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Commentary

Nutritional Composition, Anti-nutrient Factors and Antioxidant Potentials of *Momordica cymbalaria Hook F.*

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ABSTRACT

Introduction: *Momordica cymbalaria* Hook. F. is a perennial creeper and a highly acceptable wild vegetable in South India. The present research aims to investigate the nutritional composition, antinutrients, enzyme inhibitors and antioxidant activities of the tuber, leaf, and fruit of *M. cymbalaria*.

Methods: The proximate analysis was carried out using the prescribed techniques. The minerals were analysed using the Optimal Emission Spectrometer (OES) equipped with the Inductively Coupled Plasma (ICP). The amino acids were detected using the HPLC system. Antinutrients (phytate, total phenolic content, tannin), enzyme inhibitors (α-amylase and trypsin) and antioxidant activities (by DPPH, ABTS radicle scavenging and RPA assays) of the tuber, leaf, and fruit were carried out using colourimetric methods.

Results: The mean proximate components of fruit were greater than those of the leaf and tuber. The leaves are a rich source of potassium, magnesium, calcium, sodium, zinc and manganese. The leaf and fruit contained higher levels of leucine, valine, lysine, phenylalanine, and isoleucine. The leaf had higher concentrations of phytic acid (305 mg/100 g), total phenolic acid (760 mg GAE/100 g), tannins (129 mg GAE/100 g), α-amylase (39.17% inhibition) and trypsin inhibitors (21.05% inhibition). The fruit has shown the highest antioxidant activity as it contains the highest DPPH (14.36 AAE mg/100 g), ABTS (0.53 TE mg/100 g) scavenging activities and reducing power values (10.86 EC50).

Conclusion: *M. cymbalaria* can be a potential vegetable with a rich nutrient profile and antioxidant potential. Furthermore, the leaf and fruit were demonstrated as excellent sources of essential amino acids for human subjects. The leaf had higher concentrations of antinutrients i.e. phytic acid, total phenolic acid, and tannins. *M. cymbalaria* exhibited hypoglycaemic influence through an inhibitory effect on α-amylase and antioxidant activities. The rich carbohydrate profile of tubers offers potential for their use as nutraceutical and functional food.

Keywords: Amino acids; α-amylase; Antioxidant; Antinutritional factors; Momordica; Proximate analysis

INTRODUCTION

Momordica (family: Cucurbitaceae) encompasses more than 60 species. Among them, 3 selected species such as *M. charantia* (bitter melon), *M. foetida* (bitter cucumber) and *M. balsamina* (African pumpkin) are commercially cultivated and widely used as vegetables. There is a need to give considerable attention to other *Momordica* species including wild edible vegetables having significant potential to maintain livelihood, and health and contribute to food security in local communities. In addition, recent trends indicate an increased demand for underutilized and neglected vegetable species [1,2]. *M. cymbalaria* Hook. F. is a perennial creeper and widely accepted wild vegetable in South India [3]. The plant is valued for its edible fruits and leaves as a vegetable. The fruits were reported for antidiabetic, antiulcer, cardioprotective, hepatoprotective, nephroprotective activities and reproductive health-promoting properties [4]. The leaves have been used to treat fever, malaria, parasites, worms and wounds [5]. The tubers have abortifacient, antiovulatory and antidiarrheal activities [6].

1. M. tuberosa is a natural and rich source of minerals, amino

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acids and phytochemicals. The fruits are abundant in calcium, potassium, sodium, and vitamin C and have higher crude fibre content than bitter gourd [7,8]. The phytochemicals such as phenolic compounds show antioxidant activity through their potency in scavenging ROS, reducing power and/or metal-chelating activity towards ferric and ferrous ions. Like other wild vegetables, M. tuberosa is also comprised of many anti-nutrients which include tannins, phytate, enzyme inhibitors etc. One of the major antinutrients is phytate, which chelates and mainly affects the bioavailability of calcium and other micronutrients. In addition to decreasing nutrient availability, antinutrients can become toxic when present beyond a certain amount [8,9]. Many researchers have reported the nutritional and medicinal values of fruits, neglecting the investigation of leaves and tubers [4,7,8]. More research is required to detect antinutrients and enzyme inhibitors in other parts of M. tuberosa. Hence, this research aims to investigate the nutritional composition (proximate principles, mineral analysis and amino acid profiles), antinutrients (phytate, total phenolic content, tannin), enzyme inhibitors (α-amylase and trypsin) and antioxidant activities (by DPPH, ABTS radicle scavenging and RPA assays) of the tuber, leaf, and fruit of *M. cymbalaria*.

