (°C)	Pressure Max (bar)	Ramp Time (min)	Hold Time (min)	Power (%)
1	150	30	10	5	50
2	190	35	5	15	80
3	50	35	1	10	0
4	-	-	-	-	-
5	-	-	-	-	-

3.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistics 10.0 software. When the treatments were significant at the probability level of 5%, the associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Fisher's Least Significant Difference (LSD). The variable with significant ($P \leq 0.05$) treatment means were further subjected to lines of the best fit. Unless otherwise stated, only treatment means significant at 5% level of probability were discussed.

3.4 Results

3.4.1 Plant variables

The harvesting stages had highly significant ($P \leq 0.00$) effect on the measured plant variables (Appendix 3.2 – 3.5), contributing 92, 87, 85 and 81% to TTV in VL, NS, NL and LA, respectively (Table 3.2). However, no significant ($P \geq 0.5$) effect was observed in SD (Appendix 3.1), and treatment contributed only 12% to TTV in the respective variable (Table 3.2). Similarly, treatments had highly significant ($P \leq 0.00$) effect in CC and leaf colour (Appendix 3.6- 3.10), contributing 28, 33, 68, 71 and 63% to TTV in CC, brightness (L*), redness (a*), greenness (b*) and hue (h), respectively (Table 3.3).

Table 3.2 Sum of squares for stem diameter (SD), vine length (VL), number of shoots (NS), number of leaves (NL) and leaf area (LA) of *Momordica balsamina* harvested at different growth stages.

Source		SD (cm)		VL (cm)		NS		NL		LA (cm ^{2})	
	DF	SS	%	SS	%	SS	%	SS	%	SS	%
Replication	9	22.603	14	3803	2	41.27	4	5170	4	423.60	5
Treatment	5	20.432	12^{ns}	202213	92**	950.53	87**	108694	85**	7221.40	81**
Error	45	121.517	74	13343	6	95.13	9	13726	11	1288.60	14
Total	59	164.552	100	219359	100	1086.93	100	127591	100	8933.60	100

^{ns} = non-significant at P ≥ 0.05; * significant at P ≤ 0.05, ** highly significant at P≤ 0.00.

Table 3.3 Sum of squares for leaf chlorophyll content (CC), colour (brightness (L*), redness (a*), greenness (b*) and hue (h) of *Momordica balsamina* harvested at different growth stages.

Source		CC		L*		a*		b*		h	
	DF	SS	%	SS	%	SS	%	SS	%	SS	%
Replication	9	116.72	7	61.842	18	13.466	7	34.711	6	109.33	9
Treatment	5	502.91	28**	112.683	33**	142.929	68**	417.150	71**	728.03	63**
Error	45	1161.74	65	166.576	49	52.423	25	137.118	23	323.36	28
Total	59	1781.37	100	341.101	100	208.819	100	588.978	100	1160.72	100

^{ns} = non-significant at P ≥ 0.05; * significant at P ≤ 0.05, ** highly significant at P≤ 0.00.

Relative to the standard control (vegetative stage), treatments drastically increased VL by 42, 119, 154, 169, and 234% at bud development, flowering, fruit set, fruit development and physiological maturity stage, respectively (Table 3.4). Similar trend was also observed in the NS, NL and LA. Relative to the standard control, the NS increased by 10, 370, 610, 970, and 960% at bud development, flowering, fruit set, fruit development, and physiological maturity stage, respectively (Table 3.4). Similarly, growth stages highly increased in the NL by 70, 463, 638, 782, and 840% at bud development, flowering, fruit set, fruit development, and physiological maturity stage, respectively, when compared to the standard control. The leaf area was also increased by 53, 74, 77, 81, and 86% at bud development, flowering, fruit set, fruit development, and physiological maturity stage, respectively, when compared to the standard control (Table 3.4).

Harvesting stages increased CC by 22, 31, 37 and 10% at the bud development, flowering, fruit set, and fruit development stage, respectively, but decreased CC by 30% at the physiological maturity stage, when compared to the standard control (Table 3.5). In terms of leaf colour, relative to the standard control, the brightness of the leaves decreased by 1, 3, 1, 3 and 11% at the bud development, flowering, fruit set, fruit development, and physiological maturity stages, respectively (Table 3.5). The leaf colour decreased in hue by 1, 2, 3, 7 and 5% at the bud development, flowering, fruit set, fruit development, and physiological maturity stages, respectively, when compared to the standard control (Table 3.5). In contrast, the redness and greenness of the leaves had similar trend. When compared to the standard control, redness decreased by 22 and 14% at the bud development and flowering stage, however, the same variable increased by 0, 27 and 16% at the fruit set, fruit development and physiological maturity stages, respectively (Table 3.5). Similarly, relative to the standard control, the greenness of the leaves increased by 16, 73 and 46% at the bud development, flowering, and fruit set stages, respectively. However, greenness decreased by 18 and 10% at the fruit development and physiological maturity stages, respectively (Table 3.5).

Table 3.4 Responses for vine length (VL), number of shoots (NS), number of leaves (NL) and leaf area (LA) of *Momordica balsamaina* harvested at different growth stages.

Treatment	VL (cm)		NS		NL		LA (cm ^{2})	
	Variable[Y]	(%)[Z]	Variable	(%)	Variable	(%)	Variable	(%)
Vegetative	73.90^e	–	1.000^d	–	13.00^d	–	37.200^c	–
Bud development	105.15^d	42	1.100^d	10	22.10^d	70	57.000^b	53
Flowering	161.83^c	119	4.700^c	370	73.20^c	463	64.600^a	74
Fruit set	187.63^b	154	7.100^b	610	96.00^b	638	65.900^a	77
Fruit development	198.58^b	169	10.700^a	970	114.60^a	782	67.400^a	81
Physiological maturity	247.05^a	234	10.600^a	960	122.20^a	840	69.100^a	86

[Y]Column means with the same letter were not significantly different P ≥ 0.05 according to Fischer's Least Significance Difference test.

[z]Relative Impact (%) = [(treatment/control – 1) × 100].

Table 3.5 Responses for chlorophyll (CC), colour (brightness (L*), redness (a*), greenness (b*) and hue (h) of *Momordica balsamina* harvested at different growth stages.

Treatment	CC		L*		a*		b*		h	
	Variable[Y]	(%)[Z]	Variable	(%)	Variable	(%)	Variable	(%)	Variable	(%)
Vegetative	12.950[bc]	–	39.281[a]	–	9.355[c]	–	8.206[cd]	–	139.82[a]	–
Bud development	15.740[ab]	22	38.222[a]	1	7.323[d]	–22	6.738[d]	–18	139.04[a]	–1
Flowering	16.960[ab]	31	38.067[a]	–3	8.075[d]	–14	7.455[d]	–10	137.53[ab]	–2
Fruit set	17.780[a]	37	38.722[a]	–1	9.387[c]	0	9.481[c]	16	135.27[b]	–3
Fruit development	14.240[ab]	10	38.252[a]	–3	11.889[a]	27	14.223[a]	73	130.06[d]	–7
Physiological maturity	9.070[c]	–30	34.996[a]	–11	10.836[b]	16	11.973[b]	46	132.65[c]	–5

[Y]Column means with the same letter were not significantly different P ≥ 0.05 according to Fischer's Least Significance Difference test

[Z]Relative Impact = [(treatment/control – 1) × 100].

