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Nutritional Composition and Phytochemical Properties of Underutilized Indigenous Edible Plants (*Momordica balsamina* and *Hypoxis hemerocallidea*) from KwaZulu-Natal, South Africa

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Abstract

Food and nutrition insecurities remain major challenges in rural communities, highlighting the need to identify affordable and nutrient-dense indigenous food resources. Therefore, this study evaluated the nutritional composition, antioxidant activity, and phytochemical characteristics of *Momordica balsamina* and *Hypoxis hemerocallidea* collected from KwaZulu-Natal, South Africa. In this study, proximate composition, mineral content, antioxidant activity, FT-IR spectroscopy, and LC-MS analyses were conducted using standard analytical procedures. Results showed that *M. balsamina* contained significantly ($p < 0.05$) higher crude protein, fibre, ash, potassium, calcium, magnesium, total phenolics, flavonoids, carotenoids, and DPPH radical scavenging activity than *H. hemerocallidea*. In contrast, *H. hemerocallidea* exhibited higher carbohydrate and food energy content, indicating its value as an energy-rich indigenous food source. Both species demonstrated strong antioxidant activity, with DPPH inhibition exceeding 80%, confirming the presence of bioactive phytochemicals indicating antioxidant potential. FT-IR analysis identified several functional groups associated with phenolic compounds, flavonoids, and terpenes, while LC-MS analysis confirmed the presence of hypoxoside in *H. hemerocallidea* and suggested that detected ions are consistent with reported *M. balsamina* compounds without claiming confirmation of novel phytochemicals in the absence of MS/MS fragmentation and structural validation. These findings highlight the nutritional and nutraceutical potential of both plant species and support their promotion as affordable indigenous food resources capable of contributing to food, nutrition, and health security in rural communities.

Keywords: antioxidant properties; food diversification; functional foods; phytotherapeutic potential; plant metabolites; rural nutrition; traditional medicinal plants

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1. Introduction

Food and nutrition insecurity remains one of the most serious challenges affecting rural communities in developing countries [1], including South Africa (SA), where rainfed

subsistence agriculture remains the dominant farming system. These communities are characterized by high unemployment, limited access to resources, and widespread poverty, all of which reduce their ability to obtain adequate and nutritious food. Moreover, rural populations also experience an uneven burden of diet-related non-communicable diseases together with undernutrition and overnutrition, a condition commonly referred to as the triple burden of malnutrition [2,3]. Therefore, this reflects the complexity of food insecurity, where poor dietary diversity, rather than food quantity alone, contributes significantly to negative health outcomes. Historically, global food systems have relied heavily on a narrow range of staple crops, mainly wheat, maize, and rice, to address hunger and food shortages [4]. Although these crops have contributed substantially to global food production, their continued dominance is increasingly insufficient to meet the nutritional demands of a rapidly growing population [4]. Consequently, reliance on a limited number of crops contributes to hidden hunger, where caloric intake may be adequate while deficiencies in essential micronutrients and phytochemicals persist [5]. This highlights the urgent need to diversify food systems and promote alternative nutrient-rich food sources capable of improving long-term food and nutrition security.

In response to these challenges, agricultural diversification has increasingly been promoted as a strategy to improve food security, nutrition, and rural livelihoods [6,7]. Within this context, indigenous and underutilized plant species are receiving renewed attention because of their nutritional richness, adaptability to harsh environments, and long-standing use in traditional food and medicinal systems [8]. These species are naturally adapted to diverse agroecological conditions and have supported rural communities for generations through their dual role as food and medicine [6,7]. However, many of these plants became neglected following the expansion of conventional agriculture and increased dependence on synthetic pharmaceuticals, which gradually displaced indigenous knowledge systems and traditional dietary practices [8,9]. As a result, there is growing scientific interest in reevaluating indigenous edible plants because of their potential contribution to nutrition, health, and nutraceutical development.

Among these underutilized species, plants belonging to the *Momordica* genus have long been recognized for their importance in traditional and folk medicine [10]. For centuries, *Momordica* species have been used in the management of several conditions, including hypertension, obesity, and cancer [11]. Likewise, *Momordica balsamina* has been widely utilized in traditional medicine, where the fruits, leaves, and seeds are prepared as decoctions, infusions, poultices, and herbal teas for therapeutic purposes [12]. Different plant parts of *M. balsamina* have also demonstrated anti-diabetic, anti-plasmodial, antiviral, anti-inflammatory, analgesic, shigellocidal, anti-diarrheal, antiseptic, and antimicrobial activities, highlighting its broad pharmacological value [9]. In addition to its medicinal relevance, *M. balsamina* has attracted considerable interest in nutraceutical sciences because of its favorable nutritional composition. Previous studies reported that the leaves contain approximately 17 amino acids together with substantial mineral concentrations, high protein (288 g/kg), and fat (54 g/kg) contents, while maintaining relatively low fiber levels (37 g/kg) [13]. Furthermore, *M. balsamina* leaves were reported to contain 127.00 g/kg ash, 53.70 g/kg fat, 287.70 g/kg protein, and 37.20 g/kg crude fiber, emphasizing their nutritional significance [13].

Likewise, *Hypoxis hemerocallidea*, commonly known as the African potato, occupies an important place in African traditional medicine systems [14]. However, the common name "African potato" can be misleading because many people assume that the species is edible. In some parts of South Africa, particularly the Northwest Province, *H. hemerocallidea* has nevertheless been reported among indigenous and traditional food crops consumed by local communities [15]. The species may also be confused with the edible *Plectranthus esculentus*,

since both plants share the common name "African potato" [16]. Although *H. hemerocallidea* contains relatively low crude protein concentrations and limited accumulation of some essential mineral elements compared to recommended daily human requirements [17], the species contains relatively high iron concentrations, making it a potentially valuable resource for addressing iron deficiencies [18]. In addition, *H. hemerocallidea* has been used traditionally for the management of several conditions, including diabetes, arthritis, burns, tuberculosis, and certain cancers [17,19–21].

Despite the recognized nutritional and medicinal importance of both species, comprehensive information on their nutritional composition, mineral profile, antioxidant properties, and phytochemical composition remains limited, particularly for plant materials collected from KwaZulu-Natal, South Africa. Although environmental factors such as soil type, climate, rainfall, and geographical location are known to influence plant nutrient composition and secondary metabolite accumulation [22,23], the present study was not designed to evaluate these effects because samples were collected from a single location during one collection period. Therefore, future multi-location and multi-season studies are needed to quantify environmental influences on plant composition. Accordingly, the present study comprehensively evaluated the nutritional composition, mineral content, antioxidant properties, FT-IR spectra, and untargeted LC-MS metabolomic profiles of *M. balsamina* and *H. hemerocallidea* collected from KwaZulu-Natal, providing baseline comparative data for these underutilized indigenous edible plants.

2. Materials and Methods

2.1. Chemicals and Materials

All chemicals used in this study were of analytical grade. Nitric acid (70% v/v), hydrochloric acid (32% v/v), ethanol (>95%), and petroleum ether (>99%) were sourced from Sigma-Aldrich (Durban, South Africa). An ICP multi-element standard solution (1000 mg/L) prepared in 5% nitric acid was procured from Sigma-Aldrich (Johannesburg, South Africa). All chemicals were used as received without further purification. Ultra-high-purity water was obtained from a Milli-Q RO4 purification system (Millipore, Bedford, MA, USA) and used throughout all analytical procedures.

