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NUTRITIVE EVALUATION, MINERAL COMPOSITION, AMINO ACID PROFILE AND PHYTOCHEMICAL ANALYSIS OF LEAF PROTEIN CONCENTRATES OF BITTER GOURD (*MORMODICA CHARANTIA)*

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ABSTRACT: *Fresh green leaves of bitter gourd (Mormodica charantia) were harvested and processed with a view to analyzing its phytochemical constituents and nutritional potentials of the leaf protein extract. Proximate, mineral, amino and phytochemical analyses were determined using standard analytical techniques. The result showed that Mormodica charantia has a Moisture content of 5.11±0.0014g/100g, Ash content 9.66±0.00g/100g,Crude protein 21.30±0.007g/100g, Fat content 7.23±0.00g/100g and Fibre content 9.59±0.04g/100g.The mineral analysis of the sample shows that Mormodica charantia contains Zinc content of 37.55±0.1mg/100g, Magnesium content 62.75±0.14mg/100g and Potassium 26.80±0.00mg/100g. Mormodica charantia also contains Tannin, Saponnin, Alkaloids and some other phytochemical. The results of this analysis showed that this vegetable leaf protein concentrates is a good source of minerals such as Mg,Ca, S and Cu. Based on the findings from this study, Mormodica charantia has numerous nutritional potentials, this implies that it is capable of furnishing man with certain value of daily dietary nutrient requirement and medicinal potentials. It is a kind of plant that is highly indispensable for man use and can be employed for medicinal and drug processes.*

KEYWORDS*:* Mormodica charantia, nutritional potentials, phytochemical analysis, mineral, leaf protein concentrates.

INTRODUCTION

Medicinal plants and its products have continued to be an important therapeutic aid for alleviating the ailments of human kind (Joseph & Jini 2011, 2012). Herbs for diseases treatment are not new. Since ancient times, plants and plant extracts were used to combat diseases. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. The World Health Organization (WHO) has listed 21 000 plants, which are used for medicinal purposes around the world. Among them, 150 species are used commercially on a fairly large scale.

Momordica charantia (M. charantia), also known as bitter melon, karela, balsam pear, or bitter gourd, is a popular plant used for the treating some ailments like diabetes related conditions amongst the indigenous populations of Asia, South America, India, the Caribbean, East Africa and West Africa (Cefalu et al, 2008). Its fruit has a distinguishing bitter taste, which is more pronounced as it ripens, hence the name bitter melon or bitter gourd. Biochemical and animal model experiments have produced abundant data and hypotheses accounting for the antidiabetic effects of M. charantia. In comparison, clinical studies with human subjects are sparse and low

quality in design. Diabetes mellitus is well known clinical entity with various late complications like retinopathy, neuropathy, nephropathy, etc. Natural products are known to play an important role in pharmaceutical biology (Joseph & Raj, 2010). Specific plant knowledge may provide insight for strategic consumption and sustainable use. The alternate medicine system is now gaining momentum with the knowledge of active principles identified from plant species (Joseph & Jini, 2011).

Bitter lemon or bitter gourd has significant antidiabetic, antioxidant, antimicrobial, antiviral activity as well as relatively high nutritional value due to high iron and ascorbic acid content so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes, colic, to heal skin sores and wound as well as to delay the late complications of the said ailments. In the present study, we have elucidated and analyzed the nutritive values, amino acids profiles, mineral components as well as its phytochemical constituents.

MATERIALS AND METHODS

Preparation of Sample;

Fresh Broad, large sized leaves of *Mormodica charantia* were obtained from vegetable garden situated near Erelu dam at Oyo town. The leaves were washed with distilled water and pulped by passing it through the locally produced mincer (technically referred to as cell rupture). The pulps were collected and strained through a cotton cloth followed by screw press. The green juice obtained from straining the pulp through the cotton cloth, was heated between 85-90^{0}C by steam injection, which resulted in coagulation of all the protein present within the pulp. The coagulum was then centrifuge from the rest of the solution, pressed, pulverized and air-dried prior chemical analysis.

Phytochemical Analysis

Tannin, total phenol, phytate, oxalate, alkaloid, saponin and flavonoid were determined using the following method of analysis.