MATERIALS AND METHODS

Plant materials

The plant materials of *M. cymbalaria* including fresh tubers, leaves and fruits were obtained from the agricultural fields of Poidi Kalva (N-14°. 50' 41. 63", E-78°. 66' 16.77", altitude 159 m), YSR Kadapa District, and Andhra Pradesh, India from July to August 2021. The taxonomic identity was verified by comparing voucher specimens housed in the herbarium (field no. 5258) of the Department of Botany at Yogi Vemana University, located in Kadapa. Following collection, the plant materials were rinsed with a continuous tap water flow to eliminate any remaining dust particles. The tubers were peeled with a stainless steel peeler and then sliced. The excess water on the leaves was drained off by placing them on blotting papers. The pedicles of fruits were manually removed. The 3 samples were dried at 50°C ± 2°C for 72 h. Subsequently, they were subjected to an additional air drying period of 6 hours-8 hours inside the laboratory while being kept in a shaded environment. Then the dried materials were ground to a specific size of 200 µm-300 µm in a laboratory mixer. The powders derived from root tuber, leaves and fruits were carefully placed in air-tight polyethylene bags and preserved inside desiccators for future use.

Proximate principles

The moisture content, ash content, crude fibre content, and dietary fibre content were analysed using the prescribed techniques outlined in the Bureau of Indian Standards (BIS) and Indian Standards (IS) 7874-1 (1975): Methods of tests for animal feeds and feeding stuff, Part-1; General methods (IS 7874-1, 1975).

Determination of fat content

The total fat content was estimated using the method described by [10]. The powdered materials of tuber, leaf, and fruit weighing 10 g were subjected to extraction using hexane as the solvent. The resulting mixture was then centrifuged at a speed of 3000 rpm for 2 minutes. The pellets underwent 3 further hexane extractions, following the procedure outlined before. The extract from the combination of solvents was subjected to evaporation at 40°C. The lipid residue in the beaker was measured by weighing. The fat content was represented as g/100 g of plant material.

Determination of proteins

Proteins were estimated according to the method described by [11]. The absorbance was measured at a wave length of 660 nm using a Shimadzu UV-1800 UV/vis spectrophotometer, with a blank serving as the reference. The protein content was calculated from the Bovine Serum Albumin (BSA) calibration curve and the results were expressed as grams (g) of proteins per 100 g of plant material (g/100 g).

Determination of carbohydrates

Total carbohydrates were determined according to the method described by [12]. The absorbance was measured at 630 nm with a UV/vis spectrophotometer (Shimadzu UV-1800) against the blank. The amount of carbohydrates was determined using the D-glucose calibration curve and the results were reported as grams (g) of carbohydrate per 100 g of plant material (g/100 g).

Determination of starch

The amount of starch was determined according to the method described by [12]. The absorbance was measured at 620 nm using a UV/vis spectrophotometer (Shimadzu UV-1800) against the blank. The amount of starch was calculated from the D-glucose calibration curve. The data of D-glucose equivalents were subject to a multiplication factor of 0.9 to determine the starch content. The results were expressed as gram (g) of starch per 100 grams of sample (g/100 g).

Determination of reducing sugars

The contents of reducing sugars were determined according to the method [13]. The absorbance was measured at 530 nm using a UV/vis spectrophotometer (Shimadzu UV-1800) against the reagent blank. The amount of reducing sugars was calculated from the D-glucose calibration curve and the results were expressed as gram (g) per 100 grams of sample (g/100 g).

Determination of non-reducing sugars

Non-reducing sugars were estimated according to the modified method [14]. This methodology quantifies sugar content by assessing its reducing sugar and total carbohydrate components. Determining non-reducing sugar involves subtracting the amount of reducing sugar from the overall quantity of carbohydrates, and

multiplying the resulting difference by a factor of 0.95. The results were expressed as gram (g) per 100 grams of sample (g/100 g).

Mineral analysis

A microwave vessel was used to digest the powdered materials of tuber, leaf, and fruit (0.5 g ± 0.01 g) using a combination of nitric acid and hydrogen peroxide at 7:1 (v/v). The vessels were tightly capped and placed into the microwave digestion (Milestone ETHOS) system. The samples were digested in 2 steps. In the first step, the samples were subjected to a power of 1000, a temperature of 110°C, a ramp duration of 15 minutes, and a hold time of 5 minutes. In the second step, the samples were exposed to a power of 1000, a temperature of 190°C, a ramp time of 10 minutes, and a hold time of 20 minutes. The vessels were extracted from the digestor and then let to cool down to ambient temperature. The vessels were cautiously opened, and the contents were transferred into an acid-cleaned 50 mL standard measuring flask. The contents were diluted with grade-1 water to a final volume of 50 mL. The digested samples were subjected to analysis using the Optimal Emission Spectrometer (OES) equipped with the Inductively Coupled Plasma (ICP) (Perkinelmer, Optima 5300 DV). The concentrations of potassium (K), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), phosphorus (P), sodium (Na) and magnesium (Mg) contents were determined by measuring their absorbance at 766.40 nm, 317.933 nm, 259.939 nm, 213.857 nm, 257.61 nm, 589.592 nm, 279.077 nm, 324.752 nm and 213.617 nm, respectively. The mineral contents were quantified using yttrium as an internal standard. The minerals were quantified by comparing their concentrations to those of known concentrations in the working standard solutions, which were used for calibrating the device. The concentration of macro elements (K, P, Mg, Ca and Na) were expressed as g per 100 g of dry matter in the sample. Whereas the concentration of microelements (Fe, Zn, Mn and Cu) were expressed as mg per 100 g of dry matter in the sample.

