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Profiling and *In vivo* studies of Bromelain Bitter Gourd (*Momodica charantia*) seed protein hydrolysate with antidiabetic activity

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Abstract

Diabetes mellitus is a multifactorial chronic disease that affects the human population and it is the third most common cause of death worldwide. Momordica charantia is commonly known as Bitter melon, Bitter guard and used as a food and natural medicine. The scientific name, Momordica means "to bite," in Latin which is refers to the jagged edges of the leaves. Including fruits, all parts of the plant, contains a bitter compound, momordicinso and very bitter in taste. It has long been used as a traditional medicine for some ailments. Bromelain bitter guard seed protein hydrolysate was profiled by ultrafiltration and SDS-PAGE analysis revealed that the lower molecular weight peptide ≤ 25 kDa exerted the high antidiabetic activity. Spontaneously diabetic rats showed a decrease in the blood Glucose level (Glu), Glycated haemoglobin (HbA1c) level, Glycogen level and also shows lower level of Lipid profile parameters (Chol, HDL, LDL, and TG) in the serum of the diabetic rats after 21 days of oral administration of bromelain bitter guard seed protein hydrolysate at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg. However, the effect was dose-dependent. As a novel protein hydrolysate source with *invivo* antidiabetic activity, future research should aim to demonstrate the molecular mechanism of action and validate its bioactivity through human intervention trials.

Keywords: Bitter gourd (*Momordica charantia*); Bromelain bitter gourd seed protein hydrolysate; Antidiabetic activity; Biochemical parameters

1. Introduction

Diabetes mellitus is a disorder that affects the body's ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for fuel. People cannot live without insulin (A. D. A. 2007). Diabetes results in abnormal levels of glucose in the bloodstream. This can cause severe short-term and long-term consequences ranging from brain damage to amputations and heart disease (A. D. A. 2007).

Metabolic forms of diabetes *include; Type 2 diabetes:* Also known as insulin dependent diabetes mellitus (IDDM), this accounts for 90 - 95% of diabetic cases, according to the U.S. National Institutes of Health (NIH). Some of these patients have had prediabetes that went uncontrolled. Once considered a disease of middle and old age, type 2 is also becoming more common in youths as the incidence of childhood obesity grows, Autoimmune*:* The body's immune system can mistakenly destroy the insulin-producing beta cells of the pancreas. The causes of autoimmune diabetes are poorly understood, but genetics and family history play a role, and viruses or other environmental factors are believed to figure in. Autoimmune forms of diabetes include: Type 1 diabetes formerly known as juvenile diabetes, this form generally develops in children and young adults. This variation of type 1 can occur later in life. Individuals with autoimmune diabetes who overeat, are sedentary, gain weight or have certain genes can, like people with metabolic forms of diabetes, develop insulin resistance. This state is known as double diabetes. Diabetes can also result from another disease, such as pancreatitis, or even from a medical treatment, including pancreatectomy (surgical removal of the pancreas) or

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certain medications. This is known as secondary diabetes. In addition, there are uncommon inherited disorders that cause diabetes, such as maturity-onset diabetes of the young and Wolfram syndrome. Most cases of diabetes last the rest of a person's life. However, gestational diabetes generally ends when the pregnancy does, and some cases of secondary diabetes are also temporary (Cefalu *et al*., 2007). Gestational diabetes; Develops in 2 percent to 5 percent of all pregnancies but usually disappears when a pregnancy is over. Hormonal changes contribute to this condition which can develop in any previously nondiabetic woman during pregnancy, especially those who are overweight.

The root causes of diabetes are complex. Most cases begin with one of two processes which include unhealthy lifestyle factors such as overeating, physical inactivity and obesity can impair the body's ability to use insulin. This is called insulin resistance. Uncontrollable risk factors which include genetics, family history and age can also be involved. Diabetes Mellitus (DM) is a name among the first described disease and is one of the most widespread chronic endocrine disorders all over the globe. As per the survey of 2014, it has been estimated that there are 387 million of people carrier of this sweet killer disease among which 90% of the carriers are associated with Type 2 Diabetes Mellitus (T2DM). From 2012 to 2014 only diabetes is assessed to conclude in 1.5 to 4.9 million deaths each year. The global economic cost of diabetes in 2014 was estimated to be $ 610 billion USD. It is recognized as a global epidemic by the World Health Organization. It is a well-known and clear fact that managing diabetes is a complex and challenging task; therefore, over the period of last few decades, development of various anti-diabetic drugs has shown an emerging tool to tackle this over growing disease in combination with proper eating habits and physical exercises.

