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Original article

Psidium Guajava Seed Protein Hydrolysates Exhibit Invitro Antioxidant and Inhibitory activity against α- Amylase and α-Glucosidase

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Abstract

Exposure of certain amino acids in some plant peptides makes them biologically active and can be used as remedy for various diseases including diabetes. This investigation has examined the invitro α-amylase inhibitory properties and the antioxidant activities of *Psidium guajava* (guava) seed protein hydrolysate. Proteins from guava seed were isolated by precipitation using acid and then broken down by pepsin and trypsin. The degree of hydrolysis by trypsin (42.32±0.44%) was significantly higher than pepsin hydrolysis (31.85±0.32%). Tryptic hydrolysate showed the highest α-amylase inhibition (64.06±0.19%) than peptic hydrolysate inhibition (58.19±0.01%) but lower than acarbose used as standard (72.53±0.04%). All the hydrolysates show inhibitory activity as their concentration increases. The antioxidant study revealed that the hydrolysates have DPPH and H2O2 prowling activities with ferric reducing antioxidant property and none of the hydrolysates have higher antioxidant activity than the standard (Ascorbic acid). The outcomes therefore indicate the bioactivities of guava seed protein hydrolysates may make it a beneficial anti-diabetic agents.

Keywords: Hydrolysate, Trypsin, bioactive peptides, *Psidium guajava*.

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INTRODUCTION

Diabetes mellitus (DM) is an advancing ailment which is characterized by continuous hyperglycemia because of the inability of the cells of the pancreas to make insulin and/or peripheral insulin resistance (You *et al.,* 2019). Long-term hyperglycemia is linked to cumulative dyslipidemia, reactive oxygen species generation, and decreasing antioxidant status (Hu *et al.,* 2018). In line with the World Health Organisation Report, atleast 422 million persons across the globe suffer from diabetes mellitus. Most patients are diagnosed with type 2 diabetes mellitus (T2DM) that has a combined feature of hyperinsulinemia and insulin resistance. Inactive lifestyle, high energy diet and obesity are associated with the development of T2DM. A common characteristic of DM is a chronically high blood glucose level (hyperglycemia), which can result to micro and macro vascular problems such as nerve damage, neurodegenerative disorders, atherosclerosis, retinal vascular disease, end stage renal diseases, joint pain and damage etc. (Schmutterer *et al.,* 2017). DM is also complicated by highly reactive species that contains one or more unpaired electrons in their outer shell. Series of reactions occur once the reactive species are formed and these reactions can be terminated by antioxidants through various mechanisms (Hamid *et al.,* 2010; Amit and Priyadarsini, 2011). Alpha-amylase and α-glucosidase hydrolyse starch into glucose through a series of mechanisms. Substances that decrease the function of α-amylase and α-glucosidase makes the hydrolysis of starchy food take longer in the duodenum and reduce the postprandial blood glucose excursion (Kazeem *et al.,* 2013).

Psidium guajava is a tree that fits in to the family of Myrtaceae. Guava is the usual name called, for species of *P. guajava*. The guava tree is dispersed in tropical and subtropical regions of America, the Caribbean, Asia, Africa, and the Pacific islands (Elixabet *et al.,* 2017). Chronic diseases including diabetes has been treated by using *P. guajava* traditionally in China for some time (Devalaraja 2011). Some studies undertaken inside and outside of the living cells have shown the antihyperglycemic and hypoglycemic potential of the leaf and seed extracts of guava (Brahmi *et al.,* 2012). Therefore it is logical to describe these effects from the inherent bioactive compounds in guava. Phytochemical investigation of the peel, flesh and seeds of guava various parts of guava has shown it has lots of total phenolics and flavonoids, including flavanols, flavonols, tannins, and phenolic acid products (Elixabet *et al.,* 2017).