2.2. Collection of Plant Material

Momordica balsamina leaves and *H. hemerocallidea* corms were collected in January 2024 from Cedara College of Agriculture, located within the uMgungundlovu District Municipality, KwaZulu-Natal, South Africa. The precise geographical coordinates of the sampling site were recorded using a Global Positioning System (GPS) device as −29.549769, 30.256531 (Figure 1). The sampling site is characterized by a subtropical climate, with summer (wet season) temperatures ranging from 21 °C to 38 °C and winter (dry season) temperatures from 10 °C to 20 °C. Mean annual precipitation ranges from 838 to 1140 mm, providing favourable conditions for the natural growth of both plant species. Plant material was collected from 10 healthy, mature individuals of each species at the mature growth stage. The leaves of *M. balsamina* were harvested using secateurs, whereas the corms of *H. hemerocallidea* were carefully excavated using a stainless-steel digging fork to minimise physical damage. Samples from each species were pooled to obtain a representative composite sample before analysis. Following collection, the pooled samples were individually packed into clean polyethylene bags, placed in 25 L cooler boxes to maintain freshness, and transported promptly to the laboratory for further processing. All laboratory analyses were performed in triplicate.

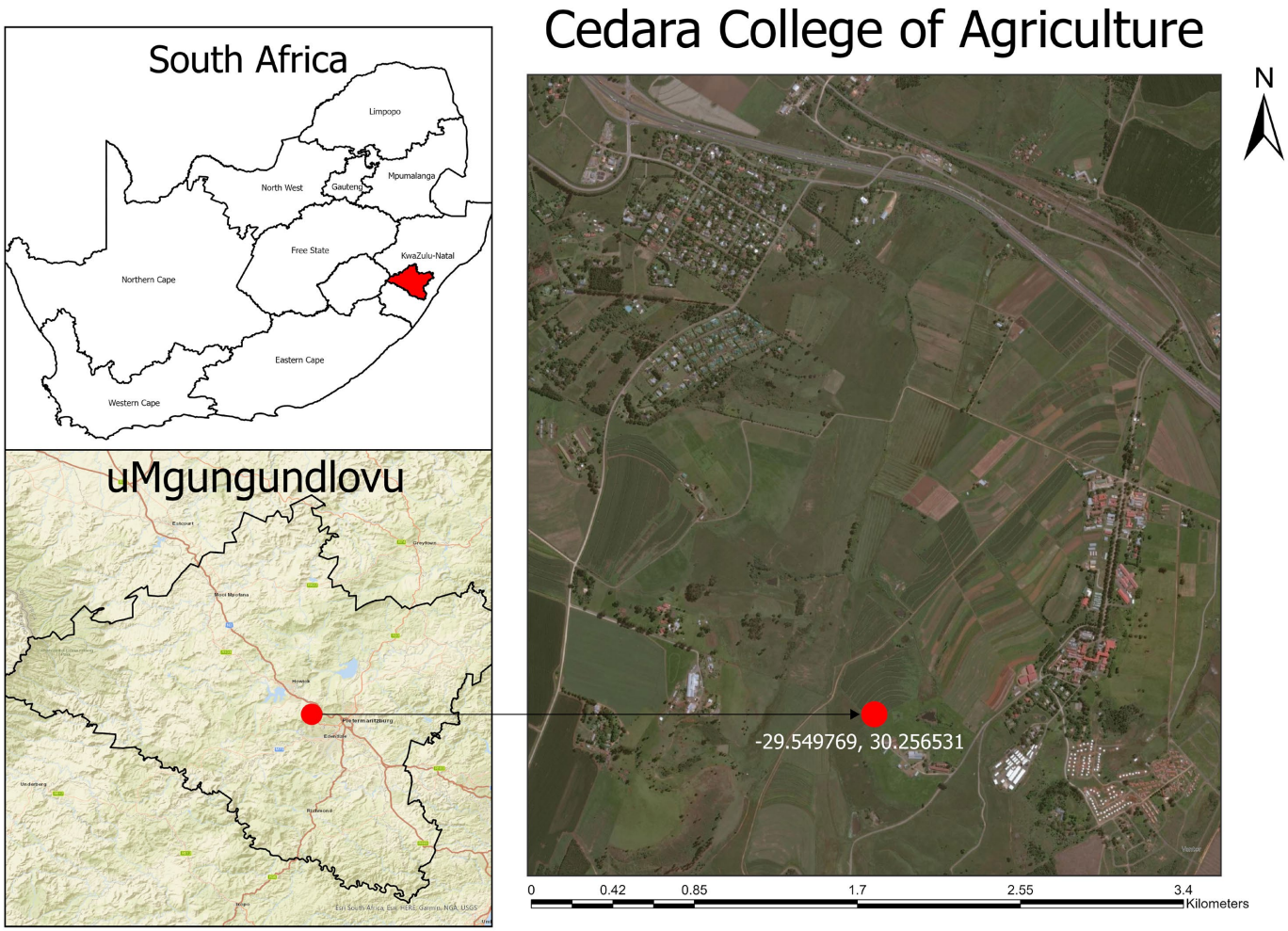


Figure 1. Location of the plant sampling site at Cedara College of Agriculture, KwaZulu-Natal, South Africa.

2.3. Nutritive Value

2.3.1. Proximate Composition

The nutritional composition of both plant species was determined through proximate analysis, encompassing crude protein, crude fat, dietary fibre, total mineral matter (ash), total carbohydrates, and food energy content. All analyses were performed in triplicate to ensure reproducibility and reliability of results. Crude protein content was determined using a LECO TruSpec Nitrogen Analyser (LECO Corporation, St. Joseph, MI, USA) following the Association of Official Analytical Chemists (AOAC) official method, 990.03 [24]. Plant samples were loaded into a combustion chamber via an autoloader and subjected to complete combustion at 950 °C. The nitrogen released was quantified and the percentage crude protein content was subsequently calculated by applying the nitrogen-to-protein conversion factor of 6.25, as expressed in Equation (1).

$$\% \text{ Crude protein} = \%\text{N} \times 6.25 \quad (1)$$

Crude fat content was determined gravimetrically using a Büchi Soxhlet fat extractor (BÜCHI Labortechnik AG, Flawil, Switzerland) with petroleum ether as the extraction solvent, following AOAC official method 920.39 [24]. The percentage crude fat was calculated using Equation (2).

$$\% \text{ fat} = \frac{\text{beaker} + \text{fat} - \text{beaker}}{\text{sample mass}} \times 100 \quad (2)$$

Dietary fibre was quantified as neutral detergent fibre (NDF) following the Van Soest method [25]. Briefly, 0.5 g of each plant sample was accurately weighed into a sintered glass crucible, to which marble buffer beads and 50 mL of neutral detergent solution (NDS) were added. The NDS was prepared by dissolving 45.3 g disodium tetraborate, 124 g ethylene diamine tetra-acetic acid (EDTA), 30.4 g disodium hydrogen phosphate, and 200 g sodium lauryl sulphate in distilled water, with 67 mL of 2-ethoxyethanol added thereafter. The crucible was placed into a digestive block maintained at 110 °C, after which 1 mL of termamyl (α-amylase) enzyme was added and the contents were allowed to digest for 70 min. The crucible was subsequently transferred to a draining rack, and the residue was washed with boiling water under vacuum suction until completely free of NDS, followed by a final rinse with acetone. The washed residue was dried in an oven at 105 °C for 4 h, cooled in a desiccator, and weighed. The NDF content was calculated using Equation (3).

$$\% \text{ Fiber} = \frac{(\text{crucible} + \text{dry residue}) - (\text{crucible} + \text{ash})}{\text{sample mass}} \times 100 \quad (3)$$

Total mineral matter (ash) content was determined by high-temperature incineration following AOAC method 942.05 [24]. Weighed samples were placed in a muffle furnace at 550 °C for 12 h to ensure complete volatilization of all organic matter. The remaining inorganic ash residue was used to calculate the ash content, as expressed in Equation (4).

$$\% \, ash = \frac{(\text{mass of the sample} + \text{crucible after ashing}) - (\text{mass of pre} - \text{dried crucible})}{(\text{mass of sample} + \text{crucible}) - (\text{mass of pre} - \text{dried crucible})} \quad (4)$$