In Nigeria, the current prevalence of Diabetes Mellitus among adults aged 20–69 years is reported to be 1.7% (I.D.F. 2018). It is widely perceived that prevalence figures reported by the IDF grossly under-report the true burden of DM in Nigeria, given that they are derived through the extrapolation of data from other countries. Various researchers have reported prevalence ranging from 2% to 12% across the country in recent years (Nyenwe *et al*., 2003). The last time a nationwide population estimate of DM was undertaken in Nigeria was during the 1992 Nigerian National Non-communicable Diseases (NCD) survey, where DM was said to occur in 2.2% of the population. There has been no nationwide health (diabetes) survey in Nigeria since then. However, it is important to determine the actual burden of DM in Nigeria to facilitate appropriate health resource allocation, advocacy, and planning. Thus, in the work reported in the present paper, we aimed to determine the prevalence of and risk factors for DM in Nigeria using a systematic review and meta-analysis. (Muhammad *et al*., 2017)

Treatment of diabetes mellitus typically includes diet control, exercise, home blood glucose testing, and in some cases, oral medication and/or insulin. Approximately 40 percent of people with type 2 diabetes require insulin injections.

Momordica charantia is commonly known as Bitter melon, Bitter guard and used as a food and natural medicine. The scientific name, *Momordica* means "to bite," in Latin which is refers to the jagged edges of the leaves. Including fruits, all parts of the plant, contains a bitter compound, momordicinso and very bitter in taste. The plant grows in tropical regions such as India, China, America Malaya, Bangladesh, tropical Africa, Thailand, Middle East. *Momordica charantia* contains a different biologically active phytochemicals, which includes proteins, triterpens, saponins, flavonoids, steroids, alkaloids, and acids. The plant is beneficial for its anti-tumorous, anti-fungal, anti-parasitic, anti-cancer, antiviral, anti-fertility, anti-bacterial and hypoglycaemic properties due to the presence of numerous phytochemicals. In traditional medication, fruits and leaves are used to cure several diseases like: gout, rheumatism, colic, worms, illness of liver and spleen. Momordica contains alkaloids and peptides which resemble like insulin and charantin, a collection of steroidal sapogenins due to which it has hypoglycemic property.

The use of synthetic antidiabetic such as Insulin, Metformin, and Glibanclamide has serious limitations due to high cost and potential sight effect on human health. Therefore, alternative medications are necessary for the production of antidiabetic compounds from natural sources without side effects on human health (Jin *et al*., 2017).

Protein hydrolysates and biopeptides have various biological activities such as antidiabetic, antioxidant, antibacterial, and antihypertensive, potential depending on amino acid composition, sequencing, hydrophobicity, and chain length (Nasri, 2017). In recent years, various food protein hydrolysates have been reported to be antidiabetic and antioxidant potential in barley (Alu'datt *et al*., 2012), egg yolk protein (Zambrowicz *et al*., 2015), pinto beans (Ngoh & Gan, 2016), and cumin seeds (Siow & Gan, 2016).

However, there are some inadequate information's about bitter gourd seed regarding the production of bioactive peptides hydrolysate and impact of the bioactive peptides as antidiabetic compound. Therefore, there is need to profile major principally active protein(s) in *Momodica charantia* especially those of seed which normally are being discarded and evaluate their antidiabetic properties which may be considered cheap, reliable, and with low side effects. This study

aimed to profile bromelain bitter gourd seed protein hydrolysate with *In vivo* antidiabetic activity which may be considered cheap, reliable, and with low side effects.

