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(RESEARCH ARTICLE)



Safety and health benefits profile studies of leaf extracts of *Momordica balsamina* Linn (Cucurbitaceae) found in North Central Nigeria

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Abstract

Momordica balsamina Linn (Cucurbitaceae) plant possesses both medicinal and nutritional properties and has been used for its nutritional and medicinal benefits. The purpose of this paper was to evaluate the safety and health benefits of the leaf extracts of the plant found in North Central Nigeria. The leaf powder was sequentially extracted with solvents of varying polarity index. The protein and amino acid contents were determined by micro Kjeldahl nitrogen methods. The lipid, carbohydrate, fibre and ash contents were determined by well-established and standardized methods. The mineral compositions were analyzed by atomic absorption spectrophotometer. The antioxidant activity was evaluated by the scavenging activity of the extracts on DPPH free radical, while the acute toxicity studies were carried out using male albino rats. Results show that the leaf contains $22 \pm 0.58\%$ of crude proteins on dry weight basis. The corresponding values for carbohydrates, lipids, crude fibre and ash are 32.5 ± 0.8 , 3.98 ± 0.12 , 16.72 ± 1 and $21 \pm 0.81\%$ respectively. The calorific value and moisture content are 201.23 Kcal /100 g and $3.77 \pm 0.22 \%$ respectively. The leaf was composed of macro- and micro nutrients. The methanolic extract possesses the highest radical scavenging activity. The median lethal dose of the aqueous extract at 5000 mg/kg body weight showed no mortality. These results indicate that the leaf of *Momordica balsamina* possesses nutritional and antioxidant properties, and is safe for consumption as herbal vegetable.

Keywords: *Momordica balsamina*; Composition; Radical scavenging; Safety; Health

1. Introduction

There is a growing interest in the use of herbs as food and medicines [1]. Some herbs such as *Bidens pilosa* serve as vegetable or remedy for diabetes and cancer [2]. Plants such as *Moringa oleifera* serve as herbs and are eaten as vegetable for nutritional needs or as a remedy for hepatitis, cardiovascular disease or cancer [3]. *Vernonia amygdalina* has antidiabetic, anti-infective, anti-cancer and anti-inflammatory properties [4]. It is important to evaluate the proximate and mineral compositions and the antioxidant properties of herbs and vegetables in order to determine their nutritional values and radical scavenging properties for human health. In a population where the consumption of these herbs and vegetables are not taken seriously, vitamins, macro- and micronutrients deficiencies may cause malnutrition, chronic and terminal diseases such as hypertension, diabetes mellitus, and cancer [5]

Momordica balsamina also known as 'Balsam apple' (African pumpkin), is an important medicinal and nutritional plant of the Cucurbitaceae family. It is an annual or perennial tendril-bearing herb native to tropical regions of Africa. In India, it occurs naturally in forest, in the rainy season. The leaves, fruits, seeds, and bark possess various constituents of medicinal and nutritional importance. It is called 'Hidden gift of Nature' [6]. 'Momordins' or 'Balsamin' is reported to inhibit the growth of human immune deficiency virus (HIV) and other viruses [6, 7]. The leaf and fruit extracts possess antiplasmodial activity and is used against malaria in African traditional medicine [8]. It has been reported that the

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extracts from parts of this plant possess shigellocidal, anti-diarrhoeal, antiseptic, anti-inflammatory, hypoglycemic and antimicrobial properties [9, 10, 11]. The purpose of this work was to evaluate the proximate and mineral compositions, free radical scavenging activity and acute toxicity of the leaf extracts of this plant. The work would highlight the nutritional values of the plant.

2. Material and methods

2.1. Materials

Momordica balsamina leaves were collected from Jos North area of Plateau State, Nigeria. The identification and authentication was done at the Federal College of Forestry, Jos, Plateau State, where voucher number 288 was deposited. The animals used were purchased from the Animal House of the University of Jos. Other materials used in the work were of analytical grade.

2.2. Methods

2.2.1. Preparation and Extraction of *Momordica balsamina* Leaves

The methods adopted for the extraction of constituents of the leaves of this plant have been reported previously [12]. The leaf powder was sequentially extracted with hexane, dichloromethane, acetone, methanol and distilled water in order of increasing polarity index. The extracts were filtered using Whatman No. 1 filter paper. Evaporation of solvent from the extract was carried out at 40 °C using rotary evaporator (Model RE 100, England). Subsequent extractions were carried out using the same procedure.