Determination of tannin content:

Tannin contents of the samples were determined using method described by price and Butter 1977. 5g of plant samples is added 30 mins after filtration, the solution is further transferred to a 30ml flask and water was added to 50ml. 5ml aliquots are finally transferred to 1 ml of 1% $K_3Fe(CN)_6$ and 1 ml of $FeCl_3$ are added and water is added to make 10ml volume. After 5 min, the solutions are measured spectrophotometrically at 720nm. The actual tannin concentrations were calculated on the basis of the absorbance values obtained for the standard solution in range 5.25mg/10ml

Determination of saponin content

The method described by Obadori and Ochuko 2001 was used for determination of Saponin. 10g of powdered leaf, root and stem of the samples are located in 150ml of 20% aqueous ethanol. The samples are located with continuous stirring at 550C for 4 hours. The mixture was filtered

and the residue is extracted with another 200ml of 20% ethanol. The combined extracts were concentrated to 40ml over water bath at about 90^0C. The concentrate was extracted with 200ml of diethyl ether. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated; 50ml of n-butanol is added. The combined n-butanol extracts are washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was located in water bath. After evaporation the sample was dried in the oven to a constant weight and the saponin content was calculated as percentage.

Determination of alkaloid

The total alkaloid contents of the samples were determined by method described by Manjunath et al., 2012. The sample extract was dissolved in 2N HCI and then filtered. 1ml of this solution was transferred to separating funnel and washed with 10ml chloroform. The pH of phosphate buffer solution was transferred to a separating funnel and the 5ml of phosphate buffer and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in 10ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470-nm.

Determination of flavonoid content

5g of *Mormodica charantia* plant was weighted into 100ml plastic bottle and extracted repeatedly with 100ml of 80% aqueous ethanol at room temperature. It was then filtered with Whitman filter paper into 100ml flask. This filtrate was transferred into a crucible dish and evaporated to dryness over a water bath. This was further dried in an oven at 60^0C for 30 minutes and later cooled in desiccators. Both the crucible and the content were weighed and recorded (Ukpabi et al., 2013).

Determination of total phenolic content

Total phenol was determined after the fat free sample was boiled with 50ml of ether for 15 minutes. 5ml of the extract each was pipette into a 50ml flask and 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated alcohol were also added. This sample was left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 500nm. A blank sample for each extract was used for background subtraction. A standard phenol was prepared as 0.005mg/l and absorbance measured the total phenolic content was expressed as mg/100g.

Proximate Analysis

The parameters determined for proximate analyses include ash, moisture, crude protein, fat, fiber and carbohydrate. All of these were carried out using the method of analyses described by the official method of the Association of Official and Analytical Chemists (AOAC 2006).

Mineral Analysis

The atomic absorption spectrophotometer (AAS) was used for the analyses of the namely mineral contents present in *Mormodica charantia*: Na, K, Ca, Mg, Zn, Fe, Cu, Mn, P and Cd. A known amount of the sample was placed in a dish and heated with burnsen burner in a fume

cupboard until the sample turned to ashes and there was no smoke emitted. The ash was transferred to the desiccator in order for it to cool after which 0.1m HCl solution was added to the ash after which few drops of Concentrated HNO_3 was followed. The resulting solution was evaporated almost to dryness in water bath, filtered and diluted with distilled water.

Amino Acids Analysis

The sample was dried to constant weight, defatted, hydrolyzed and evaporated in a rotary evaporator after PTH amino acid analyzer was used to determine the amino acid present in the sample (Benitez, 1989).

The mixture of chloroform/methanol (2:1) was used to defat the samples after weighing the sample into an extraction thimble. The extraction was carried out for 15hours in Soxhlet extraction apparatus (AOAC, 2006)

Method of calculating Amino Acid Values

An integrator attached to the analyzer calculates the peak area proportional to the concentration of each of the amino acids.

RESULTS AND DISCUSSION

Table 1: Proximate Composition of *Mormodica charantia*

Parameter	(mg/100g)
Ash	9.66 ±0.00
Moisture content	5.11 ±0.01
Crude Protein	21,30 ±0.00
Fat	7.23 ±0.00
Fibre	9.59 ±0.00
CHO	ND

Table 1 above shows the result of the proximate composition of *Mormodica charantia*. The sample *have* moisture content of 5.11 ±0.01 mg/100g. These results are lower than the present the results of proximate composition of *Daucus carota* leaf protein concentrates which contained 8.69±0.03g/100g moisture content (Sodamade, 2019). The moisture in food determines the characteristics keeping quality; it facilitates the rate of digestion assimilation and absorption within the body. The low moisture content of the samples means that there is a concentration of solutes and decreased ability to perishability (Fennema and Tannen Baum 1996).