3.4.2 Nutritional composition

The leaf harvesting stages had a highly significant ($P \leq 0.01$) effect on the nutritional quality of *M. balsamina* (Appendix 3.11 – 3.16), contributing 46, 93, 68, 93, 95, and 96% to TTV in K, Ca, Mg, Zn, Fe and P, respectively (Table 3.6). Relative to the standard control, K, P and Zn had similar responses to the treatments. Treatments increased K concentration by 56 and 95% at the bud development and flowering stages, respectively (Table 3.7). However, the same treatments decreased K concentration by 87, 91 and 84% at the fruit set, fruit development, and physiological maturity stages, respectively (Table 3.7). Similarly, P increased in concentration by 12 and 5% at the bud development and flowering stages, respectively, but the variable experienced a significant decrease of 154, 170, and 173% at the fruit set, fruit development and physiological maturity stages, respectively, when compared to the standard control (Table 3.7). Furthermore, relative to the standard control, treatments increased Zn concentration by 12 and 10% at the bud development and flowering stages, respectively, but significantly decreased by 181, 229, and 217% at the fruit set, fruit development and physiological maturity stages, respectively (Table 3.7).

Calcium and Mg concentrations experienced similar trends as well. Relative to the standard control, the Ca concentration drastically increased by 41, 101, 7406, 12033, and 11932% at the bud development, flowering, fruit set, fruit development and physiological maturity stage, respectively (Table 3.7). Similarly, the Mg concentration also increased by 101, 566, 553, 680 and 2129% at bud development, flowering, fruit set, fruit development and physiological maturity stage, respectively, relative to the vegetative stage. In contrast, relative to the standard control, treatments increased Fe concentration by 5% at the flowering stage (Table 3.7). However, similar treatments decreased Fe content by 6, 390, 488, and 508% at the bud development, fruit set, fruit development and physiological maturity stages, respectively (Table 3.7).

Table 3.6 Sum of squares for Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Phosphorus (P) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source		K (mg/L)		Ca (mg/L)		Mg (mg/L)		Zn (mg/L)		Fe (mg/L)		P (mg/L)	
	DF	SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
Replication	9	2948799	7	1387	1	356.15	6	4.886	2	9.049	2	212.4	1
Treatment	5	1.267E+07	46**	171924	93	4329.66	68**	210.125	93**	593.876	95**	22683.1	96**
Error	45	1.167E+07	26	10853	6	1666.28	26	11.199	5	21.338	3	673.7	3
Total	59	2.729E+07	100	184164	100	6352.08	100	226.211	100	624.264	100	23569.1	100

^{ns} = non–significant at P ≥ 0.05; * significant at P ≤ 0.05, ** highly significant at P≤ 0.00

Table 3.7 Responses for Potassium (K), Calcium (Ca), Magnesium (Mg), Phosphorus (P), Iron (Fe), and Zinc (Zn) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Treatment	K (mg/L)		Ca (mg/L)		Mg (mg/L)		P (mg/L)		Fe (mg/L)		Zn (mg/L)	
	Variable^{Y}	(%)^{Z}	Variable	(%)	Variable	(%)	Variable	(%)	Variable	(%)	Variable	(%)
Vegetative	619^{b}	–	6.37^{c}	–	2.896^{d}	–	–22.590^{c}	–	–1.347^{c}	–	–1.715^{c}	–
Bud development	966.3^{ab}	56	8.97^{c}	41	5.827^{cd}	101	–25.360^{c}	12	–1.269^{c}	–6	–1.924^{c}	12
Flowering	1206.2^{a}	95	12.83^{c}	101	9.556^{c}	566	–23.810^{c}	5	–1.414^{c}	5	–1.877^{c}	10
Fruit set	80.0^{c}	–87	81.43^{b}	7406	18.910^{b}	553	12.084^{b}	–154	3.902^{b}	–390	1.395^{b}	–181
Fruit development	55.0^{c}	–91	127.70^{a}	12033	22.600^{ab}	680	15.880^{a}	–170	5.220^{a}	–488	2.205^{a}	–229
Physiological maturity	97.2^{c}	–84	126.69^{a}	11932	25.190^{a}	2129	16.540^{a}	–173	5.492^{a}	–508	2.010^{a}	–217

[y]Column means with the same letter were not significantly different P ≥ 0.05 according to Fischer's Least Significance Difference test

[z] Relative Impact = [(treatment/control – 1) × 100].

3.5 Discussion

3.5.1 *Momordica balsamina* growth variables measured at different harvesting stages

Generally, significant differences were observed in all the measured growth variables namely, VL. NS, NL and LA, except for SD. The significant variables increased at an increasing rate as *M. balsamina* leafy vegetable plant developed into different growth stages in this study. Observations revealed that the VL of *M. balsamina* increased with the plant's maturity and was highest at the physiological maturity stage. In most instances vine elongation potential may be influenced by environmental factors. Kong *et al*. (2017) suggested that *M. charantia* grown in the open field had longer leader vines than those grown in high tunnels. The root to shoot ratio is believed to influence the vine potential in controlled environments. Planting in controlled environments is usually done in confined pots or bags, which limits root growth to be at its maximum potential, thus limiting the shoot length. (Kong *et al.,* 2017). However, in this study, planting *M. balsamina* in plastic pots under controlled conditions, did not affect growth and elongation of the vines as they were observed to increase at an increasing rate at all the tested growth stages.

The NS also increased with growth stages in this study. Generally, an increase in NS in a plant, is a result of an increased number of auxiliary buds formed as the plant develops into maturity stage (Le Bris *et al*., 2012). Literature has established that the formation of auxiliary buds is constant in production for both the vegetative and reproductive growth phases in a growing plant (Morrison, 2007). In this study, an increase in the NS might be attributed to the fact that each axillary bud formed has the potential to develop into a new vegetative shoot (Ngezahayo, 2014). During plant maturity, auxiliary bud development is secondarily activated by the increasing presence of cytokinins in shoot nodes when shoot length increases (Song *et al.,* 2022).

The NL in *M. balsamina* leafy vegetable also increased as the growth stages progressed up to physiological maturity. Basically, the NL on a plant is influenced mainly by the genetics of the plant which controls the arrangement of leaves on the stem (Ghosh *et al.,* 2001). However, the success of new leaf formation is encouraged by provision of optimum growing conditions. Normally, the leaves that are already present in a plant during its early vegetative phases are capable of the end formation of new leaves in preceding phases, in such that leaves produce a signal that promotes vegetative phase change. In addition, the new shoots produced at different growth stages, account for the gradient in the number of new leaves produced as it was the case with this study.