Total carbohydrate content was estimated by difference, calculated as the remainder after subtracting the percentages of crude protein, crude fat, dietary fibre, and ash from 100. Food energy was subsequently calculated from the macronutrient composition according to Equation (5) [26], where FE denotes food energy expressed in kJ/100 g and CHO denotes total carbohydrates.

$$\text{FE} = (\%\text{fat} \times 9) + (\%\text{Protein} \times 4) + (\%\text{CHO} \times 4) \quad (5)$$

2.3.2. Mineral Composition

The mineral composition of both plant species was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) following an ultrasonic-assisted acid digestion procedure [27]. Briefly, 0.5 g of each powdered plant sample was accurately weighed into a 100 mL Erlenmeyer flask, and 5 mL of concentrated nitric acid (HNO_3, 70% v/v) was added. The mixture was ultrasonicated (Science Tech, Johannesburg, South Africa) at 80 °C for 22.5 min, after which an additional 5 mL of HNO_3 was added and ultrasonication continued for a further 22.5 min. The digest was allowed to cool, filtered through Whatman 70 mm filter paper, and centrifuged at 3000 rpm for 10 min. The resulting supernatant was transferred into a 100 mL volumetric flask and made up to volume with ultrapure water prior to analysis. Mineral concentrations were quantified using a Varian 720-ES ICP-OES instrument (Varian Inc., Johannesburg, South Africa) operating with a pneumatic concentric nebulizer at 15 rpm, argon carrier gas flow of 0.75 L/min, RF power of 1.00 kW, and a frequency of 40 MHz. All results were expressed in micrograms per gram dry mass (μg g^{-1} DM).

2.4. Sample Extraction

A 0.5 g of each plant sample was accurately weighed and extracted with 7 mL of 80% ethanol. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C and the resulting supernatant was collected for the determination of total phenolic content, total flavonoid

content, and DPPH radical scavenging activity. All spectrophotometric measurements were performed using a Cary 50 UV-Vis spectrophotometer (Varian Inc., Durban, South Africa).

2.5. Total Flavonoid

Total flavonoid content was determined using the aluminum chloride colorimetric method described by Jia et al. [28] with slight modifications. An aliquot of 60 µL of plant extract was transferred into a 10 mL test tube containing 2 mL of distilled water. Subsequently, 150 µL of 5% $NaNO_2$ solution was added and the mixture was allowed to stand for 5 min. This was followed by the addition of 0.75 mL of 10% $AlCl_3$ solution and a further standing period of 6 min. Finally, 2 mL of 1.0 M NaOH was added and the mixture was vortexed for 10 s. Absorbance was measured at 510 nm against a prepared reagent blank and total flavonoid content was expressed as catechin equivalents (CE) µg g^{-1} DM. All analyses were performed in triplicate.

2.6. Total Phenolic

Total phenolic content was determined using the Folin–Ciocalteu method described by Singleton et al. [29] with slight modifications. An aliquot of 100 µL of the plant extract was added to 0.5 mL of the Folin–Ciocalteu phenol reagent diluted 10-fold with distilled water and incubated for 2 min. Subsequently, 1 mL of the 7.5% sodium carbonate solution was added, the mixture was vortexed, and the reaction was allowed to proceed for 5 min at 27 °C. Absorbance was measured at 760 nm against a methanol blank. All analyses were performed in triplicate.

2.7. DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of each plant extract was evaluated using the DPPH assay described by Brand-Williams et al. [30] with slight modifications. An aliquot of 100 µL of plant extract was mixed with 1.9 mL of DPPH solution, prepared by dissolving 4.73 mg of DPPH in 100 mL of HPLC-grade methanol. The mixture was incubated in the dark for 1 h at 27 °C to allow the scavenging reaction to reach completion. The reduction in absorbance was measured spectrophotometrically at 517 nm against a methanol blank, with 100 µL of pure methanol serving as the negative control. Results were expressed as percentage inhibition, and all analyses were performed in triplicate.

2.8. Ascorbic Acid

Ascorbic acid content was determined spectrophotometrically according to the method described by Klein and Perry [31] with slight modifications. One gram of each plant sample was extracted with 10 mL of 1% metaphosphoric acid and allowed to stand for 45 min at 27 °C. An aliquot of 1 mL of the filtrate was subsequently mixed with 9 mL of 2,6-dichlorophenolindophenol (DCPIP) dye and absorbance was measured immediately at 515 nm against a reagent blank to minimize dye oxidation. Ascorbic acid content was quantified using a calibration curve constructed from a standard L-ascorbic acid stock solution serially diluted to concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg g^{-1}. Results were expressed as µg g^{-1} DM.

2.9. Total Carotenoid

Total carotenoid content was determined following the method described by Bambalele et al. [32] with slight modifications. One gram of each plant sample was accurately weighed into a 50 mL falcon tube and mixed with 14 mL of hexane: acetone (3:2, v/v). The mixture was incubated in the dark for 90 min to prevent photo-oxidative degradation of carotenoids. The mixture was subsequently centrifuged at 10,000 rpm for 10 min at 4 °C using an Eppendorf centrifuge 5417R (Johannesburg, South Africa). The resulting

supernatant was carefully transferred into a volumetric flask and adjusted to 25 mL with the hexane: acetone solvent mixture. Absorbance was measured at 450 nm against a hexane: acetone blank using a Cary 50 UV-Vis spectrophotometer (Varian Inc., Durban, South Africa). Total carotenoid content was expresses as β-carotene (ε 139,000 L mol^{-1} cm^{-1}, MW 536.87) in μg g^{-1} DM.

2.10. Anthocyanin

Approximately 30 mg of each plant sample was accurately weighed into a 15 mL falcon tube and 400 μL of distilled water was added. The samples were boiled in a water bath at 100 °C for 30 min and subsequently allowed to cool to room temperature. An extraction buffer (2 mL) was then added to each sample, where the buffer was prepared by mixing 2 mL distilled water, 94.8 mL of 95% ethanol, and 3.2 mL of 37% hydrochloric acid per 100 mL. The sample solutions were vortexed and left agitating overnight to ensure complete extraction. The samples were then centrifuged at 13,000 rpm for 15 min and the supernatants collected. To maximize extraction efficiency, 1 mL of fresh extraction buffer was added to each pellet, vortexed, and left agitating for two hours, followed by centrifugation at 13,000 rpm for 15 min. The two supernatants were combined and subjected to a final centrifugation at 13,000 rpm for 30 min prior to spectrophotometric analysis. Absorbance was measured at 530 nm using a Cary 50 UV-Vis spectrophotometer (Varian Inc., Durban, South Africa), with the extraction buffer serving as the blank. Total anthocyanin content was expressed as cyanidin 3-glucoside equivalents (ε 26,900 L mol^{-1} cm^{-1}, MW 484.82) in μg g^{-1} DM [33].

2.11. FT-IR Spectral Analysis

Fourier transform infrared (FT-IR) spectral analysis was performed using a Bruker Alpha II FTIR spectrophotometer (Bruker Optics GmbH, Ettlingen, Germany) equipped with an attenuated total reflectance (ATR) Diamond single-bounce disc. Each plant sample was placed directly onto the sample holder and scanned over the wavenumber range of 400 to 4000 cm^{-1} at a spectral resolution of 4 cm^{-1}, with 31 background and sample scans accumulated per spectrum. Absorption frequencies were recorded and expressed as υmax cm^{-1}. The collected spectral data was subsequently processed using OPUS Spectroscopy version 8.5 Software for baseline correction, smoothing, and peak labelling.