2. Material and methods

2.1. Experimental design

2.1.1. Animals and Treatment

Sixty (60) Sprague–Dawely healthy rats aged 8-10 weeks (110–150 g) were obtained from National Veterinary Research Institute (NVRI), Vom, Jos Plateau State. The animals were housed in standard cages under proper environmental conditions, feed with a commercial diet, tap water provided *ad libitum* and kept for 2 weeks for acclimatization and maintained at 25± 1 °C with a 12 hrs dark and light cycle. Animals were randomly distributed into six (6) groups of ten (10) rats each and were treated with the Bromelain Bitter Gourd Seed Protein Hydrolysate (BGSPH) orally for 4 weeks (28 days) 7 days after intraperitoneal (IP) induction with freshly prepared Alloxan in which analyses was carried out on last day of 28 days.

2.1.2. Induction of Diabetes Mellitus

Rats were injected intraperitoneally (IP) with freshly prepared alloxan monohydrate in normal saline at a dose of 120 mg/kg b.w. Because alloxan induces fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (5–10 mL) orally after 6 hrs. The rats were then being kept for the next 24 hrs on 5% glucose solution bottles in their cages to prevent hypoglycemia. After one week, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with blood glucose levels of ≥200 mg/dL) were chosen for the experiment.

2.1.3. Measurement of blood glucose and body weight

Blood glucose concentration in all experimental groups were recorded following 12 h fasting each day, at 8:00 a.m., before feeding rats, using AccuChek glucometer (Pharmatec) by glucose oxidase peroxidase method using glucose test strips. Rats were weighed individually at weekly intervals using a Mettler Toledo® digital precision balance with a sensitivity of 0.001 g (Model MT-500D), and the body weights were recorded to calculate weekly body weight gains. The fasting body weight and blood glucose levels were estimated on 1, 7, 14, 21 and 28 days, periodically.

Table 1 Groups and doses of Bromelain Bitter Gourd Seed Protein Hydrolysate (BGSPH) administered (mg/kg body weight)

GROUPS	DOSAGE	NUMBER OF ANIMAL
Normal control	Food / water	10
Negative control	120 mg/kg Alloxan + No treatment	10
Positive control	120 mg/kg Alloxan + 50 mg/kg Metformin	10
Test 1 + Bromelain BGSPH	100 mg/kg	10
Test 2 + Bromelain BGSPH	200 mg/kg	10
Test 3 + Bromelain BGSPH	400 mg/kg	10

2.2. Determination of the *In vivo* antidiabetic activity of Bromelain Bitter Gourd Seed Protein Hydrolysate (BGSPH)

In vivo study was carried out by biochemical estimation of Glucose level, Glycogen level, Glycated Hemoglobin (HbA1c) and Serum Lipid profile.

2.2.1. Serum Preparation for Biochemical analysis

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood samples were collected in an appropriate container on decapitation and serum was separated by centrifugation. The serum samples were processed for Biochemical analysis.

Blood were allowed to clot for 30 minutes and then centrifuge at 40,000 rpm for 20 minutes and the serum were obtained which were used for Biochemical analysis.

2.2.2. Blood Glucose Level Determination

The blood glucose was determined using ACCU-CHEK Active Diabetes Monitoring Kit (Roche Diagnostics GmbH, Mannheim Germany), based on the glucose oxidase method.

2.2.3. Glycogen level determination

Glycogen Assay Kit was used to determine the glycogen levels in blood samples (calorimetrically) according to the manufactures instruction.

2.2.4. Glycated Hemoglobin (HbA1c) determination

Glycated Hemoglobin (HbA1c) was determined in the blood samples base on the World Health Organization (WHO) calorimetrically according to manufactures instruction.

2.2.5. Serum lipid profile assay

Serum lipid profile assay (Total cholesterol, HDL, LDL and Triglyceride) was determined in the serum samples using ChemRay 240 Semiautomated Chemistry analyzer.

2.3. Profiling of bitter gourd seed protein hydrolysate

2.3.1. Fractionation of Bitter gourd seed protein hydrolysates (ultrafiltration) using VivaSpin tube protein concentrator.

Fractionation of Bitter gourd seed protein hydrolysate (ultrafiltration) was carried out using VivaSpin tube with molecular weight cut-off (MWCO) of 10 kDa.

2.3.2. Determination of Amino Acid Profile and Crude Protein

Amino Acid Profile was determined by method Described by AOAC (2005) with slight modifications.