The result of the analysis shows that *Mormodica charantia* contains 9.66±0.00mg/100g. Ash is a measure of total mineral content in the samples. The result indicates that the samples could be a source of mineral elements. This value is lower than the value obtained for Daucus carota leaf protein concentrates which is 19.69±0.02g/100g, the value is also lower than 11.60g/100g and11.37g /100g reported for two varieties of *Ipomea batatas* leaf sample (Haard 1976). Also, From Table 1 the crude protein content in *Mormodica charantia* is 21.30±0.00mg/100g. The

result indicates that the crude protein content of the sample is high. Therefore, *Mormodica charantia* is a good source of protein.

The fat content in *Mormodica charantia* is 7.23±0.00mg/100g. The fact that fat supplies most of the energy required by man (Osborne and Voogot, 1978) suggests that *Mormodica charantia* is a good source of fat. Fat serves as energy store in the body. It can be broken down and used in the body to release glycerol and free fatty acids. The glycerol can be converted to glucose by the liver and used as a source of energy. The crude fibre content in *Mormodica charantia* is 9.59±0.00mg/100g.The value is lower than 14.81±0.02g/100g obtained for *Daucus Carota* leaf protein concentrates (Sodamade et al., 2019). Significant proportion of Fibre in food reduces the risk of cardiovascular disease, coronary heart disorder, obesity and gastro intestinal disorder. Crude fibre concentration of this sample is a true prediction of the concentration of mineral element in the sample. Also, the value is lower than 28.60g/400g reported for *Amaranthus cruenthus* (Oguntona 1988).

Table 2: Concentration of Mineral elements in Bitter Gourd (*Mormodica charantia*)

Minerals	Concentration (mg/100g)
Na	23.63 ±0.00
K	26.80 ±0.00
Ca	45.48 ±0.00
Mg	62.75 ±0.14
Zn	37.55 ±0.1
Fe	12.45 ±0.01
Cu	0.03 ±0.03
Mn	1.27 ±0.01
P	8.85 ±0.01
Cd	N.D

Minerals are important component of diet because of their physiological and metabolic function in the body. Table 2 shows various concentration of minerals present in Bitter Gourd (*Mormodica charantia*). From the result of the analysis, *Mormodica charantia* contains 23.63mg/100g Sodium. The values are lower than the 500mg value of *ad*ults recommended daily allowance (NRC,1989). Sodium is an important mineral that assists in the regulation of body fluid and in the maintenance of electric potential in the body tissue (Alinnor & Akalez, 2010). Also, The World health organization (WHO) recommended intake of sodium per day is 500mgper adult and 400mg for children (WHO,1973). This result indicates that sodium content of *Mormodica charantia* is below the WHO recommended standard. This indicates that the sample is good for hypertensive patient but significant quantity of the sample must be eaten if the recommended daily allowance has to be met by eating the sample alone. Sodium is an important source of electrolyte within the body but too much of sodium in combination with chloride could lead to increase blood pressure.

Mormodica charantia contains 26.80±0.00mg/100g Potassium content. The potassium content of Momordica *charantia* leaf protein concentrate is higher that of Daucus Carota which is 9.72mg/100g (Sodamade, 2019). Also, the concentration of potassium in this sample is lower than 100g reported for astragalina leaves (Gafer et al., 2011). Furthermore, Potassium concentration value in this sample is lower than 90.3±0.42mg/100g reported for Thaumatococus danieli (Sodamade, 2014). Potassium is important in the regulation of heart beat, neuro transmission and water balance of the body. The WHO recommended intake of potassium per day is 200mg for adult and 1600mg for children. This study revealed that potassium content of *Mormodica charantia is* below WHO standard.