2.12. Phytochemical Screening by Liquid Chromatography–Mass Spectrometry (LC-MS)

Phytochemical profiling of *M. balsamina* and *H. hemerocallidea* was conducted using LC-MS (Shimadzu Corporation, Kyoto, Japan) following vacuum liquid chromatography (VLC) fractionation. The LC parameters were configured with a column temperature of 50 °C and an ambient autosampler temperature of 25 °C. The mobile phase was delivered at a flow rate of 0.2 mL/min, with a total run time of 23 min. A 5 μL injection volume was used, and all samples were filtered through a 0.2 μm membrane filter prior to analysis. The MS was operated in positive electrospray ionization mode, with a mass detection range of *m/z* 50–1200. The ion source temperature was set to 100 °C, while the desolvation (evaporation) temperature was maintained at 350 °C. Low-energy scans were acquired at 4 V, whereas high-energy scans were performed over a collision energy range of 25–50 V.

Dried and pulverized plant material from both species was extracted with dichloromethane-methanol (DCM-MeOH, 1:1 *v*/*v*) to obtain crude extracts. The extracts were subsequently fractionated by VLC using solvent systems of increasing polarity, namely DCM-EtOAc, EtOAc, EtOAc-MeOH, and MeOH. Based on previously reported phytochemicals in the literature, the EtOAc and EtOAc-MeOH fractions were selected for *M. balsamina*, while the EtOAc-MeOH fraction was selected for *H. hemerocallidea*, owing to the high polarity of hypoxoside, the principal bioactive compound of this species. Sample solutions of 400 ppm

in methanol were prepared for both species and analyzed by LC-MS using an isocratic mobile phase of acetonitrile-water (70:30, *v*/*v*) over a 45-min elution period.

2.13. Statistical Analysis

All data were subjected to statistical analysis using IBM SPSS Statistics software (version 27.0, IBM Corp., Armonk, NY, USA). Prior to analysis, data normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. The data were confirmed to be normally distributed, with homogeneous variances across groups, satisfying the assumptions for parametric analysis. Consequently, one-way analysis of variance (ANOVA) was performed to determine significant differences among groups, followed by Tukey's Honest Significant Difference (HSD) post hoc test for pairwise mean comparisons. All analyses were conducted in triplicate, and results were expressed as mean values ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Nutritional Composition and Energy Content

The nutritional composition and energy content of *M. balsamina* leaves and *H. hemerocallidea* tuber are presented in Table 1. *Momordica balsamina* recorded significantly ($p < 0.05$) higher crude protein (14.51 ± 0.12%), crude fat (3.15 ± 0.04%), ash (12.17 ± 0.11%), and fibre (38.96 ± 0.05%) compared to *H. hemerocallidea*, which yielded 3.55 ± 0.03%, 1.83 ± 0.12%, 3.89 ± 0.20%, and 9.72 ± 0.15%, respectively. Contrarywise, *H. hemerocallidea* recorded a significantly ($p < 0.05$) higher total carbohydrate content (81.01 ± 1.01%) compared to *M. balsamina.* Food energy was not significantly ($p > 0.05$) between *H. hemerocallidea* (344.67 ± 0.11 kJ/100g) compared to *M. balsamina* (203.23 ± 0.20 kJ/100g).

Table 1. Mean results (±SEM) of nutritional composition and energy content of *Momordica balsamina* leaves and *Hypoxis hemerocallidea* tubers.

Items	*M. balsamina*	*H. hemerocallidea*
Ash (%)	12.17 ± 0.11 ^{a}	3.89 ± 0.20 ^{b}
Fat (%)	3.15 ± 0.04 ^{a}	1.83 ± 0.12 ^{b}
Protein (%)	14.51 ± 0.12 ^{a}	3.55 ± 0.03 ^{b}
Fibre (%)	38.96 ± 0.05 ^{a}	9.72 ± 0.15 ^{b}
Carbohydrate (%)	31.21 ± 0.01 ^{a}	81.01 ± 1.01 ^{b}
Food energy kJ/100g	203.23 ± 0.20 ^{a}	344.67 ± 0.11 ^{a}

Values with different superscript letters within the same row are significantly different ($p < 0.05$).

3.2. Method Performance

The ICP instrument was relatively sensitive towards the studied elements with the detection limits based on the amount of sample (0.5 g) and the volume (10 mL) of acid for digestion. The method detection limits for each of the studied elements in μg g^{-1} were as follows; Al (0.0012), As (0.0056), Ca (0.42), Cd (0.00032), Co (0.02), Cr (0.381), Cu (0.05), Fe (0.0083), K (0.16), Li (0.15), Mg (1.42), Mn (0.028), Na (0.0432), Ni (0.012), Pb (0.015), Se (0.14) and Zn (0.117). The coefficients of determination, R^2 for instrument calibration ranged between 0.9902 and 0.9999 demonstrating good linearity. The RSD values were within the acceptable limit of 20%. All samples were analysed in triplicate.

3.3. Mineral Composition

The mineral composition of both plant species is presented in Table 2. Potassium (K) was the most abundant mineral detected in both species, with *M. balsamina* record-

ing a higher K content of 2.940 ± 0.20% compared to 0.001 ± 0.03% in *H. hemerocallidea*. *Momordica balsamina* also recorded higher calcium (Ca) at 0.007 ± 0.01%, magnesium (Mg) at 0.001 ± 0.12%, selenium (Se) at 0.34 ± 0.03 μg g^{-1}, and zinc (Zn) at 0.31 ± 0.01 μg g^{-1} compared to *H. hemerocallidea*, which yielded 0.0003 ± 0.22%, 0.0002 ± 0.05%, 0.25 ± 0.15 μg g^{-1}, and 0.22± 0.35 μg g^{-1}, respectively. Sodium (Na) and manganese (Mn) were higher in *H. hemerocallidea* at 1.78 ± 0.20 and 0.73 ± 0.01 μg g^{-1} compared to *M. balsamina* at 0.44 ± 0.03 and 0.06 ± 0.03 μg g^{-1}, respectively. Iron (Fe) was detected at equal concentrations of 0.02 μg g^{-1} in both species. Copper (Cu) and lead (Pb) were not detected in either species.

Table 2. Mean results (±SEM) of mineral composition of *Momordica balsamina* leaves and *Hypoxis hemerocallidea tuber.*

Items	WHO Guidelines	*M. balsamina* (μg g^{-1})	*H. hemerocallidea* (μg g^{-1})
Microminerals (% DM)			
Na	-	0.044 ± 0.03 ^{b}	0.0002 ± 0.20 ^{a}
K	-	2.940 ± 0.20 ^{a}	0.001 ± 0.03 ^{b}
Mg	-	0.001 ± 0.12 ^{a}	0.0002 ± 0.05 ^{b}
Ca	-	0.007 ± 0.01 ^{a}	0.0003 ± 0.22 ^{b}
Microminerals (μg g^{-1} DM)			
Al	-	0.09 ± 0.02	0.06 ± 0.01
As	0.5	0.02 ± 0.15	0.03 ± 0.03
Cd	0.2	0.01 ± 0.02	-
Co	50	0.08 ± 0.10	0.08 ± 0.04
Cr	5	0.01 ± 0.01	0.01 ± 0.02
Cu	40	-	-
Fe	450	0.02 ± 0.02	0.02 ± 0.11
Li	-	0.24 ± 0.01	0.24 ± 0.12
Mn	500	0.06 ± 0.03 ^{b}	0.73 ± 0.01 ^{a}
Ni	67	0.06 ± 0.01	0.07 ± 0.02
Pb	0.3	-	-
Se	-	0.34 ± 0.03 ^{a}	0.25 ± 0.15 ^{b}
Zn	60	0.31 ± 0.01 ^{a}	0.22 ± 0.35 ^{b}

Values with different superscript letters within the same row are significantly different ($p < 0.05$). (-) = not detected.