- The unhydrolyzed protein and Bromalein Protein hydrolysate samples were dried at 70^0c to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Loading of the hydrolysate into analyzer

- 60microlitre was loaded into the amino acid analyser, this was dispensed into the cartridge of the analyser.
- The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

2.4. Statistical analysis

All analyses were presented as mean ± SEM. Data analysis was carried out using SPSS software (Version 20) and data of differences between samples was compared. ($p < 0.05$)

3. Results

Table 2 Amino acid composition of Crude Protein and Bromelain Protein Hydrolysate of Bitter Gourd Seed

S/N	Amino Acids	Crude Protein Amino Acid Concentration (g/100 g)	Bromelain Protein Hydrolysate Amino Acid Concentration (g/100 g)
1	Leucine	7.21±0.03	12.23±0.04
2	Lysine	3.15±0.02	5.01±0.03
3	Isoleucine	3.29±0.05	4.01±0.02
4	Phenylalanine	4.04±0.05	3.77±0.05
5	Norleucine	0.00±000	0.00±0.00

6	Tryptophan	0.82±0.03	2.42±0.06
7	Valine	3.09±0.02	3.86±0.03
8	Methionine	1.18±0.06	2.47±0.01
9	Proline	3.20±0.05	4.51±0.05
10	Arginine	4.18±0.05	6.11±0.09
11	Tyrosine	3.27±0.17	3.87±0.09
12	Histadine	2.09±0.05	2.44±0.05
13	Cystine	1.09±0.06	2.15±0.03
14	Alanine	3.91±0.04	5.06±0.06
15	Glutamic acid	10.34±0.04	16.26±0.08
16	Glycine	3.18±0.04	3.89±0.05
17	Threonine	2.89±0.03	3.62±0.02
18	Serine	3.27±0.03	3.38±0.02
19	Aspartic Acid	7.40±0.05	9.90±0.05

Values represent the mean from two replicates ± SEM

Table 2 shows that Glutamic acid and Aspartic acid are the most abundant amino acids in better gourd seed crude protein while Glutamic acid and Leucine are the most abundant amino acids in Bromelain better gourd seed protein hydrolysate, while Tyrosine and Cysteine are the least in crude protein and Bromelain better gourd seed protein hydrolysate respectively.

Table 3 Some biochemical parameters level in serum of alloxan-induced diabetic albino Rats after treated with Bromelain Bitter Gourd Seed Protein Hydrolysate (BGSPH)

Index	**Normal control**	**Diabetic**	**Dmetformin**	**D100**	**D200**	**D400**
GLU (mmol/l)	3.9±1.73$^{\#}$	13.3±1.59$^{*\$}$	4.0±0.1$^{\#}$	5.3±0.49$^{\#}$	4.6±0.17$^{\#}$	4.2±0.47$^{\#}$
HbA1c (%)	4.2±0.28$^{\#}$	10.67±0.45$^{*\$}$	3.9±0.14$^{\#}$	5.0±0.41$^{\#}$	4.9±0.64$^{\#}$	4.6±0.33$^{\#}$
Glycogen (mmol/L)	4.3±0.76$^{\#}$	11.00±0.51$^{*\$}$	4.6±0.76$^{\#}$	7.1±0.96$^{\#}$	6.2±0.48$^{\#}$	6.0±0.32$^{\#}$
CHOL (mmol/l)	1.53±0.67	1.60±0.58	1.47±0.18	1.33±0.47	1.27±0.29	1.17±0.12
HDL (mmol/l)	0.23±0.03	0.20±0.06	0.24±0.06	0.25±0.09	0.23±0.09	0.21±0.03
LDL (mmol/l)	0.43±0.03	0.43±0.23	0.37±0.03	0.50±0.07	0.48±0.15	0.47±0.07
TG (mmol/l)	0.67±0.07	0.79±0.15	0.77±0.09	0.60±0.21	0.57±0.07	0.60±0.00

Diabetic induced rats were orally administered with Bromelain bitter gourd seed protein hydrolysate at the doses mentioned earlier for 28 days. Values are given as means ± SEM (n = 10). * $p \leq 0.05$ were considered significant compared to Normal control group; # $p \leq 0.05$ were considered significant compared to diabetic rat; $ $p \leq 0.05$, were considered significant compared to diabetic rats treated with metformin.