Determination of amino acid profile

A clean sample tube was used to collect 0.25 g ± 0.01 g of powdered materials derived from tuber, leaf, and fruit. These materials were subjected to hydrolysis using 10 mL of 6N HCl solution containing 1% phenol. The hydrolysis process was carried out at 110°C for 24 hours, ensuring that no air was introduced throughout the procedure. Subsequently, the hydrolysates were cooled, followed by vacuum drying under and filtration using a 0.45 µm syringe filter. Then, 0.5 mL aliquots were diluted with 7 mL of water, followed by the pH adjustment of the resulting solution to 7-8 with NaOH (6N). The final volume was then adjusted to 10 mL using water. The reconstituted sample underwent pre-heating in a water bath at 55°C. To a volume of 20 µL of the reconstituted sample, 140 µL of AccQ*Flour Borate Buffer and 40 µL of AccQ*Flour Reagent were subsequently added. The mixture was vortexed for several seconds and hydrolyzed the surplus reagent to form 6 amino quinoline. The contents were transferred from the tube to a vial labelled as Ria. The vials were sealed with a septum lined with silicone, followed by heating in a water bath for 10 minutes at 55°C. The vials were subjected to cooling, after which the contents were transferred to a High Performance Liquid Chromatography (HPLC) vial. The blank was subjected to derivatization without heat, as previously reported.

The amino acid derivatives were separated on a Waters (e2695 module) HPLC system equipped with an auto sampler and fluorescence detector. A sample of 5 µL was injected into a Waters hydrolysate amino acid column (WAT052885; 3.9 mm × 150 mm). The temperature of the column was adjusted to 45°C. The mobile phase consisted of a buffer solution (containing 11.48 g of sodium acetate anhydrous and 1.722 g of triethylamine in 800 mL water). The pH was adjusted to 5.02 using orthophosphoric acid and the volume was 1000 mL. Additionally, the mobile phase included a combination of water and acetonitrile. The elution of samples was performed at a flow rate of 1.0 mL/min, with a total duration of 45 min. The amino acids such as aspartic acid (Asx), serine (Ser), glutamic acid (Glx), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), cysteine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Iseu), leucine (Leu), phenylalanine (Phe) were detected using fluorescence detection at 250 nm (excitation) and 395 nm (emission). Identifying the individual amino acid peaks corresponding to amino acids was accomplished by comparing their retention times with those of standards. The results were reported as milligram) of amino acid per 100 g (mg/100 g).

Tryptophan (Tyr) was determined by a base hydrolysis process using 10 mL of 6N NaOH at 110°C for 24 hours while ensuring no air was introduced. The sample was reconstitution using concentrated hydrochloric acid (HCl) at a pH of 3.0 in a water bath at 55°C. Subsequently, the sample was derivatized using methanol. The sample underwent vortexing, centrifugation, and filtration using a 0.45 µm syringe filter. The separation of amino acid derivatives was conducted using a Waters (e2695 module) HPLC system equipped with an auto sampler and fluorescence detector. A sample of 5 µL was injected into an Inertsil, Octadecysilane column with dimensions of 4.6 mm × 250 mm. The temperature of the column was adjusted to match the ambient room temperature. The mobile phase consisted of 1 mL of trifluoroacetic acid in 1 L of water and acetonitrile. The elution of the samples was conducted at a flow rate of 1.0 mL/min, and the whole process lasted for 10 minutes. Tryptophan (Try) was detected using fluorescence detection with excitation at 280 nm and emission at 356 nm. The results were reported as milligrams (mg) of amino acid per 100 g (mg/100 g).

Antinutrients

Determination of phytic acid: Phytic acid content was determined according to the method described by [15]. The absorbance of the alcoholic upper layer was measured at 465 nm

with UV/visible spectrophotometer (Shimadzu UV-1800) against a blank. The phytate content was calculated from the sodium phytate calibration curve. The results were expressed as mg of phytates per 100 g of sample (mg/100 g).

Determination of Total Phenolic Content (TPC): The total phenolic content was measured according to the method described by [13]. The absorbance of the solution was measured at 765 nm with UV/visible spectrophotometer (Shimadzu UV-1800) against the blank. The total phenolic content was measured from the Gallic Acid (GAE) calibration curve. The results were expressed as mg of GAE equivalents per 100 g of sample (mg GAE/100 g).