3.4. Antioxidant

The antioxidant capacity of both plant species is presented in Table 3. *Momordica balsamina* recorded significantly ($p < 0.05$) higher total phenolic content at 3036 ± 0.09 μg g^{-1} compared to *H. hemerocallidea* at 289 ± 0.03 μg g^{-1}. Total flavonoid content was also higher in *M. balsamina* at 3606 ± 0.12 μg g^{-1} compared to 2454 ± 0.11 μg g^{-1} in *H. hemerocallidea*. Total anthocyanin content was higher in *M. balsamina* at 101 ± 0.35 μg g^{-1} compared to 75 ± 0.20 μg g^{-1} in *H. hemerocallidea*. The DPPH radical scavenging activity was higher in *M. balsamina* at 89 ± 0.02% inhibition compared to 82 ± 0.05% in *H. hemerocallidea.*

Table 3. Mean results (±SEM) of antioxidant capacity of *Momordica balsamina* leaves and *Hypoxis hemerocallidea* tubers.

Items	*M. balsamina* (μg g^{-1})	*H. hemerocallidea* (μg g^{-1})
DPPH (% inhibition)	89 ± 0.02 ^{a}	82 ± 0.05 ^{b}
Total flavonoids (μg g^{-1})	3606 ± 0.12 ^{a}	2454 ± 0.11 ^{b}
Total anthocyanin (μg g^{-1})	101 ± 0.35 ^{a}	75 ± 0.20 ^{c}
Total phenolic (μg g^{-1})	3036 ± 0.09 ^{a}	289 ± 0.03 ^{b}

Values with different superscript letters within the same row are significantly different ($p < 0.05$).

3.5. Ascorbic Acid and Total Carotenoids

M. balsamina recorded significantly ($p < 0.05$) higher total carotenoid content at 476 ± 0.01 μg g^{-1} compared to *H. hemerocallidea* at 16 ± 0.10 μg g^{-1}. Ascorbic acid content was also higher in *M. balsamina* at 300 μg g^{-1} compared to the 268 μg g^{-1} recorded in *H. hemerocallidea*.

3.6. FT-IR Spectra

The FT-IR spectra of *M. balsamina* and *H. hemerocallidea* are presented in Figure 2 and their corresponding peak values are shown in Table 4. Both plant species displayed similar FT-IR spectra, indicating the presence of functional groups that are present in phytochemical compounds. Absorption bands attributed to O–H stretching vibrations were recorded at 3332.33 cm^{-1} for *M. balsamina* and 3267.19 cm^{-1} for *H. hemerocallidea*. C–H stretching vibrations were observed at 2917.85 cm^{-1} and 2922.05 cm^{-1} for *M. balsamina* and *H. hemerocallidea*, respectively. C≡C stretching vibrations were detected at 2057.38 cm^{-1} for *M. balsamina* and 2085.81 cm^{-1} for *H. hemerocallidea*. Bands associated with C=C stretching vibrations were recorded at 1725.67 cm^{-1} for *M. balsamina* and 1636.16 cm^{-1} for *H. hemerocallidea*. Two C–O stretching bands were identified in each species, recorded at 1243.12 and 1032.79 cm^{-1} for *M. balsamina* and 1278.55 and 1009.63 cm^{-1} for *H. hemerocallidea*.

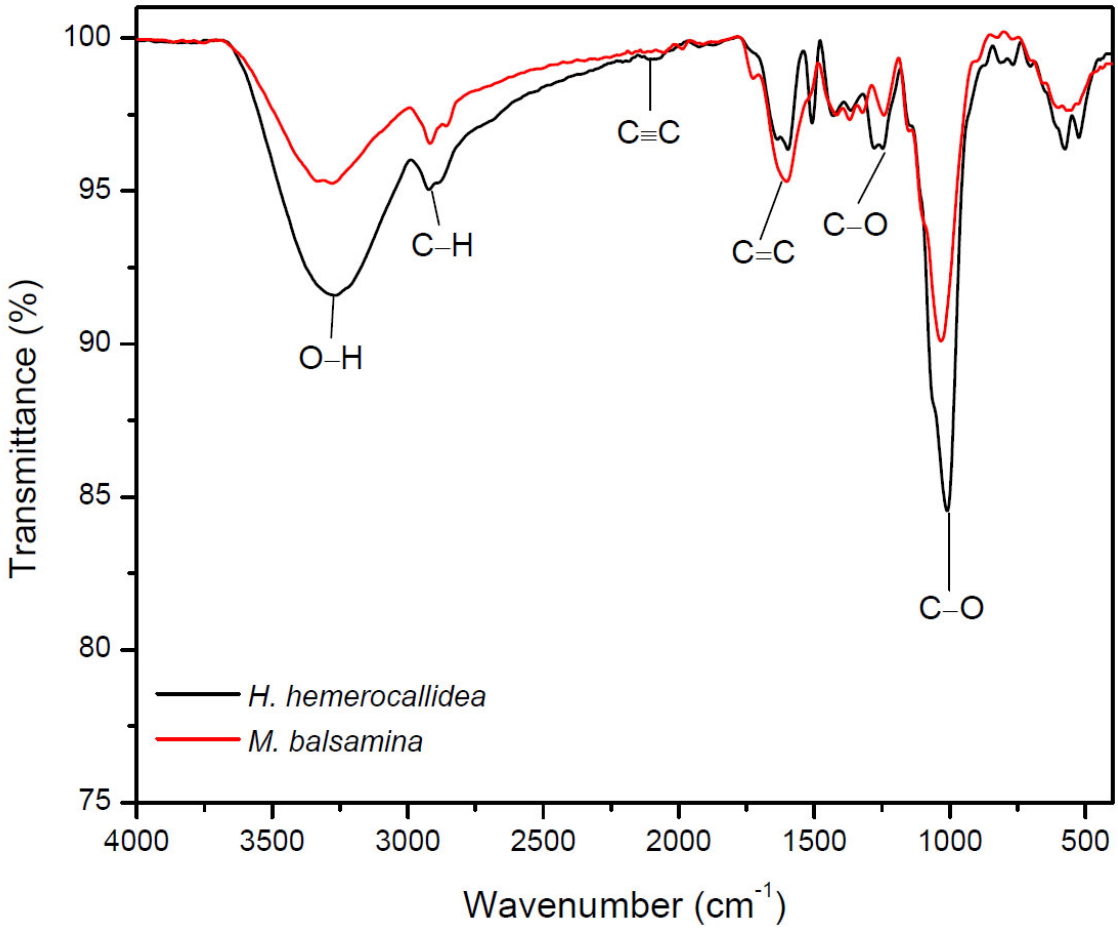


Figure 2. FT-IR spectra of *Momordica balsamina* leaves and *Hypoxis hemerocallidea* tuber.

Table 4. FTIR functional groups and peak values of *M. balsamina* and *H. hemerocallidea.*

Functional Group	Wavenumber (cm^{-1})	
	M. balsamina	***H. hemerocallidea***
O–H	3332.33	3267.19
C–H	2917.85	2922.05
C≡C	2057.38	2085.81
C=C	1725.67	1636.16
C–O	1243.12	1278.55
C–O	1032.79	1009.63

3.7. Phytochemical Screening by LCMS

A detailed analysis of the ions and ionic adducts of the detected peaks in the MS (Table 5) enabled the calculation of the molecular masses of these peaks. None of the calculated molecular masses corresponded to the compounds reported in the literature. The structures of previously isolated compounds from *M. balsamina* have multiple hydroxide groups. In the MS, these compounds will likely ionise in negative mode.

Table 5. Calculated molecular masses with their corresponding ion adducts.