The LA of *M. balsamina* showed an increase as the plant develops into various growth stages and subsequently enters physiological maturity stage in this study. Mardhiana *et al.* (2017) also witnessed an increase in LA with maturity of *Cucumis sativas* leaves. It is believed that leaf expansion and leaf area are controlled by several abiotic factors including light intensity, temperature and possibly also nitrogen supply. Reduced light intensity has been shown to retard the rate of change in leaf morphology from young to adult leaves (Niinemets, 2010). Also, nitrogen affects photosynthesis and photo-protection in leaves and as a result affecting photosynthates which will be used by plants to expand leaf size and leaf area (Meier and Leuschner (2008).

The CC and the leaf colour (brightness, redness, greenness and hue) were also highly significant. Chlorophyl content was observed to be reduced at physiological maturity, whereas the greenness and redness of the leaves were only reduced at early growth stages but started increasing when the plant reached fruit setting stages up to physiological maturity. The brightness of the leafy vegetable was evident at the bud development stage, however when flowering stage sets in, the plant lost its brightness until physiological maturity. As for the hue of *M. balsamina*, observation revealed a decrease of the variable as the plant develops into different growth stages.

Basically, the CC of *M. balsamina* experienced reduction when the plant developed fruits. The highest reduction was observed at physiological maturity. However, at juvenile stages, CC content was increased at an increasing rate up to fruit set. A similar observation was made by Khan (2011), where the lowest quantity of total chlorophyll was measured in mature leaves of bottle gourd *(Lagenaria siceraria)*. Generally, plant leaves are suggested to respond to light intensity by adjusting the CC (Zhang *et al.,* 2009). According to Lee and Richards (2002), young leaves have a more efficient distribution of chloroplasts. Juvenile stage leaves have convexly curved cells that focus light, thereby retracting light onto the chloroplasts. Focusing increases light intensity at the upper level of chloroplasts, whereas the matured leaves have flat epidermal cell walls with no focusing effect, resulting in high CC in juvenile leaves and low CC in mature leaves (Zhang *et al.,* 2009). As witnessed in this study, in young leaves, chlorophyll pigment predominated, while in mature leaves CC degradation took place as a result of senescence (Patsilinakos *et al*., 2018).

In terms of leaf colour, findings of this study revealed that the brightness and hue of the tested plant leaves reduced as the plant growth progressed up until physiological maturity. In contrast, the greenness and redness of the tested plant leaves reduced at early vegetative stages, however, when the plant progressed into fruit set stage, the two respective variables became dominant throughout the remaining growth stages up to physiological maturity. Notably, the degree of colour changes observed at different maturation stages of leaves is possibly due to the varying ratios of chlorophyll, xanthin, and anthocyanin (Zhang *et al.,* 2009). According to Cox *et al*. (2004), significant decrease in brightness and hue in leaves is mostly attributed to chlorophyll degradation, whereas leaves increase in greenness and redness is attributed to the leaves relying on the presence of carotenoids and anthocyanins in order to reduce leaf damage. In matured leaves there is greater production of anthocyanins and, to a lesser extent, higher investment in carotenoids compared to chlorophyll content. As documented by Gong *et al*. (2020), the high concentration of anthocyanins and carotenoids plays a strong photo-protective role during leaf expansion. Therefore, as the plant matures, the leaves expand, and ultimately anthocyanin production is increased, owing to the increased redness (dos Santos *et al.,* 2021).

3.5.2 Nutritional quality of *Momordica balsamina* measured at different harvesting stages

Essential nutrients are necessary for human nutrition, plant growth and development (Santos *et al*., 2014). Potassium, Mg, P, Ca, vitamins A and C, iron, amino acids, manganese and zinc are found in abundance in the leaves of *M. balsamina*. All the measured nutritional quality of the test crop were highly affected by the harvesting stages. At bud development and flowering stages both K and P where increased, respectively. *Mormordica balsamina* leaves were found to accumulate highest concentrations of K content, during early growth stages, which is required for plant development (Kopsell *et al.,* 2013). Bindraban *et al.,* (2015) reported that K is available in abundance in many plant cells and tissues and the concentration is high during the early stages of plant development due to its active role in photosynthesis, respiration, and water control mechanisms. In human nutrition K is beneficial for the management of cardiovascular diseases such as high blood pressure (Thakur *et al*., 2009). Potassium (K) is also very important in the prevention and treatment of high blood pressure, which is caused by excessive salt intake and hypertension. It is an effective treatment of diarrhoea in infants and adults and also effective for headache treatment and headache–causing allergies (Sawka, 2005). The recommended daily dosage by the dietary goals for the United States (DGUS) is 1200–2000 mg for children and adults (Kirschmann, 2007). Harvesting *M. balsamina* at the flowering stage may provide the necessary quality and quantity of K, as K values are shown to be the highest at the flowering stage.

Generally, tender vegetables are frequently sought as a base component of mixed salads, soups, main dishes, and sandwiches, particularly the ready–to–eat types, whose consumption is steadily increasing because of their nutritional composition (Saini *et al.,* 2017). Highest concentration of P in leaves of *M. balsamina* at early growth stages was in line with findings by Waterland *et al*. (2017), whereby it was documented that P is required for early developmental stages of the seedlings. Nutritionally, P is important in the human body to speed up the healing process in bone fractures and treat mental stress, which causes arthritic symptoms such as aching of joints. It treats teeth and gum disorders and is a remedy for mental fatigue, and relieves muscle cramps and pains (Mensah *et al.,* 2008). The DGUS recommends 800 mg of P for adults from 25 years and 1200 for younger children and pregnant females (Kirschmann, 2007). *Momordica balsamina* is established not to be a good source of P at any stage as P values showed to be less than the recommended amount.

The Ca content in *M. balsamina* increased with the growth stages with the highest accumulation noticed at fruit development stage. White and Broadley (2009), reported a significant amount of Ca in matured and senescing organs, the Ca content increased from bud development to physiological maturity stage. Biondo *et al*. (2014) also reported higher Ca concentration in beetroot (*Beta vulgaris*) as the plant underwent the various developmental stages (Biondo *et al*., 2014). In other studies, microgreens were found to contain lower Ca content than physiologically matured pigweed *(Amaranthus retroflexus*) and kale (*Brassica oleracea var. sabellica*) (Waterland *et al.,* 2017), while matured lettuce (*Lactuca sativa*) had higher Ca content than its microgreens (Pinto *et al.,* 2015).

Generally, Ca is a natural tranquilizer that calms the nerves and promotes deep sleep. It can be used for muscle cramp relief and the prevention and treatment of arthritis (aching bones) (Pravina *et al.,* 2013). It is also another element that lowers blood pressure and cholesterol. The Nation Research Council (NRC) recommends 800 mg daily and 1200 mg for pregnant and lactating women (Kirschmann, 2007). None of the harvesting stages complied with the recommended dosages in this study. The Ca values were lower than the recommended intake levels. Therefore, *M. balsamina*, in this instance, is not a suitable source of Ca.