Calcium concentration of *Mormodica charantia* is 45.48±0.00mg/100g. According to the recommended daily intake of calcium by WHO is 800mg for both adult and children. This value is lower than 49.30mg/100g obtained for Calcium concentration of *Daucus carota* leaf protein concentrate. Calcium is an important mineral required for bone formation and neurological function of the body. It is also required for skeletal and muscular development. However, care must be taken in choosing calcium rich foods because approximately 5% kidney stores are composed predominantly of calcium compounds. The concentration of Magnesium in *Mormodica charantia* is 62.75±0.14mg/100g. Magnesium plays essential role in calcium metabolism in bones and also involved in prevention of circulatory diseases. It helps in regulating blood pressure and insulin releases (Umar et al,, 2005). Recommendation dietary allowance for magnesium in adults is 350mg/day while children is 170mg/day. The result reveal that the results obtained for *Mormodica charantia* is below the recommended values. Therefore, *Mormodica charantia* cannot be regarded as rich source of magnesium. Also, from Table 2 the Zinc content for *Mormodica charantia* is 37.55±0.1mg/100g The WHO recommended dietary allowance for Zn in adult and children are 15mg/day and 10mg/day respectively. The value sample is higher than the recommended dietary intake. This study indicates that *Mormodica charantia is* highly rich in Zinc. Zinc is an essential micronutrient associated with a number of enzymes, especially those associated with synthesis of ribonucleic acid (Guil-guerrero et al,1998). Zinc deficiency reduces the rate of recovery for protein energy in malnourished children (Hambrigde, 1986). The result of this study shows that Iron content in *Mormodica charantia is* 12.45 ± 0.01mg/100g. The recommended dietary allowance for Iron in adult and children is 10mg/day, while female adult is 15mg/day. This study indicates that the Zinc content is above the recommended standard, Iron is essential for blood formation and it is important for normal functioning of the central nervous system (Adeyeye and Fagbohun, 2005). Furthermore, the Copper content in *Mormodica charantia* is 0.03±0.03mg/100g.The recommended standard is 3mg/100g for adults and 2mg/day for children. From the result of the analysis, the copper content of *Mormodica charantia* is far below the recommended standard.

Copper is required in the body for enzyme production and biological electron transport. Manganese concentration in *Mormodica charantia* is 1.27±0.01mg/100g the value is lower compared to 2.50±0.88mg/100g reported for Thaumatococcus danielli leaf protein concentrates by Sodamade (2014). Manganese assist in the regulation of blood sugar level, it is involved in the cell production, energy and reinforce the immune system (Deborah 2008).

The Phosphorous concentration in is 8.85±0.01mg/100g while Cadium concentration was not detected.

Table 3: Essential Amino acids concentrates in *Mormodica charantia*

Amino Acid	**Concentration**
Leucine	8.29
Lysine	7.46
Isoleucine	5.50
Phenylalanine	5.29
Tryptophan	1.10
Valine	1.57
Methionine	4.35
Tyrosine	3.54
Histidine	2.47
Threonine	3.57

Table 4: Non-Essential Amino Acid concentrates in

Amino Acid	Concentration
Proline	4.35
Arginine	5.54
Cystine	3.54
Alanine	4.46
Glutamic acid	13.87
Glycine	3.82
Serine	3.08
Aspartic acid	8.59

Amino acid composition both Essential and Non-Essential of *Mormodica charantia* was presented in Tables 3 and 4 respectively. Protein biological and nutritive value of is dependent upon its constituent amino acids and its tendency to meet the nitrogen and essential amino acids requirements. Aside Methionine and Tryptophan, the concentrations of other amino acids (both essential and non-esssential amino acids) detected in *Mormodica charantia* are appreciably normal.

Leucine and valine are branched chain amino acid that critically enhances protein synthesis in the body, they join to assist in muscle and worn out tissue repair in the body. Leucine works closely with insulin to regulate the blood sugar levels stimulates wound healing while valine promotes hormones that are responsible for growth (Fitch & King, 1987). The recommended daily allowance of valine is 24mg per day while recommended daily allowance of leucine is 42mg per day (Forman 1986). *Mormodica charantia* can contribute between 15.16% to 17.32% of leucine and 14.13 to 15.68% of valine respectively.

Methionine is needed for the synthesis of choline which in turn forms lecithin and other phospholipids in the body (Olusanya, 2008). Phenylalanine is the precursor of some hormones and the pigment melanin in hair, eyes and tanned skin (Quadri & Musa, 2015) while Aspartic acid is needed in the body to generate adenosine triphosphate (ATP), the fuel that powers all cellular activity. Lysine is helpful in the absorption and production of calcium; it is required for protein synthesis hormone and energy production. The lysine concentration is lower than the recommended daily allowance of lysine (38mg per day) (Formon, 1974). Significant quantity of this sample will be utilized to meet up with recommended daily allowance.

Histidine is required for the production of histamine, the neurotransmitter that is vital to immune response, digestion sexual function and sleep wake cycles (Formon 1974). The recommended daily intake of histidine is 14mg per day. It maintains myelin sheath that forms a protective barrier for the nerve cells (FAO 1970).