Retention Time (min)	MM	*m/z* (+)	*m/z* (−)
ESI-MS of EtOAc VLC fraction of *M. balsamina*			
6828	286	287_$[M+H]^+$	285_$[M-H]^-$ 571_$[2M-H]^-$
13,081	513	514_$[M+H]^+$ 536_$[M+Na]^+$	-
17,117	513	514_$[M+H]^+$ 536_$[M+Na]^+$	-
ESI-MS of EtOAc_MeOH VLC fraction of *M. balsamina*			
4298	286	287_$[M+H]^+$	285_$[M-H]^-$ 571_$[2M-H]^-$
4635	*	268, 309 449_$[M+H]^+$	215, 279, 437, 567
4905	448	471_$[M+Na]^+$ 487_$[M+K]^+$	447_$[M-H]^-$
6.695 7008	436	437_$[M+H]^+$	-
7308	634	657_$[M+Na]^+$ 673_$[M+K]^+$	633_$[M-H]^-$ 679_$[M+HCOO]^-$
7667	*	657	-
8596	*	437, 597, 655, 696 437	677, 719, 745
8994	662	685_$[M+Na]^+$ 701_$[M+K]^+$ 437	707_$[M+HCOO]^-$
10,698	628	629_$[M+H]^+$ 651_$[M+Na]^+$ 667_$[M+K]^+$	
12,247	642	643_$[M+H]^+$ 665_$[M+Na]^+$ 681_$[M+K]^+$	-
13,078	472	419, 437 495_$[M+Na]^+$ 511_$[M+K]^+$	-

Table 5. *Cont.*

Retention Time (min)	MM	*m*/*z* (+)	*m*/*z* (−)
17,128	472	437 495_$[M+Na]^+$ 511_$[M+K]^+$	-
30,855	*	279, 342, 437, 523	-
ESI-MS of hypoxoside from *H. hemerocallidea*.			
4.205	606	629_$[M+Na]^+$	605_$[M-H]^-$

* MS too complex to calculate *m*/*z* values.

4. Discussion

4.1. Proximate Composition and Energy Content

Plants serve as invaluable components of both the human diet and traditional medicine systems, with their nutritional and therapeutic properties fundamentally rooted in their chemical composition [18]. Evaluating the proximate composition of medicinal and edible plants is therefore essential not only for understanding their nutritional value but also for elucidating their potential therapeutic properties [34]. In this study, *M. balsamina* was more nutritious than *H. hemerocallidea* across most proximate parameters, which is consistent with the well-established understanding that photosynthesis occurs predominantly in the chloroplast-rich tissue of leaves, resulting in higher autotrophic nutritional accumulation compared to underground storage organs [35].

The elevated protein observed in *M. balsamina* is attributable to the high natural synthesis of aromatic amino acids through the chorismate pathway in the leaves, which serve as precursors for a broad range of secondary metabolites and underpin the high phytochemical activity of this species [36,37]. This characteristic has prompted the promotion of *M. balsamina* as a protein supplement for cereal-based diets in resource-limited rural communities [13]. Although the protein content of *H. hemerocallidea* tuber was low, it still contributes meaningfully to dietary protein requirements. The National Research Council [38] classifies crude protein below 20% as indicative of low protein value; however, even plant parts with modest protein content can contribute to meeting daily protein requirements when consumed regularly. The protein content of *M. balsamina* compares favorably with values reported for *Coriandrum sativum* (3.5%), *Hibiscus sabdariffa* (1.8%), and *Trigonella foenum-graecum* (5.4%) [39], while remaining lower than the 23.36% reported for *Ipomoea hederacea* seeds [40]. A previous study conducted in Nigeria reported a lower protein content of 11.29% for *M. balsamina* leaves [41], while Flyman and Afolayan [42] recorded a higher value of 28.8% in Botswana, alongside fibre, fat, and ash values of 3.7%, 5.4%, and 12.7%, respectively. These variations across studies highlight the well-documented influence of agro-ecological factors, including soil type, climate, and geographic location, on the nutritional composition of plant species [43].

In this study, the crude fat content of both species was notably low, with *M. balsamina* and *H. hemerocallidea* yielding 3.15% and 1.83%, respectively, consistent with reports for most vegetables, which are generally regarded as poor sources of lipids [44]. The fat content of *M. balsamina* was slightly lower than values reported for *M. charantia* leaves (3.68% and 3.03%) [45,46] and *Rumex crispus* L. (2.01%) [47], though comparable to those of *Solanum nigrum* L. (1.90%) and *Celosia argentea* L. (0.50%) [48]. The low fat content of both plants makes them potentially beneficial for individuals managing weight, hyperlipidaemia, obesity, and cardiovascular complications [44]. A profiling of the fatty acid composition of the small fat fraction present may nonetheless prove valuable, as it could reveal the

presence of essential fatty acids with health-promoting properties supported by previous studies rather than conclusions derived from the present work.

The ash content, a proxy indicator of total mineral matter, was significantly higher in *M. balsamina* leaves compared to *H. hemerocallidea* tuber comparing favorably with recognised medicinal herbs such as *Origanum majorana* and *Salvia officinalis* [39] and significantly exceeding ash values reported for *Solanum nigrum* L. (3.80%), *Celosia argentea* L. (3.60%), and *Alternanthera sessilis* (1.50%) [48], further highlighting the mineral richness of *M. balsamina*. The noticeably higher fibre content of *M. balsamina* (38.96%) relative to *H. hemerocallidea* (9.72%) is nutritionally significant, as dietary fibre plays a well-established role in reducing the risk of colorectal cancer, cardiovascular disease, and type 2 diabetes [13]. On the other hand, the higher total carbohydrate content (81.01%) and food energy (344.67 kJ/100 g) of *H. hemerocallidea* reflects its nature as a starchy underground storage organ, where carbohydrate accumulation is a primary physiological function. Previous work documented carbohydrate and energy values of 83.4% and 381.5 kJ/100 g for *M. balsamina* seeds [26], which are higher than those obtained in this study for leaves, differences that may be attributed to the plant part analyzed, varying agro-ecological growing conditions, and developmental stage at the time of harvest.

4.2. Mineral Composition

Dietary minerals are essential to human health, which play critical roles in physiological, biochemical, and structural functions throughout the body, and their adequate intake through plant-based foods is particularly important for rural communities with limited access to animal-derived dietary sources [49,50]. Among the minerals detected in both *M. balsamina* and *H. hemerocallidea*, calcium (Ca), magnesium (Mg), and potassium (K) were the most abundant, collectively reflecting the broad nutritional significance of these two underutilized species as accessible sources of essential macrominerals. Calcium is required by plants to develop strong cell walls and membranes [51], and its importance in human nutrition is equally well established. The Ca content of *M. balsamina* (0.007%) was higher than that recorded for *H. hemerocallidea* (0.0003%), and the presence of Ca in both species suggests that their regular consumption could contribute meaningfully to mediating vascular contraction, nerve-impulse transmission, and glandular secretions, while also reducing the risk of diabetes, osteoporosis, and bone fractures in consumers [52]. These findings are nevertheless lower than the considerably higher Ca concentration of 222 $\mu g\ g^{-1}$ previously reported for *M. balsamina* by Flyman and Afolayan [42], and such differences are largely attributable to agro-ecological factors, including soil composition, geographic location, and prevailing plant growth conditions [53].

The study found that K was the most abundant mineral detected in both species. Within the plant, K is essential for enzyme activation and for promoting protein, starch, and adenosine triphosphate (ATP) production [54], while in human nutrition, adequate K intake is associated with a reduced risk of coronary heart disease and stroke, bone and cardiac health, and the management of hypertension and other cardiovascular diseases [13]. Critically, K content was noticeably higher than sodium (Na) content in both species, yielding a favourable low Na/K ratio, which has been reported to be beneficial for the maintenance of acid–base balance and the reduction in hypertension incidence [55,56]. The Na content was comparatively higher in *H. hemerocallidea* (0.0002%) than in *M. balsamina* (0.044%), which is expected given the direct and prolonged contact of root and tuber vegetables with the soil mineral matrix. Magnesium was detected in both species, with *M. balsamina* yielding a higher Mg content (0.001%) compared to *H. hemerocallidea* (0.0002%). Magnesium serves as the central core of the chlorophyll molecule in plant tissues [57] and plays an important role in fighting heart disease and stroke and facilitating cell repair in

humans [58]. The presence of Mg in both plant species could therefore partly account for the documented use of these plants in the traditional management of cardiovascular conditions. Furthermore, Mg and Ca are known to antagonize each other in absorption, inflammation, and various other physiological activities, and the low K/Ca/Mg ratio observed in this study implies that the bioavailability of dietary magnesium, calcium, zinc, and phosphorus will not be compromised upon consumption, thereby reducing the associated risk of mortality [59].