Momordica balsamina contained higher mg content which was increasing with the developmental stages. The highest was achieved in the matured stage compared to the bud development stage. This difference could be attributed to the different growth periods, which could have affected the mineral composition (Xiao *et al*., 2016). Magnesium is involved in the biosynthesis of pigments and proteins in plants and serving as an enzyme cofactor during carbon metabolism (Singh *et al*., 2013). Results of this study was consistent with the findings of Waterland *et al.,* (2017) where the three mature kale had higher Mg concentration. Magnesium is a popular treatment for ailments such as hypertension, diarrhoea, vomiting, and toothache resulting from tooth decay. Chronic alcoholism symptoms, emotional liability and irritability, and hyperreflexia are caused by Mg deficiency (Hans *et al*., 2002). The NRC recommends an intake of 350 mg of Mg intake for adults, 320 mg for pregnant women, and just 80 mg for children (Trumbo *et al.,* 2001). *Momordica balsamina*, in this instance, does not supply the relevant quantities of Mg at different harvesting stages, as Mg values were found to be lower than the required level.

In this study, the high content of Fe was observed during the flowering stage only. The amount of iron needed during the flowering process, which was justifiable in a previous study by Bernier *et al.,* (2018). In the absence of Fe, cocklebur (*Xanthium pensylvanicum)* consistently responded abnormally to photo-induction; it appears reasonable to suggest that Fe is important in the flowering process (Bernier *et al.,* (2018). Our data also agrees with that of Waterland *et al*. (2017), where Fe content was higher in microgreens compared to mature kale. Iron is involved in the synthesis of chlorophyll in plants, and it is required for plant respiration and the proper structure and function of photosynthetic pigments (Rout and Sahoo, 2015). Iron is beneficial in the treatment of anaemia, expressed by easy fatigue and less disease resistance. It is necessary for menstruation and required mainly by adolescent girls, as during adolescence, there is an accelerated demand for Fe to compensate for its losses through menstruation (Fasuyi, 2006). The recommended dosage for female adolescents is between 5 mg to 28 mg of Fe, 9 mg for pregnant and lactating females, and 14 mg to 28 mg for female adults (Trumbo *et al.,* 2001). In this study, the highest Fe values were found at the physiological maturity stage. Therefore*, M. balsamina* may provide an essential amount of Fe at the physiological maturity stage for female adolescents between the age of 10–12 who require 5–10 mg Fe.

Concentrations of Zn were increased at early growth stages of *M. balsamina* in this study. Our findings are consistent with Waterland *et al*. (2017), who found that the Zn concentration of three kale cultivars (*B. oleracea* var. *acephala*, B. oleracea var. acephala, and *B. napus), at* two different microgreen stages (kale with two fully expanded cotyledons, and kale with two fully expanded true leaves), was higher than that of mature kale. Zinc is a micronutrient required for plant growth and development. The nutrient act as a cofactor for antioxidant systems, which protect the human body from illnesses caused by free radical damage (Trumbo *et al.,* 2001). Zinc is essential in treating ulcers, acne, and sickle cell anaemia. It also can treat herpes, high cholesterol, and rheumatoid arthritis (Bhowmik and Chiranjib, 2010). World Health Organisation recommends that adult males take 2.2 mg per day of Zn; however, children and pregnant women need more (Trumbo *et al.,* 2001). In this instance, for males, *M. balsamina* at the fruit development stage may comply with the prescribed dosage of Zn, as the Zn values were the highest at the fruit development stage.

3.5.3 The effect of different harvesting stages on plant nutritional quality

The appropriate stage of harvesting and consumption of plant material should be based on nutritional information since the harvesting stage affects the nutritive value of the plant material. When plant material is harvested at the appropriate stage of maturity, optimum nutritional benefits can be obtained from the ingredient (Ncube *et al.,* 2015). Harvest maturity is a stage of plant development at which quality attributes preferred for consumption (volume, flavour, appearance, chemical composition, and good shelf life) are at maximum (Mamboleo, 2015). The plant's age at harvest is known to affect the nutritional composition of leafy vegetables. The harvesting method can determine the extent of variability in maturity and consequently influence the nutritional composition of fruits and vegetables (Lee and Kader, 2000). Biologically active compounds vary during maturity, depending on different biosynthesis pathways and mechanisms of metabolic control. The mineral composition of plant organs at different growth stages are determined by a sequence of events that begin with the bioavailability of soil mineral nutrients, their membrane transportation in and from roots, their translocation to vegetative organs, and finally, their deposition in various cellular compartments (Flyman and Afolyan, 2008).

The uptake rate of many nutrients depends on the nutrient's demand for growth, determined by the role the nutrients play in plant growth. Most Ca and P functions are structural components of macromolecules. Potassium is required for cell expansion, protein synthesis, and stomatal regulation, while Mg is the central atom in the chlorophyll molecule (Makokha *et al.,* 2019). The uptake of N, P, K, Ca, and Mg had been established to be higher during the vegetative stage than in the reproductive stages. It was believed that nutrients are partitioned towards the reproductive organs during the reproductive stage (Bender *et al*., 2015). In addition, translocation of nutrients N, P, and K in the form of amino acids from shoots to roots in the absence of soil nutrient replenishment and a decrease in demand for nutrients for new growth as plants age accounts for the decrease in the levels of nutrients in African leafy vegetables with increasing plant age (Makokha *et al.,* 2019). In this study, it was observed that different harvesting stages had a significant effect on the various nutrient elements assessed, where nutrient concentrations of *M. balsamina* were different at various harvesting stages. The flowering stage was shown to have the highest K quality, whereas the fruit development stage had the highest Ca and Zn quality, and the physiological maturity stage had a high Mg, P, and Fe quality.

3.6 Conclusion

Harvesting of *M. balsamina* leaves at different growth stages influenced the nutritional quality of the leaf powder. Therefore, the null hypothesis of this objective is rejected. The accumulation of K, P, Fe, and Zn in *M. balsamina* leaves was found to be high at early growth stages, but were reduced at mature stages. However, Ca and Mg, were increasing from early vegetative stage with the increasing growth stages. A noticeable reduction in most nutrients (K, P, Fe, and Zn) occurred from the fruit set stage. The flowering stage was found to be the appropriate stage where all the tested nutrients can be found in sufficient quantities.

CHAPTER 4

BIOCHEMICAL COMPOSITION OF *MOMORDICA BALSAMINA* LEAF POWDER HARVESTED AT DIFFERENT GROWTH STAGES OF THE PLANT

4.1 Introduction

Momordica species have been used for primary healthcare to manage different disease conditions in various communities in Africa, Asia, Australia, and India (Nagarani *et al*., 2014; Thakur *et al.,* 2009). Researchers have reported biological activities such as antioxidant, anti-diabetic, and cardioprotective properties (Olalere *et al.,* 2019). The potential antioxidant activity of *Momordica balsamina* has already been reported, and its antioxidant components are polyphenols, inhibiting the 5-lipo-oxygenase enzyme responsible for inflammation (Akula and Odhav, 2008). In addition, the antioxidant potential of *Momordica* species is attributed to the contribution of total phenolic compounds (TPC) and ascorbic acid (AA) (Perumal *et al*., 2021).