Aside the structural functions, amino acids are the main precursors for the manufacture of many important substances in the body of living organisms and could also serve as valuable sources of energy especially in the absence of carbohydrate and fats in the body (Olusanya 2008).

Table 5: Phytochemical Analysis of *Mormodica charantia*

Phytochemicals	**Concentration mg/100g**
Tannine	6.45±0.01
Total phenol	9.54±0.01
Phytate	26.09±0.10
Oxalate	3.28±0.53
Alkaloids	6.45±0.02
Saponin	3.89±0.69
Flavonoid	3.12±0.01

The phytochemical contents of ***Mormodica charantia*** were presented in Table 5, the presence of Tannin, Phenol, Phylate, Oxalate, Alkaloids, Saponin and Flavonoids in this plant may account for its therapeutic properties.

Tannins hasten healing of wounds and inflamed mucous membranes (Edeoga et al 2005). Tannins, which denature protein constituents of the intestinal mucosa and form protein- tannates causes astringent activity (Edeoga et al 2005). They can also be used medicinally as antidiarrheal, hemostatic, and antihemorrhoidal agents (Cushinie, et al 2005), Tannins have astringent properties and so are remarkable in the treatment of stomach ulcers and diarrhoea. They form a protective layer over wounds and so preventing it from infections (Ashok and Upadhyaya, 2012). The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Tannins also participate in

oxidation–reduction reaction of ascorbic acid and its antibacterial properties have been reported (Cushinie, et al 2005).

Alkaloids and their synthetic derivatives are used as basic medicinal agents and have been implicated to possess anti-malaria, analgesic properties (Edeoga et al 2005), anti-cholinergic and anaesthetic properties (Okwu et al, 2006). The presence of alkaloid in the plant supports its use in the possible treatment of malaria, hypertension and cancer (Achi et al., 2017). Due to their free radical scavenging activities, flavonoids have been associated with the prevention of diseases that involve oxidative stress (Huang et al., 2010). Flavonoids protect against allergies, platelet aggregation, ulcer, tumor and viruses (Amakoha et al, 2002). The rejuvenating effects of flavonoids on cells and tissues can also contribute a substantial role to the fertility enhancing potentials of the plant. Flavonoids have also been reported to possess many pharmacological properties such as: anti-oxidant, anti- cancer, anti-inflammatory and anti- microbial activities (Okwu et al, 2005; Joy et al, 1998; Shoskes et al, 1999).

Saponins are natural antibiotics which fight infections and microbial invasions. They also have hypocholesterolemic properties, which could offer some chemoprotection against heart diseases to human consumers (Okwu & Emenike, 2006). The most striking prospect for saponins is how they inhibit the growth of cancer cells without posing any significant risk on normal cells, as is the mode of some cancer-fighting drugs Saponins possess aphrodisiac properties and act as a source or precursor of sexual hormones like testosterone, corticosterone, aldosterone, progesterone, estrogens and androgens. The saponins present may be responsible for the medicinal properties accorded the plant (Abo-Dorna et al, 1991). Cancer cells have more cholesterol-type compounds on their membranes than normal cells. Saponins bind these cholesterol-like compounds and thus interfere with their growth and division (Okwu, 2005).

The anti-nutrients phytate (26.09±0.10Mg/100g) as reported in this study have been reported to have complicated effects in human system including indigestion of food and flatulence (Maynard et al, 1994). It has also been known to exert substantial effect on bioavailability of minerals in foods by forming complexes with minerals (such as Ca, Zn and Mg), thereby preventing efficient absorption by the body systems (Oboh, et al, 2003). However, extensive and proper processing methods are needed to reduce some of these anti-nutrients. The presence oxalate (3.28±0.53Mg/100g) concentration as reported in this study is known to cause great risk of renal absorption and also possess the ability to chelate divalent minerals and prevent their absorption by the body systems. However, the levels of oxalate in the leaves is moderate and can still be reduced when subjected to Heat treatment like boiling and frying etc.

The results obtained in this study thus suggest the identified phytochemical may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit so it can be subjected to drug and nutritional processes.

CONCLUSION AND RECOMMENDATIONS

Mormodica charantia shows viable amount of nutritional value, minerals, amino acid profile and its phytochemical constituents which implies that it is capable of furnishing man with certain value of daily dietary nutrient requirement and medicinal potentials. It is a kind of plant that is highly indispensable for man use and can be employed for medicinal and drug processes. However, further research can be done to investigate the level of its active compound and their mechanical action.

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