Determination of tannins: Tannin was determined according to the method described by [16]. The absorbance was measured at 605 nm with a UV-visible spectrophotometer (Shimadzu UV-1800) against the blank. Tannin content was measured from the Gallic Acid (GAE) calibration curve. The results were expressed as mg of GAE equivalents per 100 g of sample (mg GAE/100 g).

Enzyme inhibitors

α-amylase inhibition activity: The determination of α-amylase inhibitory activity was conducted using the methodology described by [17]. The absorbance was measured at 580 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The results of α-amylase inhibition activity were expressed in terms of inhibition percentage.

Trypsin inhibition activity: It was determined using the methodology outlined by [18]. The absorbance was measured at 280 nm using a UV-V is spectrophotometer (Shimadzu UV-1800) against a blank. The experiment without the plant filtrate was considered 100% enzyme activity. The results of trypsin inhibition activity were expressed in terms of inhibition percentage.

Antioxidant assays

Free radicle scavenging activity on DPPH: It was used to measure the free radical scavenging activity of the extracts derived from tubers, leaves and fruits by [19]. The absorbance of DPPH radical was measured at 517 nm using i mark microplate reader against a blank. The concentration of DPPH* was determined using a calibration curve from the Ascorbic Acid (AA). The results were expressed as Milligrams (mg) of Ascorbic Acid (AA) Equivalents (E) per g of sample (mg AAE/g).

ABTS radical scavenging assay: The ABTS (2,2-azanobis-(3-ethylbenzothiazoline-6-sulfonate)) free radical scavenging activity of extracts from tubers, leaves, and fruits was assessed using the methodology outlined by [20]. The ABTS working solution was added and mixed with the different aliquots of plant extracts. The reaction mixture was incubated for 30 in at room temperature. The absorbance was recorded at 750 nm using an i mark microplate reader (Shimadzu UV-1800) against a blank. The measurement of ABTS radicle concentration was obtained from the Trolox calibration curve. The results were expressed as Milligrams (mg) of Trolox (TE) equivalents per g of sample (mg TE/g).

Reducing Power Assay (RPA): Reducing power assay of tuber, leaf and fruit extracts was performed according to the method described by [21]. The absorbance was measured at 700 nm using a UV/visible spectrophotometer (Shimadzu UV-1800), against a blank. Ascorbic acid was used as a standard antioxidant for calibration and the results were expressed as EC50, the effective concentration at which absorbance was 50.

Statistical analysis: All experiments were carried out in triplicates (n=3) and the results were expressed as the Mean ± Standard Deviation (SD). The data was subjected to statistical analysis using one-way ANOVA by Tukey's Multiple Comparison Test for all the experiments. The mean values were considered significant when $p \leq 0.05$. The statistical analyses were conducted using GraphPad Software (version 8.0).

RESULTS AND DISCUSSION

Proximate composition

The proximate analysis of the tuber, fruit, and leaf of M. *cymbalaria*, based on the dry weight, has been reported in Table 1. The fruit had the highest mean moisture content (84.30%), while the tuber had the lowest (78.31%). The level of moisture in a food product serves as an indicator of its texture and ability to store for a long time. Tuber has a less significant potential for prolonged shelf life than the leaf and the fruit. The leaf had the highest total ash content (20.76 ± 0.01 g/100 g), followed by fruit (10.54 ± 0.01 g/100 g) and tuber (4.08 ± 0.01 g/100 g). The ash's presence in the material indicates its mineral composition. The results suggest that wild and underutilized vegetable has the potential to be a good source of essential minerals in human diets [22].

Table 1: Proximate composition of dried tuber, fruit and leaf of M. cymbalaria*

Sample	Moisture %	Total ash (g/100 g)	Total dietary fiber (g/100 g)	Crude fiber (g/100 g)	Fats (g/100 g)	Proteins (g/100 g)
Fruit	84.30d ± 0.01	10.54d ± 0.01	37.27d ± 0.01	24.17d ± 0.01	6.06d ± 0.01	8.38d ± 0.01
Leaf	81.37d ± 0.01	20.76d ± 0.01	36.47d ± 0.01	6.49d ± 0.01	2.75d ± 0.01	10.5d ± 0.01
Tuber	78.31d ± 0.01	4.08d ± 0.01	23.38d ± 0.01	6.18d ± 0.01	3.34d ± 0.01	10.35d ± 0.01
*values are mean of triplicate determination (n=3) ± standard deviation; ns, non-significant; statistically significant at ($p \leq 0.05$) where a>b>c>d						