Antioxidants may be an essential property of plant medicines associated with treating several ill-fated diseases. The main characteristic of an antioxidant is its ability to trap free radicals or its antioxidant activity. Free radicals generated in biological systems may oxidize nucleic acids, proteins, and lipids and initiate degenerative disease. Oxidative stress is implicated in many diseases such as dyslipidaemia, atherosclerosis, obesity, Type II diabetes, arthritis, cancer, cardiovascular diseases, and neurodegenerative diseases (Nagarani *et al*., 2014). Most natural antioxidants have been declared phenolic (Ho, 1992). Phenolic compounds are secondary metabolites found in plants and are known to have antioxidant effects (de Oliveria *et al*., 2018). They can be classified into water-soluble and insoluble compounds (Rispail *et al.,* 2005). Ascorbic acid, or vitamin C, is a water-soluble non-enzymatic antioxidant that protects cellular components from free radical damage. Ascorbic acid has been shown to scavenge free radicals directly in the aqueous phases of cells and the circulatory system (Beyer, 200). Various types of antioxidants accumulate at different concentrations during the growing period of plants (Moyo *et al*., 2013). Therefore, it is imperative to investigate the variations of

*M. balsamina l*eaves biochemicals involving antioxidant activities at different growth stages.

4.2 Materials and methods

4.2.1 Experimental site

Momordica balsamina plants were raised under similar location and conditions as described earlier (Chapter 3). Freshly harvested leaves were transported in a cool storage box containing ice blocks, to the Post-harvest Technology Laboratory at the Tshwane University of Technology, Gauteng province (25°73'22"S, 28°16'19"E),, where the biochemical evaluation experiment was conducted.

4.2.2 Treatments and research design

Same six growth stages, namely: vegetative, flower-bud development, flower initiation, fruit set, fruit ripening, and physiological maturity, served as treatments as explained in chapter 3. The experiment was replicated five (5) times (n=30). The vegetative stage served as the reference. The experiment was laid out in a complete randomised design (CRD).

4.2.3 Sample preparation

The sample preparation procedure followed the same route as in the sample preparation for the nutritional analysis in chapter 3 (3.4.2.2) prior to extraction procedures.

4.2.3.1 Sample extraction procedure

A 100 mg of *M. balsamina* ground powder was weighed into 2 ml Eppendorf tubes. An 80/20 methanol solvent was added using a 1000 μL pipette. The mixture was vortexed and then centrifuged for 20 minutes. The supernatant was derived from the centrifuged mixture.

4.2.4 Data collection

<u>Total phenolic content (TPC)</u>: The total amount of phenols in each plant extract was determined using the Folin-Ciocalteu (FC) method (Tinyane *et al*., 2013). A 10% FC reagent was prepared by adding 80 ml of FC solution in a volumetric flask filled up to

the mark with distilled water. A 15 mL 700mM sodium carbonate (Na_2CO_3) was prepared with 1.113 g in 15 mL water. Concentrations of 100 µL of each sample extract and blanks were added to 2ml Eppendorf microtubes. A measurement of 200 µL of 10% FC reagent was added and vortexed thoroughly, and then followed by the addition of 800 µL of 700 mM Na_2CO_3 to each tube. A standard was prepared using a serial dilution of gallic acid (1 mg gallic acid powder in 10 ml 80/20 methanol/water) in place of the extract. The samples were incubated in the dark at room temperature for 2 hours. A transfer of 200 µL of the reaction mixture was made to a transparent 96-well-plate. Absorbance was taken at 700 nm using a UV visible spectrophotometer. The results were expressed as mg of GAE/g of the sample.

<u>Ascorbic acid (AA)</u>: Ascorbic acid content was determined using 2,6-dichlorophenolindophenol dye in a titrimetric method (Ntsoane *et al.,* 2016). A 500 mg of dried *M. balsamina* leaf sample powder was mixed with 7.5 ml meta-phosphoric acid (HPO_3) and placed in tubes where it was centrifuged for 10 minutes at 6000 rpm. An ascorbic acid standard was prepared by dissolving 0.05 g of into 200 ml distilled water, mixed with 7.5 ml HPO_3. About 20 ml of the extracted supernatant and standards were placed in flasks. Dye was filled in a burette and used to titrate the standards until a purplish colour was observed. Titration of the samples was titrated up until an established colour that matched the standards was obtained. The results will be expressed as mg/100g on a dry weight basis.

<u>DPPH radical scavenging activity</u>: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Tinyane *et al.,* 2013) was used to determine the free-radical scavenging activity. For DPPH, the sample extracts were diluted with an extraction solvent to obtain solutions of 40, 60, 80, and 100 mg mL^{-1}. Solution (0.01 mg mL^{-1}) of 10 ml of DPPH mother solution added to 5 ml methanol was prepared immediately before the analysis and was used as a positive control. The capacity to scavenge the "stable" free radical DPPH was monitored according to Du Toit *et al*. (2001). About 150 µL aliquots of 0.013 ml 1, 1-diphenyl-2-picrylhydrazyl prepared in methanol were mixed with 10 µL of the test sample in a 96 well microplate. The control samples contained all the reagents except the extract or positive control antioxidant. The mixtures were then incubated for 20 minutes in a dark environment, and the absorbance was measured

at 517 nm using a UV/visible spectrophotometer (Zenyth 200rt Microplate Reader, UK-Biochrom Ltd.). USA). The results were expressed as IC50.

4.2.5 Data analysis

The data analysis was conducted as described in the previous chapter (Chapter 3).

4.3 Results

The leaf harvesting stages had a highly significant (P≤ 0.01) effect on the TPC, AA and antioxidant activity (DPPH) (Appendix 4.1 – 4.3), contributing 96, 98, and 97% to total treatment variation (TTV) on the respective variables (Table 4.1).

Total phenolic content and AA had similar trends. Relative to the vegetative stage (reference), treatments decreased TPC by 25, 40, 38, 26% at bud development, flowering, fruit set and fruit development stages, respectively (Table 4.2). However, treatments increased TPC by 17% at the physiological maturity stage. Similarly, relative to the vegetative stage, harvesting at different growth stages also decreased AA concentration decreased by 32, 43, 49, 58, and 57% at bud development, flowering, fruit set, fruit development, and physiological maturity stages, respectively (Table 4.2). In contrast, the same treatments increased DPPH by 42, 36, 33, 38, and 23% at the bud development, flowering, fruit set, fruit development and physiological maturity stages, respectively (Table 4.2).

Table 4.1 Sum of squares for total phenolic content (TPC), ascorbic acid (AA) and radical scavenging activity (DPPH) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source		TPC (mg/100g)		AA (mg/100g)		DPPH IC50 (mg/g)	
	DF	SS	TTVT %	SS	TTV %	SS	TTV %
Replication	4	484.8	1	7.03	1	0.01365	2
Treatment	5	72815.4	96**	1529.83	95**	1.42315	91**
Error	20	2907.4	3	70.17	4	0.11179	7
Total	29	76207.6	100	1607,03	100	1.54859	100

^{ns} = non-significant at P ≥ 0.05; * slightly significant at P ≤ 0.5, ** highly significant at P≤ 0.00

Table 4.2 Responses for total phenolic content (TPC), ascorbic acid (AA), and radical scavenging activity (DPPH) of M*omordica balsamina* leaf powder harvested at different growth stages.