2.2.2. Moisture content determination

A 2.5 g of size-reduced dry *Momordica* leaf was weighed into a dry silica dish of known weight. The dish and its contents were placed in a hot air oven at 60°C for 1 h, and cooled in a desiccator for 10 min. The procedure was repeated until a constant weight was achieved. The moisture content was calculated according to the formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{W_1} \times 100 \dots\dots\dots (1)$$

Where W_1 = Weight of *Momordica* leaf before drying and W_2 = Weight of *Momordica* leaf after drying.

2.2.3. Micro Kjeldahl nitrogen (crude protein) determination

A 2 g size-reduced sample of *M. balsamina* leaf was placed in Kjeldahl flask. Then 200 mg of catalyst mixture (copper and titanium sulphate) and 2 g of sodium sulphate were added. A 10 ml quantity of concentrated sulphuric acid was added to the contents of the flask and heated gently for a few minutes until frothing ceased. The heat was increased and the sample digested for 3 h, cooled and the volume made up to 100 ml with distilled water. A 10 ml aliquot was pipetted into the chamber of micro Kjeldahl distillation apparatus, and 10 ml of 40% sodium hydroxide solution was added. The ammonia produced was distilled into 10 ml of 2% boric acid containing mixed indicator. The excess ammonia was neutralized by titration with 0.1N hydrochloric acid which produced a grey colour as the end point. The percentage nitrogen content of the sample was calculated according to the formula:

$$\% N = \frac{(a - b) \times 0.01 \times 14.0057 \times c}{d \times e} \times 100 \dots\dots\dots (2)$$

Where a = titre value of the sample, b = titre value of the blank, c = dilution factor, d = aliquot taken for distillation, e = weight of dried sample (mg). The percentage crude protein was obtained by multiplying the percentage nitrogen by 6.25.

2.2.4. Ash determination

The residue (1.5 g) from the moisture content determination was charred in a muffle furnace at 500 °C for 12 h. The grey ash produced was cooled and weighed.

2.2.5. Fat determination (ether-extract)

A Soxhlet extractor fitted with a reflux condenser and a small flask which was previously dried in the oven and weighed was used. A 2 g of sample of the ether extract (oil) was weighed and transferred to a fat-free extraction thimble, and plugged lightly with cotton wool. The thimble was placed in a 100 ml extractor. Then 150 ml of petroleum ether (boiling point 60-80 °C) was added until it siphons over. More ether was added until the barrel of the 100 ml extractor was half full. The extraction was allowed to proceed gently for 8 h. When the ether was just short of siphoning over, the flask was detached and the contents were siphoned into the ether stock bottle. After draining, the thimble was removed and dried in the oven. The condenser was replaced and distillation continued until the ether in the flask was practically dry. The flask containing the extracted oil was detached; the exterior was cleaned and dried in the oven to constant weight. The percentage ether extract was calculated as follows:

$$\text{Ether extract} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100 \dots\dots\dots (3)$$

2.2.6. Crude fibre determination

The trichloroacetic acid (TCA) method for crude fibre determination was used. A 20 g of trichloroacetic acid was dissolved in a mixture consisting of 500 ml glacial acetic acid, 450 ml water and 50 ml concentrated nitric acid. A 1g quantity of the defatted *M. balsamina* leaf was weighed into a 250 ml conical flask. Then 100 ml of the TCA mixture was transferred into the flask and refluxed for 40 min. The flask was disconnected and allowed to cool. The material was filtered through a 15cm Whatman No 4 filter paper previously dried and weighed. It was washed 10 times with hot distilled water, and once with industrial absolute ethanol and dried in an oven at 105°C for 12 h. The dried material was transferred to a desiccator and weighed after cooling. The filter paper and the residue were transferred into a previously weighed crucible and heated at 500 °C for 12 h. The percentage crude fibre was calculated.

2.2.7. Determination of amino acid profile

The amino acid profile of the leaf powder was determined using the method reported by Ekeanyanwu [13]. A 3 g quantity of the dried sample was defatted using chloroform/methanol mixture in the ratio of 2:1 in a Soxhlet extractor. A 300 mg weight of the defatted sample was weighed into a glass ampoule. Then 7 ml of 6N hydrochloric acid (HCl) was added. Oxygen was expelled by bubbling nitrogen into the ampoule. The sealed ampoule was heated in an oven at 105± 5oC for 22 h, cooled and the contents filtered to remove the humins. The filtrate was evaporated to dryness. The residue was dissolved in 5 ml acetate buffer (pH 2.0), packed in plastic specimen bottles and frozen. A 60µl quantity was dispensed into the cartridge of an amino acid analyser (Applied Biosystems PTH Amino Acid Analyzer, Model 120 A, USA) designed to separate and analyze acidic, neutral and basic amino acids of the hydrolysate. The energy value of *M. balsamina* leaf was estimated in calories (kcal) using Atwater Specific Factors for Selected Foods Table [14].