The fruit had the highest levels of dietary fibre (37.27 ± 0.01 g/100 g) and crude fibre (24.17 ± 0.01 g/100 g), with the leaf and tuber showing lower amounts. Plant materials contained more dietary fibre than crude fibre. These results demonstrate that the crude fibre in *M. cymbalaria* is 3.7 times higher than that found in bitter gourd [7]. According to the National Research Council (NRC 1989), an inverse correlation exists between the consumption of diets abundant in plant fibre and the occurrence of cardiovascular diseases, colon cancer, and diabetes. The total fat content was highest in the fruit (6.06 ± 0.01 g/100 g), followed by the tuber and leaf. However, the present findings of fat content were higher than reported fat values in other *Momordica spp* [23]. The leaf exhibited the highest protein content (10.5 ± 0.01 g/100 g), followed by the tuber (10.35 ± 0.01 g/100 g) and fruit (8.38 ± 0.01 g/100 g) (Table 1). In general, *Momordica spp*. does not possess high protein content. This finding is consistent with the earlier reports [8,23].

The carbohydrate profiling of different plant materials varied significantly for starch, total soluble carbohydrates, reducing and non-reducing sugars (Table 2). The highest values were found in the tubers, and the lowest values were obtained in the leaf, followed by the fruit. The tubers of *M. cymbalaria* mainly contained starch (42.08 g/100 g), together with total soluble carbohydrates (19.45 g/100 g), non-reducing sugars (17.58 g/100 g) and reducing sugars (0.94 g/100 g). The starch content of the fruits of *M. dioica* (8.72 g/100 g), *M. cochinchinensis* (7.8 g/100 g) and *M. subangulata* subsp. *renigera* (8.25 g/100 g) was found to be lower than the fruit of *M. cymbalaria* (9.52 g/100 g) [24].

Table 2: Carbohydrate profiling of dried powdered materials tuber, fruit and leaf of M. cymbalaria*

Sample	Starch (g/100 g)	Total soluble carbohydrates (g/100 g)	Reducing sugars (g/100 g)	Non-reducing sugars (g/100 g)	Fats (g/100 g)	Proteins (g/100 g)
Fruit	9.52b ± 0.01	4.07d ± 0.01	0.77d ± 0.01	5.13d ± 0.01	6.06d ± 0.01	8.38d ± 0.01
Leaf	10.01b ± 0.01	6.76d ± 0.01	0.65c ± 0.01	8.41d ± 0.01	2.75d ± 0.01	10.5d ± 0.01
Tuber	42.08d ± 0.01	19.45d ± 0.01	0.94d ± 0.01	17.58b ± 0.01	3.34d ± 0.01	10.35d ± 0.01

*values are mean of triplicate determination (n=3) ± standard deviation; ns, non-significant; statistically significant at ($p \leq 0.05$) where a>b>c>d

Mineral composition: The leaf exhibits significantly ($p \leq 0.05$) the highest potassium, magnesium, calcium, sodium, zinc, and manganese content (Table 3). The fruit has significantly ($p \leq 0.05$) the highest range of iron and copper. Both leaf and tuber have the highest phosphorous content ($p \leq 0.05$). The mineral content of the leaves of *M. cymbalaria* was significantly higher than that of *M. balsamina* [25]. The fruit of *M. cymbalaria* has higher levels of minerals than the fruit of *M. charantia*, *M. dioica*, *M. subangulata subspp. renigera*, and *M. cochinchinensis* [7,24,26]. Among the minerals that subjected to analysis, potassium (3.05 ± 0.01 g/100 g) was found to be the highest, followed by magnesium (1.08 ± 0.01 g/100 g), calcium (0.89 ± 0.01 g/100 g), phosphorous (0.45 ± 0.01 g/100 g), sodium (0.38 ± 0.01 g/100 g), iron (35.46 ± 0.01 mg/100 g), manganese (12.67 ± 0.01 mg/100 g), zinc (3.68 ± 0.01 mg/100 g) and copper (1.64 ± 0.01 mg/100 g). The results obtained in this study are consistent with previous research conducted on the fruits of other *Momordica* species [26].

Table 3: Mineral composition of dried powder materials of tuber, fruit and leaf of M. cymbalaria*

Sample	Macro minerals (g/100 g)					Micro minerals (mg/100 g)			
	K	P	Mg	Ca	Na	Fe	Zn	Mn	Cu
Fruit	2.46d ± 0.01	0.45a ± 0.01	0.39b ± 0.01	0.35d ± 0.01	0.35a ± 0.01	35.46d ± 0.01	3.37a ± 0.01	2.26b ± 0.01	1.64d ± 0.01
Leaf	3.05d ± 0.01	0.33d ± 0.01	1.08d ± 0.01	0.89d ± 0.01	0.38ns ± 0.01	22.70d ± 0.01	3.68c ± 0.01	12.67d ± 0.01	1.03d ± 0.01
Tuber	0.80d ± 0.01	0.45d ± 0.01	0.29d ± 0.01	0.25d ± 0.01	0.23a ± 0.01	10.10d ± 0.01	1.54d ± 0.01	0.78d ± 0.01	0.62d ± 0.01