Treatment	TPC (mg/100g)		AA (mg/100g)		DPPH IC50 (mg/g)	
	Variable[Y]	(%)[Z]	Variable	(%)	Variable	(%)
Vegetative	366.00[a]	–	36.038[a]	–	1.5520[d]	–
Bud development	275.71[c]	–25	24.381[b]	–32	2.2020[a]	42
Flowering	218.24[d]	–40	20.495[c]	–43	2.1100[ab]	36
Fruit set	227.50[d]	–38	18.362[c]	–49	2.0680[b]	33
Fruit development	272.50[c]	–26	15.238[d]	–58	2.1380[ab]	38
Physiological maturity	303.78[b]	17	15.467[d]	–57	1.9140[c]	23

[Y]Column means with the same letter were not significantly different P ≥ 0.05 according to Fischer's Least Significance difference test

[Z] Relative Impact % = [(treatment/control – 1) × 100]

4.4 Discussions

4.4.1 Effect of different growth stages on the biochemical quality of *Momordica balsamina*.

Vegetables serve as a source of phenolic antioxidants for humans. Several plant preparations and isolated compounds have been reported to have medicinal properties owing to the presence of phytochemical components. Olalere and Gan (2020) have reported the antioxidant activities of eleven isolated phenolic compounds from *M. balsamina*. Our study investigated the amount of AA, TPC, and radical scavenging activity (DPPH) present at different growth stages of *M. balsamina* leaves*.*

The TPC decreased across all growth stages, except for the physiological maturity stage. These findings are in agreement with those of Zhang *et al*. (2009), who observed a decreasing trend in TPC across different growth stages of 'balsam pear' (*M. charantia).* Similarly, Cheptoo *et al.* (2019) observed higher concentrations of TPC in mature leaves than the young leaves in stinging nettle (*Urtica dioica*), Amaranth (*Amaranthus dubius)* and black nightshade (*Solanum scabrum*). A decrease in phenolic content may be attributed to the fact that metabolism of the plant is minimised by concentrating and retrieving metabolites from aging leaves and transporting them to seeding or fruiting organs (Masetla *et al*., 2022). The observed Increase at physiological maturity could be attributed to the extent by which biosynthesis pathways of phenolic compounds are affected during ripening. Total phenolic compounds play an important role in plant signalling. Leaf phenolic allelochemicals include *p*-hydroxybenzoic acid, *p*-coumaric acid stored in the vacuoles of plant encounter the cytoplasmatic proteins and form polyphenols-protein complex. This complex aids in the senescence of plant tissues (Pratyusha, 2022).

The highest reduction of TPC was noticed at the flowering stage. Kirruti *et al*. (2021) also observed a high TPC reduction at the flowering stage of dried Chia *(Salvia hispanica)* leaves*.* Jimoh *et al. (2009)* observed similar results in tassel flower *(Amaranthus caudatus)* leaves. The observed could be due to the hypothesis that at the flowering stage, differentiation dominates over the synthesis of secondary metabolites (Riipi *et al.,* 2002). Protein bound phenols and tannic polyphenols in leaves decrease as plants approach reproductive stages (Riipi *et al.,* 2002). In *M.*

balsamina leaves, the anti–inflammatory activity is said to be attributed to the potent free radical scavenging activity of the phenolic compounds. Inflammation is part of the body's response to aggression by viruses, bacteria or fungi, cancer, ischemia, allergen, toxic compounds, and tissue lesion (Ramalhete *et al*., 2022). The highest TPC of *M. balsamina* in this instance may better be obtained from leaves at the physiological maturity stage.

The AA decreased throughout the maturation of the plant with the highest reduction at the physiological maturity stage. Findings on AA are in agreement with Jeong *et al.* (2016) who also reported a decline of AA in *M. charantia* leaves as plant growth progressed. Another study by Bergquist *et al. (*2006) reported that significant levels of AA can be found in immature baby spinach (*Spinacia oleracea*) leaves than in mature leaves, indicating a significant reduction of AA. Onyango *et al*. (2011) also presented that AA in amaranth (*Amaranthus hypochondriacus*) leaves decreased with more advanced maturation and senescence. Since *M. balsamina* is a vining leafy vegetable, it is suggested that the increased plant foliage with maturation reduces the light intensity onto the leaves, thereby reducing accumulation of AA in the shaded plant parts (Bartoli *et al*., 2006). Reduction of AA may also be attributed to the fact that senescence at physiological maturity involves chlorophyll degradation due to the action of free oxygen radicals. Ascorbic acid constitutes the defence mechanism against these radicals in the chloroplasts as a result, a decrease in chlorophyll with maturation implies a decline of some AA contents (Ivanov, 2014).

Ascorbic acid is generally required for the maintenance of healthy skin, gums, and blood vessels (Lee and Kader, 2000), the maintenance of a healthy immune system, the reduction of colds by preventing secondary viral or bacterial infections, and the prevention of cardiovascular diseases and some forms of cancer (Eichholzer *et al.,* 2001). The recommended daily allowances (RDAs) for adult men are 40 mg/day in UK; 90 mg/day in USA; 100 mg/day in Germany; 70 mg/day in Netherland (Locato *et al*., 2013). Moreover, in order to enhance health benefits due to vitamin C intake, the scientific community is suggesting increasing its RDA to 200 mg/day. Epidemiologic studies have revealed that AA intake over the current RDA has a significant impact in reducing the risk of diseases such as respiratory tract infections, cardio-vascular diseases and cancer. (Locato *et al*., 2013) The AA levels in the various harvesting stages were lower than the suggested RDAs, implying that the leaves of *M. balsamina*, considering each growth stage, are a poor source of AA, except at the vegetative stages (reference).

The radical scavenging activity (DPPH) in leaves increased with the progression of growth of *M. balsamina*. The highest accumulation occurred during the bud development stage when compared to the physiological maturity stage. Petropoulos *et al*. (2018) observed similar results of an increment in antioxidant activity at the 3rd growth stage (stage before flowering) which is reckoned to be highly associated with the increased content of bioactive compounds, such as AA, phenolic acids and TPC. Interestingly, variation of TPC at different levels in this study suggests that different stages may have varied biological activities, since there is a link between TPC and AA (Sun *et al*., 2002). Antioxidant activity is increased in plants with a higher phenolic content (Sun *et al.,* 2002). The ability of ALVs to produce high levels of antioxidants during early plant growth stages highlights their adaptation to harsh environments where conditions are usually unfavourable for early growth and development (Tesfay *et al*., 2016)**.** By illustration, grape (*Vitis vinifera)* seed extract's antioxidant activity against 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), an equivalent of DPPH, was connected to its TPC (Sung *et al*., 2010).

Cucurbitane triterpenoids found in *M. balsamina* are medicinal agents considered as antioxidant triterpenoids due to their outstanding AA. These compounds may serve as antioxidant agents for treating reactive oxygen species (ROS) induced diseases. (Liu *et al.,* 2010). The radical scavenging antioxidant activity Observed in *M. balsamina* may, as a result, be linked with various pharmacological activities. This was supported by Kaushik *et al*. (2017), who established that three cucurbitane–type triterpenoids were associated with the antidiabetic pharmacological effect of *M. balsamina* extracts in rats. In support of that, Mokganya and Tshisikhawe (2019) expressed that the decoction of *M. balsamina* leaves is orally taken to stabilize blood sugar levels, showing an antidiabetic effect. This current study suggests that the bud development is the best growth stage to harvest *M. balsamina* leaves to obtain high antioxidant activity.