Table 3 showed the results of Glucose level (Glu), Glycated haemoglobin (HbA1c) level, Glycogen level, and Lipid profile level (Chol, HDL, LDL, and TG) in the serum of normal rats, diabetic rats and the effect of oral intake of Bromelain bitter gourd seed protein hydrolysate. The results showed that the Glucose level (Glu), Glycated haemoglobin (HbA1c) level, and Glycogen level was significantly ($P < 0.05$) higher in the diabetic control (13.3±1.59, 10.67±0.45 and 11.00±0.51 respectively) than the other groups. Also, significant ($P < 0.05$) difference was present between normal control and diabetic control groups (Negative control). The treatment of diabetic rats with Bromelain bitter gourd seed protein hydrolysates significantly ($P < 0.05$) reduces the level of Glucose (Glu), Glycated haemoglobin (HbA1c) level, and Glycogen.

The results also showed that the lower level of cholesterol (CHOL), high density lipoprotein (HDL), low density lipoprotein (LDL), and triglyceride (TG) was found in serum of the diabetic control group. The treatment with Bromelain bitter gourd seed protein hydrolysate did not cause significant ($P > 0.05$) change between all the groups.

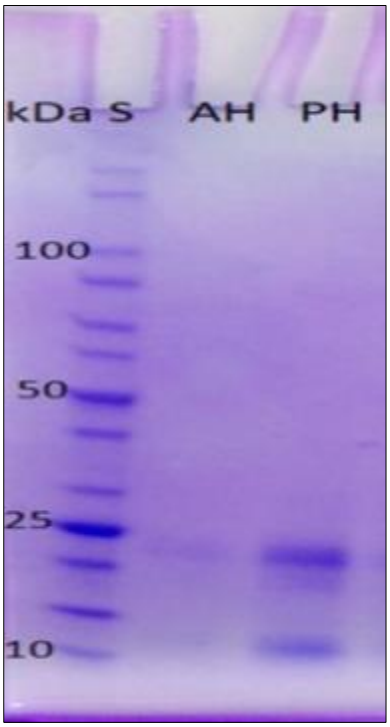

Figure 1 SDS-PAGE of Bitter gourd seed protein concentrate (AH), Bromelain Bitter Gourd Seed Protein Hydrolysate (PH) and Standard lane (S)

Protein profile of bitter gourd seed extracted and hydrolysates is shown in Figure 1. Several polypeptides ranging from 25 kDa and below was seen in crude protein concentrate and Bromelain Bitter Gourd Seed Protein Hydrolysate (PH). Prominent among them were 25 kDa, and 10 kDa proteins in Bromelain bitter gourd seed protein hydrolysate.

4. Discussion

The amino acid composition was analyzed to assess the protein profile of crude better gourd seed protein and bromelain better gourd seed protein hydrolysate. Essential amino acids (EAA) such as Valine, Leucine, and Isoleucine are associated with enhancing muscle protein synthesis, and consuming protein sources rich in those amino acids could be considered an effective strategy to counteract skeletal muscle loss among sedentary and elderly individuals (Brennan *et al*., 2019). Bromelain better gourd seed protein hydrolysate contains appreciable amounts of EAA with a high protein content while better gourd seed crude protein low amount of EAA thus Bromelain better gourd seed protein hydrolysate derivatives could be an interesting source of protein for human nutrition. Better gourd seed protein hydrolysate also contained considerable amounts of Sulphur amino acids (Cysteine and Methionine), higher than some of the legume, cereal, and nut-based sources, including soybean, faba bean, rice, and peanut (Sá *et al*., 2020)

Glycogen is the primary intracellular form in which glucose is stored and glycogen levels in various tissues, particularly the liver, are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Because alloxan causes selective destruction of beta-cells in the islets of Langerhans, resulting in a marked decrease in insulin levels, it follows that glycogen levels in the liver decrease because they depend on insulin for the influx of glucose (Golden *et al.,* 2012). Oral administration of bromelain better gourd seed protein hydrolysate significantly improved hepatic glycogen levels in the present study. This is possibly due to the reactivation of the glycogen synthase system as a result of increased insulin secretion following bromelain better gourd seed protein hydrolysate treatment of diabetic rats.