*values are mean of triplicate determination (n=3) ± standard deviation; ns: Non-significant; K: Potassium, P: Phosphorous, Mg: Magnesium, Ca: Calcium, Na: Sodium, Fe: Iron, Zn: Zinc, Mn: Manganese, Cu: Copper; statistically significant at ($p \leq 0.05$) where a>b>c>d

Amino acid profile: The total amino acid content of the plant materials of *M. cymbalaria* varied between 130.1 to 232.1 mg/g. The leaves (232.1 mg/g) exhibited significantly ($p \leq 0.05$) the highest content of total amino acids, followed by fruit (201.3 mg/g) and tuber (130.1 mg/g). 18 amino acids were identified in the fruit, whereas the leaf and tuber samples included 17 amino acids, as shown in Table 4. The absence of glutamine and asparagine was seen in the fruit, leaf, and tuber samples, suggesting a possible conversion into glutamic and aspartic acids, respectively [27]. The presence of tyrosine was observed only in the fruit but not in the tuber and leaf. Tyrosine (Tyr) may be synthesized from the amino acid L-phenylalanine. The leaf and tuber tissues may impair the conversion of phenylalanine to tyrosine [28]. Reported that the seeds and pericarp of *M. charantia* exhibited a deficiency in threonine and leucine. The results also indicated that Essential Amino Acids (EAA) concentrations are comparatively lower than non-essential amino acids (N-EAA). The present study demonstrates that the ratio of EEA to N-EAA in leaf, fruit, and tuber samples was 0.83, 0.68, and 0.53, respectively. Previous studies have shown a greater concentration of both essential and non-essential amino acids in the mature fruit and the seed of *M. charantia* [29].

Table 4: Amino acid composition of dried powdered tuber, fruit and leaf of *M. cymbalaria**

Amino acid	Fruit	Leaf	Tuber
Essential amino acids (mg/g)			
Histidine	3.4ns ± 0.01	4.2d ± 0.01	3.1ns ± 0.01
Isoleucine	7.8a ± 0.01	10.9a ± 0.01	5.4d ± 0.01
Leucine	13.3a ± 0.01	18.8a ± 0.01	8.1d ± 0.01
Lysine	10.7a ± 0.01	14.9a ± 0.01	7.3d ± 0.01
Methionine	0.2ns ± 0.01	0.5d ± 0.01	0.5ns ± 0.01
Phenylalanine	8.0a ± 0.01	13.1d ± 0.01	6.5d ± 0.01
Threonine	6.3a ± 0.01	9.7a ± 0.01	4.8d ± 0.01
Tryptophan	1.8d ± 0.01	3.7ns ± 0.01	1.7a ± 0.01
Valine	11.3a ± 0.01	16.3d ± 0.01	7.8a ± 0.01
Non–essential amino acids (mg/g)			
Alanine	11.2a ± 0.01	16.9ns ± 0.01	9.0a ± 0.01
Arginine	17.1d ± 0.01	14.1d ± 0.01	26.8d ± 0.01
Aspartic acid	23.1d ± 0.01	28.3a ± 0.01	14.3d ± 0.01
Cystine	3.2d ± 0.01	5.1d ± 0.01	3.0d ± 0.01
Glutamic acid	3.81a ± 0.01	10.6a ± 0.01	14.8a ± 0.01
Glycine	8.9a ± 0.01	11.3a ± 0.01	3.8a ± 0.01
Proline	9.6d ± 0.01	13.7d ± 0.01	6.0d ± 0.01
Serine	8.4a ± 0.01	9.9a ± 0.01	7.0a ± 0.01
Tyrosine	6.3 ± 0.01	----	----
Total amino acids	201.3d ± 0.01	232.1d ± 0.01	130.1d ± 0.01
*values are mean of triplicate determination (n=3) ± standard deviation; ns, non-significant; statistically significant at ($p \leq 0.05$) where a>b>c>d			

The leaf, fruit and tuber contained significantly ($p \leq 0.05$) higher levels of 5 EAAs, including leucine (8.1 mg/g to 18.8 mg/g), valine (7.8 mg/g to 16.3 mg/g), lysine (7.3 mg/g to 14.9 mg/g), phenylalanine (6.5 mg/g to 13.1 mg/g) and isoleucine (5.4 mg/g to 10.9 mg/g). The leaf significantly exhibited higher levels of N-EAA, including aspartic acid, alanine, arginine, proline and glycine. In the fruit, the content of N-EAA, including aspartic acid, arginine, alanine, proline and glycine, was significantly ($p \leq 0.05$) higher in comparison. The range of N-EAA, including arginine, glutamic acid, aspartic acid, alanine, and serine, was significantly ($p \leq 0.05$) considerably higher in the tuber. The testified that the bitter gourd is a good source of most essential amino acids, except for lysine, cysteine and methionine [30].