4.4.2 Accumulation of biochemical composition at different plant growth stages

The biosynthesis of phytochemicals in a plant is majorly dependent on its genetic make–up. Plants synthesize and gather various low and high molecular weight secondary metabolites, which have been proven to possess an antioxidant capacity. These naturally occurring biologically active compounds belong to different chemical groups such as phenols, flavonoids, anthocyanins, diterpenes, and isoflavones (Saxena *et al*., 2013). However, the concentration of many oxidative phytochemicals is significantly affected by farming practices and other environmental stress conditions such as seasonal changes, geographical location, plant maturity, soil type, and post–harvest processing, to mention just a few (Adegbaju *et al.,* 2020).

Leaves are the main organs of plants for photosynthesis and play an essential role in the life of plants. These leaves are used as synthetic and storage organs for secondary metabolites. Leaf age, harvesting season, and growth–stage all affects the distribution of secondary metabolites in medicinal plant leaves (Li *et al.,* 2020). Biosynthesis of phytochemicals starts from primary pathways, such as the glycolysis or shikimic acid pathways, and subsequently diversifies, mainly depending on cell type and developmental stage (Patra *et al.,* 2013). These compounds are widely distributed in plant cells, tissues, and organs. However, different cells, tissues, and organs of medicinal plants may possess different medicinal properties at different developmental stages (Bartwal *et al*., 2013). Developmental factors influence the initiation and subsequent differentiation of particular cellular structures involved in the biosynthesis and storage of secondary metabolites (Broun *et al.,* 2006). In addition, plant growth and development are usually elicited or inhibited by different environmental conditions. Therefore, adapting plant morphology, anatomy, and physiological functions to the changes in biotic and abiotic factors may influence the accumulation of secondary metabolites (Ma *et al.,* 2010).

Abiotic and biotic factors are the primary triggers of the biosynthesis of plant phenolics. Light is a primary factor affecting phytochemical accumulation during plant growth. Light exposure (daylength and amount of sunlight) induces the synthesis of phenolic compounds and, consequently, antioxidant activity in several leafy vegetables (Machado *et al.,* 2018). Accumulating metabolites during growth often occurs when plants are subjected to stresses involving various elicitors or signal molecules (Tesfay *et al.,* 2016). Although synthetic antioxidants have been extensively used, safety issues have been raised. The possible association of the long–term intake of these antioxidants with health issues, including skin allergies, gastrointestinal tract problems, and in some cases, increasing the risk of cancer are concerns. Antioxidants from natural sources still represent an excellent alternative to synthetic ones, despite the need for more studies to thoroughly assess their safety (Faujdar *et al.,* 2013).

4.5 Conclusion

The harvesting of *M. balsamina* leaves at different growth stages influenced the dietary antioxidant potential of *M. balsamina* leaves. Therefore, the null hypothesis of this objective is rejected. The accumulation of TPC and AA in *M. balsamina* leaves were found to be high at early growth stages but were reduced as the plant matured. However, the accumulation of DDPH was found to be high at mature growth stages. The bud development stage is considered the best stage where an adequate quality of antioxidant TPC, AA, and free-radical scavenging activity can be harmoniously attained for medicinal purposes.

CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSION

5.1 Summary of the findings

The study investigated the effect of harvesting *Momordica balsamina* at different growth stages on the nutritional and biochemical quality of its leaf powder, with focus on selected mineral nutrients, namely, Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), and Phosphorus (P) and the phytochemical properties, namely, total phenolic content (TPC), ascorbic acid (AA), and free-radical scavenging activity (DPPH). Treatments comprised six (6) harvesting stages, namely: vegetative, flower-bud development, flower initiation, fruit set, fruit ripening, and physiological maturity, served as treatments and replicated ten (10) times (n=60) for both objective 1 and 2 of the study. The vegetative stage served as the standard control. The experiment was laid out in a randomised complete block design (RCBD) and data was subjected to ANOVA. Plant growth variables measured on greenhouse grown *M. balsamina* African leafy vegetable included stem diameter (SD), vine length (VL), number of leaves (NL), leaf area (LA), chlorophyll content (CC), leaf colour [brightness (L*), redness (a*), greenness (b*) and hue (h)]. The mineral nutrients and phytochemicals were determined and recorded.

Highly significant effect was observed on the measured plant variables (VL, NS, NL and LA). However, no significant effect was observed in SD. Similarly, harvesting at different growth stages also had highly significant effect in *M. balsamina* CC and leaf colour. Treatments highly increased VL, NS, NL and LA in *M. balsamina* indigenous leafy vegetable at all developmental stages. Chlorophyll content increased at all growth stages but experienced a decline at maturity stage. With regard to the leaf colour, L* and h of the leaves also declined with the growth stages. In contrast, a* and b* of *M. balsamina* were reduced at early developmental stages, but when the plants started setting fruits, the two variables increased up until physiological maturity.

Harvesting of *M. balsamina* leaves at different growth stages again influenced the nutritional quality of the leaf powder. The accumulation of K, P and Zn in *M.*

balsamina leaves were found to be high at early growth stages but were reduced at mature stages of the leafy vegetable. Interestingly, Fe was increased only when the leaves were harvested at the bud development stage but was reduced in all the other stages. Contrary to the latter, Ca and Mg, were increased from the early vegetative stage throughout to physiological maturity. *Momordica balsamina* leaves showed higher contents of K, Ca and Mg. Contrarily, contents of P, Fe and Z were lower in *M. balsamina* leaves as compared to most consumed African leafy vegetables.

The tested biochemical compounds of *M. balsamina* leaves in this study, were also highly affected by harvesting the leafy vegetable at different growth stages. The accumulation of TPC and AA was decreasing as the plant developed into other growth stages, except for TPC at the physiological maturity stage. Contrarily, *M. balsamina* leaves showed some retention of the radical scavenging activity (DPPH) at every developmental stage.

5.2 Significance of the findings

Informally, *M. balsamina* indigenous leafy vegetable crop is known and utilised as a leafy vegetable and medicinal plant in many African rural communities, from the knowledge that was passed down from earlier generation's to-date. Somehow, it is known to users, that at a particular growth stage, a certain quantity of *M. balsamina* concoctions and relishes may be consumed successfully or cause unfortunate incidences of diarrhoea or stomach cramps. However, no empirical evidence has been established for a harmonious and safer plant stage for harvesting the crop for consumption, either for nutraceutical or pharmaceutical purposes. A case of severe epigastric pain and haematemesis in a 40-year-old man was previously documented with a history of within half an hour of drinking about half a litre of homemade *Momordica charantia* liquid extract for blood purification (Nadkarni, 2010). Such reports can express the importance of the understanding of the concentration and quality of various nutritional elements and biochemical qualities that can significantly set a tone, for the correct dosage recommendations for the consumption of indigenous leafy vegetables like *M. balsamina*, especially for effective medicinal purpose, to avoid any unfortunate body reactions. The findings of this study will contribute to the pharmaceutical and nutraceutical industry, by knowing at what stage to source various components from this crop, in order to assist modern and traditional health practitioners for the safe recommendation and consumption of *M balsamina* at a particular stage according to empirical findings. Most importantly, findings of this study will be disseminated in journals and presented at various national and international scientific conferences in order to encourage the domestication, subsistence and commercial farming of the leafy vegetable. Backyard gardens will also be motivated in most households and subsequently the appropriate harvest at a particular descriptive stage.