2.2.8. Quantitative determination of tryptophan

A 300 mg sample was dried to constant weight, defatted, and hydrolyzed with 4.2 M Sodium hydroxide [15]. The hydrolysed sample was evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer PTH (Model 120 A, USA).

2.2.9. Mineral composition

A 2 g quantity of the powdered sample contained in a crucible was digested in 30 ml of concentrated nitric acid on a hot plate placed inside a fume cupboard until digestion was complete. The digested sample was diluted with 50 ml of warm distilled water and filtered into a 100 ml volumetric flask. The metallic elements of the filtrate were analyzed using Atomic Absorption Spectrometer (AAS) (Thermo Scientific ICE 3000 series, USA). The extracts were prepared in sterile water as 5, 10 and 20 mg/ml respectively.

2.2.10. DPPH radical scavenging activity

The antioxidant activity of the extracts on the stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method reported by Brand-Williams [16]. Dichloromethane, methanol, acetone and aqueous extracts of plant material in concentrations of 0.98 – 500µg/ml in methanol were prepared. A 2 ml quantity of the methanolic dispersion of each extract was mixed with 4 ml of 50µM/ml DPPH solution in methanol. The mixture contained in test tubes was vortexed for 10s and incubated for 30min at room temperature of 28°C in the dark. The absorbance of the supernatant was measured at 515nm using UV-Vis spectrophotometer (Shimadzu, 1620 Japan). Low absorbance value indicates high free radical scavenging activity. Ascorbic acid was used as standard in concentrations of 0.195 – 100

$\mu\text{M}/\text{ml}$ in methanol. A blank solution was prepared by mixing 2 ml of methanol with 4 ml of $50\mu\text{M}/\text{ml}$ DPPH solution in ethanol. The differences in the absorbance values of the test samples and control (DPPH in methanol) were calculated and expressed as the percentage of scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \dots\dots\dots (4)$$

Finally, the IC_{50} value, defined as the concentration of the sample causing a 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression plots of the mean percentage of the antioxidant activity against concentration of the test extract ($\mu\text{g}/\text{ml}$).

2.2.11. Acute toxicity test

The Lorke's acute toxicity test method reported by Deora, [17] and Parasuraman, [18] was adopted with slight modification. Eighteen (18) male albino rats (117.3 – 180 g) were used, after obtaining approval from the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, University of Jos. In the first phase of the tests, the animals were grouped in 3's according to their body weight, kept in cages and given standard food and water *ad libitum* for a week. Group I (131.5 ± 5.26 g), II (140 g), and III (145.6 ± 2.3 g) were administered 10, 100, and 1000 mg/kg body weight of the water extract orally respectively after overnight starving. The animals were closely monitored within the first 24 h and daily for 2 weeks. In the absence of death from the groups, the second phase of nine rats [Group I (167 ± 12), II (142 ± 5.3), and III (129.16 ± 10)] were administered orally 1600, 2900 and 5000 mg/kg body weight of the water extract respectively and observed as described above. The median lethal dose (LD_{50}) was calculated as the geometric mean of the least dose that produced mortality, and the highest dose that produced no mortality.

3. Results and discussion

3.1. Nutritional properties

The hexane extract was oily and yellowish in colour. Those of dichloromethane, acetone and methanol were greenish in colour and sticky. The water extract was dark brown in colour and hygroscopic. Table 1 shows that the moisture content of *Momordica balsamina* dried leaves was 3.77 ± 0.22 %. Low moisture content of vegetable drugs minimizes microbial and chemical degradation of the constituents. The crude protein content was 22 ± 0.58 %. This is higher than the 11.29 % reported by Hassan and Umar [10] for leaves of the same plant collected from Kebbi State, Nigeria. Plants that possess more than 12 % of crude protein are considered as good source of protein [10]. The high protein content of *M. balsamina* makes it useful in managing malnutrition among indigent communities in Africa. The percentage ash content was 21 ± 0.81 %. Ash content is an index of the mineral composition. The crude lipid content is 3.98 ± 0.12 % DW. This is lower than the 5.4 % reported by Flyman and Afolayan [19], but higher than the 2.66 % reported by Hassan & Umar [10] for *M. balsamina* obtained from Botswana and Jega community in South Africa and Nigeria respectively. The result indicates that *M. balsamina* leaf is poor in lipid content. This is advantageous in the management of obesity and cardiovascular diseases. The carbohydrate content is 32.5 ± 0.8 %DW. Although this value did not meet the recommended dietary allowance (RDA) of 130 g in children and adults; 175 g in pregnant women; and 210 g in lactating mothers [10], it is suitable for people who need to reduce their intake of calories, especially in the management of obesity and other cardiovascular diseases.