Iron (Fe) was detected at equal concentrations in both species (0.02 μg g^{-1}). Iron is a fundamental component of myoglobin and hemoglobin, essential for the transport of oxygen and carbon dioxide in the body [60], and while the Fe concentrations obtained in this study were lower than the 140 μg g^{-1} previously reported for *M. balsamina* by Flyman and Afolayan [42], both species nonetheless contribute to dietary iron intake. Manganese (Mn) was notably higher in *H. hemerocallidea* (0.73 μg g^{-1}) compared to *M. balsamina* (0.06 μg g^{-1}), consistent with the known ability of tuber plants to produce low molecular weight organic acid exudates that stimulate Mn uptake from the soil [61]. Adequate manganese intake in humans promotes normal organ and cell function and has been associated with preventing conditions ranging from pregnancy complications to menopause and pre-menstrual syndrome in women [62]. Copper (Cu), zinc (Zn), and manganese (Mn) are essential trace elements required by the human body in minute but critical amounts for key biochemical functions, playing important and closely interrelated roles in reproduction and immune function [63,64]. The presence of these trace elements in both plant species, particularly Zn (0.31 μg g^{-1} in *M. balsamina*; 0.22 μg g^{-1} in *H. hemerocallidea*), suggests that both plants could serve as useful dietary sources for supporting body building, pro-fertility, and immune system function. Importantly, all detected mineral concentrations in both species remained well below the maximum permissible levels (MPLs) established by the World Health Organization guidelines [65], confirming that both plants pose no health risks to consumers. The observed variability in mineral content between species and relative to previously published values is attributable to differences in geographical location, soil type, pH, organic matter content, and other environmental parameters [53,66].

4.3. Antioxidant

Due to their potential phytotherapeutic qualities, the antioxidant activity of indigenous plant species has attracted considerable scientific interest in recent decades [67]. Antioxidant activity in plants is mainly attributed to the presence of aromatic phenolic groups, which facilitate the stabilization and relocation of unpaired electrons [68]. Moreover, these compounds can donate hydrogen atoms and electrons from their hydroxyl groups, thereby neutralizing free radicals [69]. In this study, *M. balsamina* produced a higher amount of polyphenolic compounds compared to *H. hemerocallidea*, indicating the presence of potent reducing agents capable of scavenging free radical activity through electron donation, which consequently explains the high DPPH inhibition capacity of 89% recorded for *M. balsamina* (Table 3). Flavonoids were the largest group of naturally occurring phenolic compounds obtained in both plants, occurring both in free state and as glycosides, which is consistent with the findings of Laczkó Zöld et al. [70]. It should be noted that high flavonoid of both species is significant, as flavonoids and other polyphenolic compounds are well documented to confer anticancer, antidiabetic, hepatoprotective, immunomodulatory, antimicrobial, anti-inflammatory, and antioxidant activities [71]. Compared to other wild edible plants, for instance *Asystasia ganjetica*, which was reported to have a total flavonoid content of 20.12 μg g^{-1} [72], both *M. balsamina* and *H. hemerocallidea* demonstrated considerably higher flavonoid concentrations, further confirming their superior phytotherapeutic potential. A previous study reported a lower total phenolic concentration of 204.45 μg g^{-1}

in *H. hemerocallidea* tuber [73], which is considerably lower than the 289 μg g^{-1} recorded in the present study, with the difference likely reflecting the influence of geographical location, seasonal variation, and plant developmental stage on secondary metabolite accumulation. The bioactive composition of these indigenous plants is significantly influenced by the genetic makeup of the plant species, plant tissue type, developmental stage, and environmental factors such as temperature, water stress, and light conditions [74]. Therefore, endorsing the production and consumption of these plants with high bioactive contents could help reduce the development of chronic diseases while simultaneously providing nutrition in developing countries, particularly in rural communities.

4.4. Ascorbic Acid and Total Carotenoids

Indigenous plants are consumed in both processed and fresh forms, where they are infused or added as fresh or dried ingredients in domestic food processing [75]. The two plant species investigated in this study were collected from the same vicinity, making their agro-environmental conditions identical, and any observed differences in ascorbic acid and carotenoid content are therefore attributable to the abiotic stress and environmental defense mechanisms between the two plants rather than environmental variability. Beta-carotene (β-carotene), a precursor to vitamin A, is an essential vitamin at any age and the predominant carotenoid terpenoid in most plants. The higher carotenoid content recorded in *M. balsamina* compared to *H. hemerocallidea* is attributable to the higher exposure of leaves to ultraviolet B (UV-B) radiation, which stimulates the production of secondary metabolites and consequently contributes to plant defense mechanisms against UV-induced oxidative stress [76]. Nicolle et al. [77] reported a lower total carotenoid content of 397.8 μg g^{-1} in carrots from the South of France, with the authors attributing such variations to geographic location, climate conditions, and genetic variability among cultivars. The higher ascorbic acid content recorded in *M. balsamina* relative to *H. hemerocallidea* is consistent with established reports indicating that leafy vegetables and fruits generally present higher amounts of ascorbic acid compared to tuber crops [78,79]. Ascorbic acid is an essential cofactor that the human species is unable to synthesize endogenously and must therefore obtain exclusively through dietary sources, making the ascorbic acid content of both species nutritionally significant. A previous study reported a considerably lower ascorbic acid content of 44.4 μg g^{-1} in *M. balsamina* [26], which is significantly lower than the concentration obtained in the present study, with the difference likely reflecting the influence of geographical location, plant tissue type, and agro-ecological growing conditions on ascorbic acid accumulation.

4.5. FT-IR Spectra

Infrared spectroscopy is one of the most powerful analytical techniques for functional group identification, providing rapid and useful information about the structural composition of molecules. It is particularly valuable in plant characterization as it simultaneously reveals group presence in both inorganic and organic compounds, and the functional groups identified serve as indicators of the medicinal properties and biological activities present in the plants [80]. The similar FT-IR spectra observed for both *M. balsamina* and *H. hemerocallidea* imply that they share comparable essential functional groups. The absorption bands at 3332.33 cm^{-1} for *M. balsamina* and 3267.19 cm^{-1} for *H. hemerocallidea* could denote O–H stretching vibrations characteristic of phenolic compounds [80,81], confirming that both species possess antioxidant properties consistent with high polyphenolic activity (Table 3). The C–H stretching vibrations observed at 2917.85 and 2922.05 cm^{-1} for *M. balsamina* and *H. hemerocallidea* respectively could be ascribed to CH_2 and CH_3 groups, indicating the presence of terpenes in both species [82]. The C≡C stretching vibrations

at 2057.38 cm^{-1} for *M. balsamina* and 2085.81 cm^{-1} for *H. hemerocallidea* may indicate the presence of alkynes in both plants. The bands at 1725.67 cm^{-1} for *M. balsamina* and 1636.16 cm^{-1} for *H. hemerocallidea* could be associated with C=C stretching vibrations in deformed aromatic rings, amino acids, and flavonoids [80], further corroborating the high flavonoid content detected in both species. The C–O stretching bands at 1243.12 cm^{-1} for *M. balsamina* and 1278.55 cm^{-1} for *H. hemerocallidea* could be attributable to vibrations of the C–O group of polyols such as hydroxyflavonoids [80] while the bands at 1032.79 and 1009.63 cm^{-1} for *M. balsamina* and *H. hemerocallidea* respectively could be related to secondary alcohols and C–O stretching of ester groups [80].