5.3 Recommendations

Momordica balsamina amongst other African leafy vegetables can potentially make a considerable contribution towards the requirements for nutrients and can offer a great antioxidant which are micronutrients of public health significance in South African healthcare. According to the findings in this study, it can be recommended that the early stages, particularly the vegetative, bud development and flowering stages are appropriate stages to consume the *M. balsamina* leaf powder as an effective medicinal plant to provide nutritional stability and manage various ailments, as it offers a well-balanced quality of all the respective nutritional and biochemical components. This recommendation is gratified towards consumption by rural community members, and it is to scientifically back up prescriptions made by health practitioners. The leaves at the early growth stages of *M. balsamina* could effectively be incorporated into the production of value-added nutraceuticals for better therapeutic activity, and effective nutraceutical *M. balsamina* powder can be developed from leaves harvested from early growth stages before its fruit setting stage.

5.4 Conclusion

In conclusion, different plant morphological growth stages do indeed entail different concentrations of various chemical properties. Harvesting at different growth stages of a plant are significant in providing a visual indication of the correct time, in which the concentration of a particular health beneficial chemical compound is at peak, average or at its lowest, to facilitate effectiveness and safe utilisation of a particular crop. Different stages are shown by distinctive morphological characteristics, such as buds, flowers, fruits, and measurable traits, such as number of leaves, and their leaf colour. Nutrient and biochemical concentrations of *M. balsamina* were influenced by the plant's developmental growth stages when harvested. These findings depicted that there were significant changes in the level of selected nutrient elements and biochemical components. It could be that the leaves at the early growth stages of *M. balsamina* effectively incorporated into the production of value-added nutraceuticals for better therapeutic activity according to dosage recommendations. However, it is essential to note that the required minimum daily amounts of antioxidants are yet to be investigated. Although the potential biochemical effectivity (highest) of *M. balsamina* leaf powder at various stages is suggested, its safety is still in question. Different growth conditions may vary the nutritional content. Future studies may invest in establishing the influence of growth stages on the active medicinal agents and antinutrients that possibly contribute to toxicity of *M. balsamina.*

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APPENDICES

Appendix 3.1: Analysis of variance for stem diameter (cm) of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	22.603	2.51144		
Trt	5	20.432	4.08631	1.51	0.2047
Error	45	121.517	2.70039		
Total	59	164.552			

Appendix 3.2: Analysis of variance for vine length (cm) of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	3803	422.5		
Trt	5	202213	40442.6	136.40	0.0000
Error	45	13343	296.5		
Total	59	219359			

Appendix 3.3: Analysis of variance for number of shoots of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	41.27	4.585		
Trt	5	950.53	190.107	89.92	0.0000
Error	45	95.13	2.114		
Total	59	1086.93			

Appendix 3.4: Analysis of variance for number of leaves of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	5170	574.5		
Trt	5	108694	21738.9	71.27	0.0000
Error	45	13726	305.0		
Total	59	127591			

Appendix 3.5: Analysis of variance for leaf area (LA) (cm^2) of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	423.60	47.07		
Trt	5	7221.40	1444.28	50.44	0.0000
Error	45	1288.60	28.64		
Total	59	8933.60			

Appendix 3.6: Analysis of variance for chlorophyll content of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	116.72	12.969		
Trt	5	502.91	100.583	3.90	0.0051
Error	45	1161.74	25.816		
Total	59	1781.37			

Appendix 3.7: Analysis of variance for brightness (L*) of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	61.842	6.8714		
Trt	5	112.683	22.5365	6.09	0.0002
Error	45	166.576	3.7017		

Total	59	341.101			

Appendix 3.8: Analysis of variance for redness (a*) of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	13.466	1.4962		
Trt	5	142.929	28.5859	24.54	0.0000
Error	45	52.423	1.1650		
Total	59	208.819			

Appendix 3.9: Analysis of variance for greenness (b*) of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	34.711	3.8568		
Trt	5	417.150	83.4299	27.38	0.0000
Error	45	137.118	3.0471		
Total	59	588.978			

Appendix 3.10: Analysis of variance for hue of *Momordica balsamina* harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	109.33	12.148		
Trt	5	728.03	145.606	20.26	0.0000
Error	45	323.36	7.186		
Total	59	1160.72			

Appendix 3.11: Analysis of variance for Potassium (K) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	2948799	327644		
Trt	5	1.267E+07	2533636	9.77	0.0000
Error	45	1.167E+07	259414		
Total	59	2.729E+07			

Appendix 3.12: Analysis of variance for Calcium (Ca) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	1387	154.1		
Trt	5	171924	34384.7	142.57	0.0000
Error	45	10853	241.2		
	59	184164			

Appendix 3.13: Analysis of variance for Magnesium (Mg) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	356.15	39.572		
Trt	45	4329.66	865.932	23.39	0.0000
Error	59	1666.28	37.028		
Total	59	6352.08			

Appendix 3.14: Analysis of variance for Zinc (Zn) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	4.886	0.5429		
Trt	5	210.125	42.0251	168.86	0.0000
Error	45	11.199	0.2489		
Total	59	226.211			

Appendix 3.15: Analysis of variance for Iron (Fe) (mg/L) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	9.049	1.005		
Trt	5	593.876	118.775	250.48	0.0000
Error	45	21.338	0.474		
Total	59	624.264			

Appendix 3.16: Analysis of variance for Phosphorus (P) (mg/L) of *Momordica*

balsamina leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	9	212.4	23.60		
Trt	5	22683.1	4536.62	303.03	0.0000
Error	45	673.7	14.97		
Total	59	23569.1			

Appendix 4.1: Analysis of variance for total phenolic content (TPC) (mg/100g) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	4	484.8	121.2		
Trt	5	72815.4	14563.1	100.18	0.0000
Error	20	2907.4	145.4		
Total	29	76207.6			

Appendix 4.2: Analysis of variance for ascorbic acid (AA) (mg/100g) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	4	7.03	1.758		
Trt	5	1529.83	305.965	87.20	0.0000
Error	20	70.17	3.509		
Total	29	1607.03			

Appendix 4.3: Analysis of variance for antioxidant activity (DPPH) (mg/g) of *Momordica balsamina* leaf powder harvested at different growth stages.

Source	DF	SS	MSS	F	P
Rep	4	0.01365	0.00341		
Trt	5	1.42315	0.28463	50.92	0.0000
Error	20	0.11179	0.00559		
Total	29	1.54859			