Antinutrients

Phytates: It form complexes with metal ions with positive charges, including zinc, iron, magnesium, and calcium. These complexes subsequently lead to decreased bioavailability of these ions due to lowered absorption rates [31]. The phytic acid concentration in *M. cymbalaria* plant materials varied from 106.0 mg to 305.0 mg per 100 g (Table 5). The fruit had the lowest measured values (106 mg/100 g), followed by the leaf and tuber (305 mg/100 g). The observed a lower concentration of phytates (41.37 mg/100 g) in the leaves of *M. balsamina*. Based on the results, it may be concluded that the comparatively low quantities of phytic acid present in *M. cymbalaria* leaves, fruit, and tubers should not interfere with human digestion and that *M. cymbalaria* might be utilised as an ingredient in the food [32].

Table 5: Anti-nutrient content of dried powdered tuber, fruit and leaf of M. cymbalaria*

Sample	Phytic acid (mg/100 g)	Total Phenolic compounds (mg GAE/100 g)	Tannins (mg GAE/100 g)	α-amylase inhibitors (% inhibition)	Trypsin inhibitors (% inhibition)
Fruit	106d ± 0.01	230d ± 0.01	101d ± 0.06	3.73d ± 0.02	10.52d ± 0.01
Leaf	305d ± 0.01	760d ± 0.01	129d ± 0.03	39.17d ± 0.01d	21.05d ± 0.01
Tuber	305ns ± 0.01	280d ± 0.02	8.0d ± 0.05	4.48d ± 0.01d	15.78d ± 0.01
*values are mean of triplicate determination (n=3) ± standard deviation; GAE, gallic acid equivalents; %, inhibition. ns, non-significant; statistically significant at ($p \leq 0.05$) where a>b>c>d					

Total phenolic compounds: Phenolics can hinder the function of digestive and hydrolytic enzymes, including amylase, trypsin, chymotrypsin, and lipase. However, phenolic compounds are the primary dietary antioxidants associated with a reduced risk of developing cancer, cardiovascular disease, and type-II diabetes. While phenolics were formerly classified as antinutritional factors, they are now recognized as nutraceuticals and are associated with many health advantages [33]. There was a statistically significant difference ($p<0.05$) in the Total Phenolic Content (TPC) observed among the various plant parts of *M. cymbalaria*. The fruit had a lower TPC of 230 ± 0.01 mg GAE/100 g, in contrast to the tuber, which had a TPC of 280 mg GAE/100 g, and the leaf with the highest TPC of 760 mg GAE/100 g. Previous research investigations have documented the TPC in several fruits, specifically reporting values of 26.08 mg GAE/100 g, 29.09 mg GAE/100 g, and 106.89 mg GAE/100 g for M. cochinchinensis, *M. subangulata subspp. renigera*, and *M. dioica*, respectively [26]. This research further demonstrated that *M. cymbalaria* had elevated amounts of TPC compared to other species within the *Momordica* genus [23,32].

Tannins: Tannins are a class of water-soluble phenolic compounds with anti-nutritional effects *via* their ability to form complexes with proteins, carbohydrates, and mineral elements. These complexes cause the inactivation of several digestive enzymes, resulting in reduced protein digestibility and diminished absorption and utilization of other nutrients [33]. The tannin concentration in the plant material of *M. cymbalaria* varied from 8.0 mg TE/100 g to 129 mg TE/100 g (Table 5), with significantly ($p \leq 0.05$) highest tannin content in leaves (129 mgGAE/100 g), followed by the fruits (101 mgGAE/100) and tubers (8.0 mgGAE/100). Tannins are oligomers of flavan-3-ols and flavan-3, 4-diols. Previous studies on *M. charantia* have shown that the leaf, stem and fruit fractions contain higher amounts of flavan-3-ols, flavanols and phenolic acid [34,35]. The observed a reduced presence of tannins (41.37 mg/100 g) in the leaves of *M. balsamina*. This research further demonstrated that *M. cymbalaria* had comparatively higher quantities of tannins than other species within the *Momordica* genus [32].

Enzyme inhibitors

α-amylase enzyme inhibitors: Plant-derived foods include several enzyme inhibitors that impact the activities of α-amylase and trypsin, which are crucial in almost all plant diets [9]. The action of α-amylase enzyme inhibitors in the plant materials ranged from 3.73% to 39.17% (Table 5). The fruit had the lowest observed values (3.73%), followed by the tuber (4.48%) and leaf (39.17%). The ethanol leaf extract of *M. dioica* and *M. charantia* had 73.5% and 35% α-amylase inhibition activity, respectively [29]. The inhibitory activity of the trypsin enzyme in *M. cymbalaria* varied from 10.52% to 21.05%. The fruit had the lowest level of trypsin inhibitor activity at 10.52%, followed by the tuber at 15.78% and the leaf at 21.05% (Table 5).