A previous report in albino Wistar rats has indicated that, in diabetes, protein synthesis is decreased in all tissues, which is due to the relative deficiency of insulin and to depressed synthesis of Hb (Pari *et al.,* 2015). Elevated HbA1c levels and

decreased Hb have also been reported in experimental diabetes (Muruganandan *et al*., 2016). In the present study, treatment of diabetic rats with bromelain better gourd seed protein hydrolysate resulted in a significant decrease in HbA1c levels. This was more prominent in the 400 mg/ kg bromelain better gourd seed protein hydrolysate treated group than in groups treated with either 100 or 2000 mg/ kg bromelain better gourd seed protein hydrolysate.

Type 2 diabetes mellitus is associated with profound alterations in the plasma lipid and lipoprotein profile. The levels of CHOL, HDL, LDL, and TG decreases non-significantly in the groups treated with bromelain better gourd seed protein hydrolysate compared to the normal control group. The obtained data proved that bromelain better gourd seed protein hydrolysate couldn't alter lipid profile level in the serum significantly at a high dose of 400 mg/kg, which is not in agreement with those obtained by Senanayake *et al*., (2018), by using methanol to extract the active components from bitter melon. However, serum cholesterol levels in various experimental groups had a slight difference from those of Ahmed *et al*., (2015).

SDS-PAGE analysis (Figure 1) was performed to assess the protein profile of the Crude bitter gourd seed protein and Bromelain bitter gourd seed protein hydrolysate. It was noticed that different protein profiles in the bitter gourd seed hydrolysate, with proteins of different molecular weights ranging from 25 to 10 kDa. At the end of hydrolysis of bitter gourd seed using bromelain, lower molecular weight bands (peptides) were observed (< 25 kDa).

5. Conclusion

The present study showed that bromelain bitter gourd seed protein hydrolysate could be used as hypoglycemic agents due to its potency as antidiabetic. Oral administration of bromelain bitter gourd seed protein hydrolysate and the presence of bioactive peptides in bromelain bitter gourd seed protein hydrolysate, which might attribute to the significant decrease in blood glucose, glycogen and glycated hemoglobin but non-significant decrease in lipid profile in alloxan induced diabetes rats. These findings also provided more information on antidiabetic effect of bitter gourd seed as a suitable functional food to relieve symptoms of diabetes. Further studies on this protein hydrolysate, mechanisms of action and clinical trials to validate these data, which may lead to the development of more potent antidiabetic formulations, is recommended to be carried out in the future.

Compliance with ethical standards

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Disclosure of conflict of interest

The author reports no conflicts of interest. The author alone is responsible for the content and writing of the paper.

Statement of ethical approval

This study was approved and ethical clearance was granted by Gombe State University, Nigeria, Animal care and use research ethics committee.

References

[1] Agrawal, R. C., Pratibha, B., Sanjeev, S., Jit, R. G., Kavita, G., Mishra, D. S., & Karihaloo, J. L. (2006). Report on Establishment of the National Information Sharing Mechanism on the Implementation of the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture in India. National Bureau of Plant Genetic Resources, New Delhi, India.

[2] Ahmed, I. Lakhani, M.S. Gillett, M. John, A. Raza, H. (2015). Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic Momordicacharantia (karela) fruit extract in streptozotocin-induced diabetic rats. Diabetes Res. Clin. Pract. 51, 155–161.

[3] Alu'datt, M. H., Rababah, T., Ereifej, K., Alli, I., Alrababah, M. A., Almajwal, A., ... & Alhamad, M. N. (2012). Effects of barley flour and barley protein isolate on chemical, functional, nutritional and biological properties of Pita bread. Food Hydrocolloids, 26(1), 135-143.

[4] Brennan, C. A., & Garrett, W. S. (2019). Fusobacterium nucleatum—symbiont, opportunist and oncobacterium. Nature Reviews Microbiology, 17(3), 156-166.

[5] Cefalu, W. T. (2007). Pharmacotherapy for the treatment of patients with type 2 diabetes mellitus: rationale and specific agents. Clinical pharmacology & therapeutics, 81(5), 636-649.

[6] Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W., & Malanda, B. I. D. F. (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes research and clinical practice, 138, 271-281.