Table 1 Per cent Proximate Composition of *Momordica balsamina* Leaf (Dry matter)

N =3						
Crude protein	Carbohydrate	Ether extract	Crude fiber	Moisture	Ash	Calorific value (Kcal/100 g)
22 ± 0.58	32.5 ± 0.8	3.98 ± 0.12	16.72 ± 1	3.77 ± 0.22	21 ± 0.81	201.23

The crude fibre content of the sample was 16.72 ± 1 %DW. This is higher than the 5.4 % obtained for *M. balsamina* from Botswana [19], but lower than the 29 % obtained from Jega community, Nigeria. Dietary fibre may reduce the risk of coronary heart disease (CHD) by improving blood lipid and glucose profiles, lowering blood pressure, and improving

insulin sensitivity and fibrinolytic activity [10, 20]. The RDA values of crude fibre for children, adult, pregnant and lactating mothers are 19 – 25, 21 -38, 28 and 29 % respectively [10]. *M. balsamina* leaves can serve as a source of dietary fibre in human nutrition. The calorific value of 201.23 kcal/100 g (DW) obtained from the sample is higher than the 189.22 Kcal/ 100 g DW reported for that obtained from Jega community in Nigeria. Vegetables are expected to possess low calorific value [10]. The percent protein content of the sample was approximately 22 %. Table 2 shows the amino acid composition of *M. balsamina* leaf. The essential amino acids constitute 43.8 % of the total amino acids. They include phenylalanine, valine, threonine, tyrosine, isoleucine, methionine, cysteine, leucine and lysine. The remaining 56.2 % of amino acids belong to the non-essential amino acids comprising proline, arginine, histidine, alanine, glutamic acid, glycine, serine and aspartic acid. The most prominent essential amino acid was leucine (7.94 g), followed by phenylalanine (4.43 g). The most prominent non-essential amino acid was glutamic acid (11.66 g), followed by aspartic acid (8.25 g). These results are similar to those reported by Hassan and Umar [10] for the plant. Methionine is a precursor of cysteine, and cysteine is a precursor of glutathione, a free radical scavenger [21]. The presence of these essential amino acids in *M. balsamina* leaf may reduce the depletion of glutathione store in the body, thereby contributing to general wellness through this antioxidant effect. Phenylalanine is known for its anti-sickling effect in the management of sickle cell disease [22]. Phenylalanine, tyrosine and tryptophan in medicinal plants have been involved in the management of sickle cell anaemia [22].

Table 2 Amino acid Composition of the Plant Leaf

Amino acid	Concentration (g/100 g protein)
*Leucine	7.94
*Lysine	4.03
*Isoleucine	3.34
*Phenylalanine	4.43
Tryptophan	0.94
*Valine	3.98
*Methionine	1.12
Proline	3.25
Arginine	4.13
*Tyrosine	3.10
Histidine	2.17
*Cysteine	0.97
Alanine	4.17
Glutamic acid	11.66
Glycine	3.99
*Threonine	3.27
Serine	3.73
Aspartic acid	8.25

*Essential amino acid

Table 3 shows the mineral contents of the sample. The sample contains 416.86 mg/l of potassium, 396 mg/l of calcium, and 97.35 mg/l of magnesium. These are the major macro elements present. The main micro elements include manganese (1.97 mg/l), zinc (0.89 mg/l) and copper (0.25 mg/l). Macro elements are minerals that are needed by the body in large quantity while trace elements are needed in small quantity. This result is in conformity with those reported for the plant from Jega community in Nigeria [10]. The role of calcium and magnesium in bone and teeth development, muscles function and body metabolic processes cannot be overemphasized. Almost 60 % of magnesium in the body is stored in the bones. Consequently, magnesium deficiency contributes to osteoporosis [23]. Intake of calcium and vitamin D prevents osteoporosis. Calcium and magnesium in diets are very important in bone development and maturity.