4.6. Phytochemical Screening by LCMS

M. balsamina, from Mozambique, has been extensively investigated and twenty-two cucurbitane-type triterpenoids were isolated (Table 6) [9,83,84]. In this study, these compounds could not be detected from the LCMS analysis of the EtOAc and EtOAc-MeOH VLC fractions from *M. balsamina*. It was observed that most of the compounds were ionizing in positive mode. This might be an indication that the compounds detected are structurally different from the previously reported compounds. Another possible reason for the difference in the phytochemical content might be the geographical location. *H. hemerocallidea* is a plant species known to contain hypoxoside, a glycoside of rooperol. The compound could be detected both in the positive mode and the negative mode of the MS in our analysis. To justify these findings, the isolation and characterization of the detected compounds from the LCMS should be further investigated.

Table 6. Molecular formulae, masses, and structures of previously isolated compounds from *M. balsamina* [83,84].

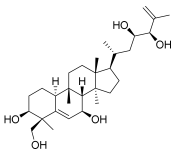
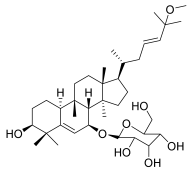
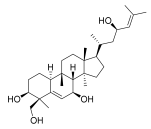
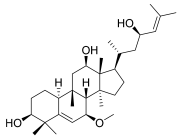
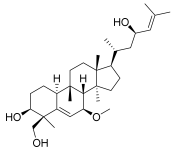
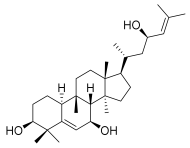
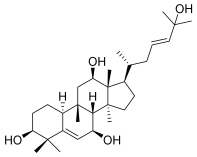
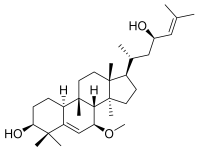
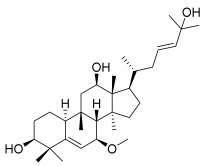
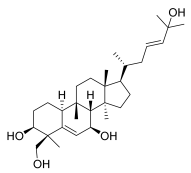
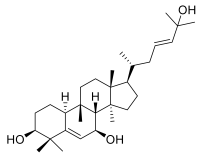
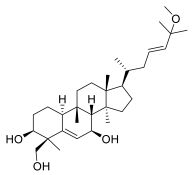
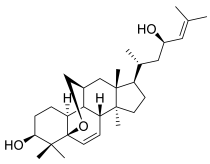
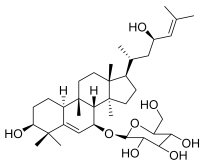
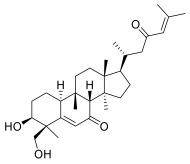
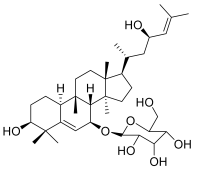
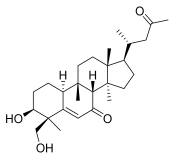
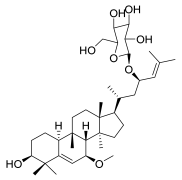
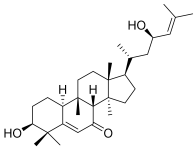
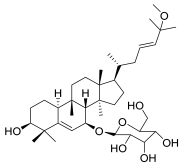
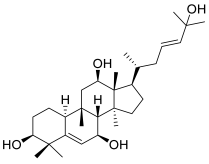
Molecular Formula	Mass (amu)	Structures	Molecular Formula	Mass (amu)	Structures
$C_{30}H_{50}O_5$	490		$C_{37}H_{62}O_8$	634	
$C_{30}H_{50}O_4$	474		$C_{31}H_{52}O_4$	488	
$C_{31}H_{52}O_4$	488		$C_{30}H_{50}O_3$	458	
$C_{30}H_{50}O_4$	474		$C_{31}H_{52}O_3$	472	
$C_{31}H_{52}O_4$	488		$C_{30}H_{50}O_4$	474	

Table 6. *Cont.*

Molecular Formula	Mass (amu)	Structures	Molecular Formula	Mass (amu)	Structures
$C_{30}H_{50}O_3$	458		$C_{31}H_{52}O_4$	488	
$C_{30}H_{48}O_3$	456		$C_{36}H_{60}O_8$	620	
$C_{30}H_{46}O_4$	470		$C_{36}H_{60}O_8$	620	
$C_{27}H_{42}O_4$	430		$C_{37}H_{62}O_8$	634	
$C_{30}H_{48}O_3$	456		$C_{37}H_{62}O_8$	634	
$C_{30}H_{50}O_4$	474				

4.7. Limitations of the Study

This study has several limitations that should be considered when interpreting the findings. First, plant materials were collected from a single geographical location during one collection period; therefore, the results do not capture spatial or seasonal variation in nutritional composition, mineral content, antioxidant properties, and phytochemical profiles. Second, although untargeted LC-MS enabled the detection of metabolites consistent with compounds previously reported in *M. balsamina* and *H. hemerocallidea*, metabolite identification was based on accurate mass and comparison with public databases and the literature, without confirmation using tandem mass spectrometry (MS/MS) or authentic reference standards. Consequently, the detected metabolites should be regarded as putatively identified. Future studies should incorporate multi-location and multi-season sampling, together with comprehensive metabolite confirmation using tandem mass spectrometry and authentic standards, to improve the robustness and generalizability of the findings.

5. Conclusions

The study demonstrated that both *M. balsamina* and *H. hemerocallidea* possess valuable nutritional and phytochemical properties, highlighting their potential as underutilized functional food resources for improving food and nutrition security in rural communities.

It was observed that *M. balsamina* consistently exhibited superior nutritional quality, particularly in crude protein, fibre, ash, essential minerals, phenolic compounds, flavonoids, carotenoids, and antioxidant activity, indicating strong nutraceutical potential. In contrast, *H. hemerocallidea* contained higher carbohydrate and energy content, suggesting its importance as an energy-rich indigenous food source. In this study, both species demonstrated strong antioxidant capacity, with DPPH radical scavenging activity exceeding 80%, confirming the presence of biologically active phytochemicals capable of contributing to the prevention of oxidative stress-related diseases. On other hand, FT-IR and LC-MS analyses further confirmed the occurrence of important bioactive compounds, including *hypoxoside* in *H. hemerocallidea*, while the phytochemical profile of *M. balsamina* suggested the possible presence of unidentified metabolites detected using these analytical approach employed; however, definitive structural confirmation will require additional analyses, including MS/MS fragmentation, molecular formula prediction, database matching, and structural elucidation. Therefore, future research should focus on the isolation and structural characterization of these unidentified phytochemicals, assessment of their bioavailability and toxicity, and evaluation of their therapeutic potential through in vivo studies. In addition, agronomic and domestication studies are needed to support the sustainable cultivation, conservation, and integration of these indigenous plants into food systems and community nutrition programs in South Africa.

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Abbreviations

The following abbreviations are used in this manuscript:

ATR	Attenuated Total Reflectance
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
Ca	Calcium
CE	Catechin Equivalent
Cu	Copper
DCM	Dichloromethane
DCPIP	2,6-Dichlorophenolindophenol
DM	Dry Matter
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EDTA	Ethylene Diamine Tetra-Acetic Acid
ESI-MS	Electrospray Ionization Mass Spectrometry

EtOAc	Ethyl Acetate
FE	Food Energy
FT-IR	Fourier Transform Infrared Spectroscopy
GPS	Global Positioning System
HPLC	High-Performance Liquid Chromatography
HSD	Honest Significant Difference
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
K	Potassium
LC-MS	Liquid Chromatography–Mass Spectrometry
Mg	Magnesium
Mn	Manganese
MPLs	Maximum Permissible Levels
Na	Sodium
NDF	Neutral Detergent Fibre
NDS	Neutral Detergent Solution
Pb	Lead
RF	Radio Frequency
SA	South Africa
SEM	Standard Error of the Mean
UV-Vis	Ultraviolet–Visible Spectroscopy
VLC	Vacuum Liquid Chromatography
WHO	World Health Organization
Zn	Zinc

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