[7] Muhammad, F. Y., Gezawa, I. D., Uloko, A., Yakasai, A. M., Habib, A. G., & Iliyasu, G. (2017). Metabolic syndrome among HIV infected patients: a comparative cross sectional study in northwestern Nigeria. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *11*, S523-S529.

[8] Golden, S. H., Brown, A., Cauley, J. A., Chin, M. H., Gary-Webb, T. L., Kim, C., ... & Anton, B. (2012). Health disparities in endocrine disorders: biological, clinical, and nonclinical factors—an Endocrine Society scientific statement. The Journal of Clinical Endocrinology & Metabolism, 97(9), E1579-E1639

[9] Jin, K. H., McCann, M. T., Froustey, E., & Unser, M. (2017). Deep convolutional neural network for inverse problems in imaging. IEEE Transactions on Image Processing, 26(9), 4509-4522.

[10] Lee, J., Durst, R. O. B. E. R. T., & Wrolstad, R. O. N. A. L. D. (2005). AOAC official method 2005.02: total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method. Official methods of analysis of AOAC International, 2.

[11] Mudaliar, S., Polidori, D., Zambrowicz, B., & Henry, R. R. (2015). Sodium–glucose cotransporter inhibitors: effects on renal and intestinal glucose transport: from bench to bedside. Diabetes care, 38(12), 2344-2353.

[12] Muhammad, F. Y., Gezawa, I. D., Uloko, A., Yakasai, A. M., Habib, A. G., & Iliyasu, G. (2017). Metabolic syndrome among HIV infected patients: a comparative cross sectional study in northwestern Nigeria. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, 11, S523-S529.

[13] Muruganandan, S., Jeffery, E., McIntyre, C., & Lee, Y. G. (2016). Nutrition, exercise, and complementary medicine: potential role in mesothelioma?. Current Pulmonology Reports, 5, 20-27.

[14] Nasri, M. (2017). Protein hydrolysates and biopeptides: Production, biological activities, and applications in foods and health benefits. A review. Advances in food and nutrition research, 81, 109-159.

[15] Ngoh, Y. Y., & Gan, C. Y. (2016). Enzyme-assisted extraction and identification of antioxidative and α-amylase inhibitory peptides from Pinto beans (Phaseolus vulgaris cv. Pinto). Food Chemistry, 190, 331-337.

[16] Nyenwe, E. A., Odia, O. J., Ihekwaba, A. E., Ojule, A., & Babatunde, S. (2003). Type 2 diabetes in adult Nigerians: a study of its prevalence and risk factors in Port Harcourt, Nigeria. Diabetes research and clinical practice, 62(3), 177-185.

[17] Pari L, Amarnath SM. (2015) Antidiabetic activity of Boerhaavia diffusa L.: effect on hepatic key enzymes in experimental diabetes. J Ethnopharmacol. 91: 109–13.

[18] Sá, A. G. A., Moreno, Y. M. F., & Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. Trends in Food Science & Technology, 97, 170-184.

[19] Senanayake, G.V.K. Maruyama, M. Shibuya, K. Sakono, M.; Fukuda, N. Morishita, T., Yukizaki, C. Kawano, M. Ohta, H. (2018). The effects of bitter melon (Momordica charantia) on serum and liver triglyceride levels in rats. J. Ethnopharmacol. 91, 257–262.

[20] Siow, H. L., & Gan, C. Y. (2016). Extraction, identification, and structure–activity relationship of antioxidative and α-amylase inhibitory peptides from cumin seeds (Cuminum cyminum). Journal of Functional Foods, 22, 1-12.

[21] Zambrowicz, A., Pokora, M., Setner, B., Dąbrowska, A., Szołtysik, M., Babij, K., ... & Chrzanowska, J. (2015). Multifunctional peptides derived from an egg yolk protein hydrolysate: isolation and characterization. *Amino acids*, *47*, 369-380.

[22] Zhu, Y., Dong, Y., Qian, X., Cui, F., Guo, Q., Zhou, X., ... & Xiong, Z. (2012). Effect of superfine grinding on antidiabetic activity of bitter melon powder. International Journal of Molecular Sciences, 13(11), 14203-14218.