Table 3 Mineral Composition of *Momordica balsamina* Leaf

Mineral	Concentration (mg/L)
Magnesium	97.35
Sodium	10.25
Potassium	416.86
Calcium	396.00
Zinc	0.89
Manganese	1.97
Copper	0.25
Lead	*Nd

*Nd = Not detected

The macro- and micronutrients in *M. balsamina* make it useful when considering herbs for management of mineral deficiencies, and in the management of hypertension and cardiovascular diseases [19]. Minerals play significant role in blood pressure control in the human body. Perez and Chang [24] reported that sodium: potassium ratio is more strongly associated with blood pressure outcomes in adults than sodium or potassium alone. People who consume diets with low-sodium/high potassium ratio have controlled blood pressure compared to those in the opposite group [24]. High consumption of fruits and vegetables in some isolated societies resulted in only 1% of the population having hypertension, while those in industrialized countries that consume diets high in processed foods and large amount of dietary sodium, 1 out of 3 persons has hypertension [24]. Primitive diets are known to be high in potassium but low in sodium [24]. Houston and Harper [25] reported that low potassium intake in the United States contributed to the prevalence of hypertension in the region. The authors [24, 25] also found that the administration of combinations of magnesium, calcium and potassium in their natural form led to effective reduction of blood pressure (BP). The roles of manganese, zinc and copper in immune functions and diabetes management have been reported [26, 27].

3.2. Antioxidant properties

Table 4 shows the antioxidant activity of the extracts. The high concentration of flavonoids in the methanolic extract with IC₅₀ of 141.90 ± 16.31 µg/ml is consistent with its high antioxidant activity. The corresponding IC₅₀ values for dichloromethane, acetone and aqueous extracts were 255.43 ± 4.41, 277.83 ± 34.05, and 826.62 ± 55.97 µg/ml respectively. Ascorbic acid had an IC₅₀ value of 0.397 µg/ml. Extracts with IC₅₀ values ≤ 100 µg/ml are active antioxidants, while those with values ≥ 200 µg/ml are not [28]. On the basis of these, the

Table 4 Antioxidant Property of *Momordica balsamina* leaf Extracts

Solvent Extracts	Total Flavonoid Content (mg/g)	Total Phenol Content (mg/g)	IC50 (µg/ml)
Dichloromethane	142.88 ± 10.28 *	9.70 ± 2.35	255.43 ± 4.41
Acetone	122.46 ± 4.42	4.37 ± 1.70	277.83 ± 34.05
Methanol	232.46 ± 33.81	6.14 ± 0.00	141.90 ± 16.31
Water	3.19 ± 0.64	9.23 ± 1.02	826.62 ± 55.97
Standard	-	-	0.397 ± 0.00

*Mean ± standard deviation

Methanolic extract possessed fairly active antioxidant activity, while the other extracts did not. Aerobic organisms are exposed to ROS (*reactive oxygen species*) formation. Antioxidant activity is required in stressful conditions such as pathogen attacks, wounds, herbivore feeding, UV light, heavy metals and others. The antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups which are able to bind iron and copper [29, 30, 31]. All the extracts possessed some level of antioxidant capacity because of the presence flavonoids which are phenolic in nature. The antioxidant activity of *M. balsamina* may be due to the presence of phenolic compounds which protect the cells from ROS.

3.3. Acute toxicity

Table 5 shows the result of the acute toxicity test of crude water extract of *Momordica balsamina* leaf given orally in rats. There was no death in Phases I and II of the experiment even at a concentration of 5000 mg/kg. This agrees with the work of Kurami *et al* [32]. Hodge and Sterner toxicity scale states that LD₅₀ ≥ 5000 mg/kg body weight is practically non-toxic [33].

Table 5 Acute Toxicity Test of the Crude Water Extract of *Momordica balsamina* Administered Orally to Rats

Phase	Group	No of Rats	Dose (mg/kg body wt)	No of Death
1	A	3	10	0/3
1	B	3	100	0/3
1	C	3	1000	0/3
2	A	3	1600	0/3
2	B	3	2900	0/3
2	C	3	5000	0/3

The toxicity profile and the therapeutic dose of a drug substance assist in the determination of effective dose of the drug substance. They aid in the determination of minimum and maximum non-lethal dose which provide information on effective dose or overdose. The result shows that *M. balsamina* is a safe herbal vegetable.

4. Conclusion

On the basis of the results of this study, the plant possesses nutritional and antioxidant properties, and is safe for consumption. It could be used in the management of some mal-nutritional problems and preventive health care.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest

Statement of ethical approval

The animal study was carried out after meeting the conditions of the Institutional Animal Care and Use (IACU) in collaboration with the Office of Laboratory Animal Welfare (OLAW) with the issuance of Ethical Clearance Certificate, Reference number: F 17 – 00379.

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