The α-amylase enzyme catalyses the hydrolysis of α-D-(1,4) the glycosidic bonds in the carbohydrates, converting polysaccharides into oligosaccharides. The studies have shown that inhibiting the activity of the α-amylase enzyme might potentially provide health advantages in mitigating the risk of developing type-2 diabetes and obesity, primarily by impeding glucose oxidation [36]. Trypsin is an enzyme involved in the breakdown of many different proteins. Trypsin inhibitors can modify trypsin and partially impede the enzymatic activities of chymotrypsin in the gastrointestinal tract, hence hindering the process of protein digestion [37]. The present study's findings revealed that the plant parts of *M. cymbalaria* exhibited a comparatively lower level of α-amylase inhibitory activity when compared to *M. dioica*. In contrast, a higher activity level than *M. charantia* and *M. balsamina* [29].

Antioxidant activities: Table 6 presents the DPPH, ABTS radicle scavenging activities and reducing power values of the tuber, leaf, and fruit of *M. cymbalaria*. The fruit (14.36 mgAAE/g ± 0.16 mgAAE/g) has shown the highest DPPH scavenging activity ($p \leq 0.05$), with the tuber (13.4 mgAAE/g ± 0.13 mgAAE/g) and leaf (1.94 mgAAE/g ± 0.08 mgAAE/g) exhibiting lower levels of activity. The fruit had shown significantly ($p \leq 0.05$) higher ABTS scavenging capacity (0.53 mgAAE/g ± 0.14 mgTE/g) than the tuber (0.47 mgAAE/g ± 0.12 mgTE/100 g) and leaf (0.29 mgTE/g ± 0.13 mgTE/g). The various plant parts exhibited distinct variations in their reducing capacities, with the fruit demonstrating a higher reducing power (10.86 EC 50) than the tuber and leaf (Table 6).

Table 6: *In vitro* antioxidant activity of dried powdered tuber, fruit and leaf of *M. cymbalaria**

Sample	DPPH (mg AAE/g)	ABTS (mgTE/g)	RPA (EC50)
Fruit	14.36d ± 0.16	0.53c ± 0.14	10.86d ± 0.02
Leaf	1.94d ± 0.08	0.29c ± 0.13	2.28d ± 0.05
Tuber	13.14d ± 0.13	0.47d ± 0.12	5.19d ± 0.01

*values are mean of triplicate determination (n=3) ± standard deviation; AAE, Ascorbic Acid Equivalents; TE, Trolox equivalents; EC50, the effective concentration at which absorbance was 50. ns, non-significant; statistically significant at ($p \leq 0.05$) where a>b>c>d

The present study reveals antioxidant activities of plant materials were in the following order, from highest to lowest: Fruit>tuber>leaf. The findings of this study agree with the research conducted by various researchers [29,32,38]. The variation has been ascribed to disparities in the number of extractable phytochemicals, the number of aromatic rings present, how hydroxyl groups are substituted, and the formation of complexes with other metabolites [29]. The antioxidant activity in several vegetables is mainly attributed to total phenolics, tannins, and flavonoids, which are considered significant phytochemicals [39]. The current investigation found no correlation between

ABTS, DPPH, and RPA scavenging levels and the total phenolic and tannin contents. The findings of this study agree with the research conducted [40]. Multiple studies have demonstrated that in addition to phenolics, flavonoids, and tannins, various metabolites such as triterpenes, polysaccharides, fatty acids, essential oils, and ascorbic acid also play a role in contributing to the antioxidant activity found in *Momordica spp* [29,41].

CONCLUSION

This study highlighted *M. cymbalaria* as a potential vegetable crop, where the proximate composition, carbohydrate profiling and mineral content provided deep insights. Furthermore, the leaf and fruit were demonstrated as excellent sources of Essential Amino Acids (EAAs), including leucine, valine, lysine, phenylalanine, and isoleucine as for human subjects. The leaf had higher concentrations of anti-nutritional factors i.e. phytic acid, total phenolic acid, and tannins. *M. cymbalaria* exhibited hypoglycemic influence through an inhibitory effect on α-amylase and antioxidant activities. Nevertheless, no correlation has been found between the scavenging levels of DPPH, ABTS, and RPA and the overall phenolic and tannin contents. Therefore, future research on *in vivo* antioxidant and antidiabetic activities and metabolite profiling would be necessary to support our *in vitro* studies. The rich carbohydrate profile of tubers offers potential for their use as nutraceutical constituents and functional food items.

CONFLICT OF INTEREST

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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