Protein hydrolysates are products of proteolysis that are easily digested and serve as macronutrients. Among the approaches used for splitting of bonds in protein, enzymatic method is the desired approach for making hydrolysates because the process conditions are not so harsh and produces enormous products of great value (Kose and Oncel, 2015). Enzymatic splitting of bonds gives rise to amino acids and small peptides from the complete proteins, which augment its nutritive value. Protein hydrolysates and peptides have been extensively looked into as a result of their potential health importance related to high bioactivities, low molecular mass, straightforward absorption, and less

harmful (Agyei *et al.,* 2016, Chakrabarti *et al.,* 2014). In recent years, plant derived peptides with antidiabetic properties and other therapeutic effect have been identified from different plant such as the potato (Shibu *et al.,* 2019), white sorghum flour (Shiwei *et al.,* 2019), *Cucurbitaceae* seed (Cesar and Ma 2017), carrot seed (Nanhui *et al.,* 2018), and from other agricultural crops (Aoife *et al.,* 2013). Despite the presence of many known antidiabetic drugs and antioxidant in the market, free radical and related ailments kept being a major challenge. Management of these complications without side effects is still a problem for the medical community. Thus, this study investigated the inhibitory activity of guava seed protein hydrolysate against α-amylase and its antioxidant property.

MATERIALS and METHODS

Materials

Ripe *P. guajava* fruits were obtained from Gombe metropolis in Gombe state, Nigeria and a sample was authenticated at the Botany section the Department of Biological science, Gombe State University with a voucher number of 43 deposited. The seeds were carefully removed from the fruit, dried under shade and pulverized with a blender. Without any purification, analytical grade reagents were used all through. α-amylase, trypsin (from bovine pancreas), pepsin (from porcine gastric mucosa), dinitrosalicylic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Acarbose and Starch were products of Sigma-Aldrich (USA), Trizma-base, n-hexane, trichloroacetic acid (TCA) products of BDH Chemical Limited (Poole, England).

Methods

Preparation of defatted P. guajava seed Powder

The defatted *P. guajava* seed powder was produced as highlighted by Aisami *et al.* (2020) with minor modification. *P. guajava* seeds were ground in a mill. Extraction using n-hexane was performed twice by the use of 1g of the flakes to 10ml of the hexane with continuous magnetic shaking for an hour. The defatted powder were spread on a large tray and kept under a fume cupboard for 6 hours to dry and to remove any remaining solvent. The flakes were poured in plastic containers and kept at a low temperature in a refrigerator

Isolation of P. guajava Seed Protein

The isolation was done by the procedure highlighted by Aisami *et al.* (2020). Defatted *P. guajava* seed meal was mixed with 0.1M NaOH (1:10), pH 12.0, agitated for an hour and spun at 18 °C and 3000 rpm for 10min. The process was repeated twice and each time the supernatant was pooled and kept. The pH of the supernatant was adjusted to 4.0 with 1M Hydrochloric acid solution and the precipitate obtained was pulled out by centrifugation, the pH of the precipitate was adjusted to 7.0, freeze dried and stored in the refrigerator. It was labeled *Psidium guajava* seed protein isolate (PSPI).

Determination of Protein Yield of Isolate

Following isolation, the protein yield was obtained as described by Aisami *et al.* (2020) using the expression shown below:

$$Protien\ Yield(\%) = \frac{Mass\ of\ protein\ isolate\ (g)}{quantity\ of\ Defatted\ \ meal\ of\ seed\ (g)}\ X\ 100\%$$

Preparation of Seed Protein Hydrolysates

The PSPI was broken down as outlined by Arise *et al.,* (2016). Hydrolysis was undertaken using each of pepsin (pH 2.2, 37ºC) and trypsin (pH 8.0, 37ºC). PSPI (5%w/v) was solubilized in the right buffer (PO_4^{-3} buffer, pH 8.0 for trypsin and glycine buffer, pH 2.2 for pepsin) and for every 1g of the enzyme used, 100g of the substrate was hydrolysed. Digestion took place for 5h after which it was inserted into a beaker of 100ºC water for 15min. The pH was made to 4.0 and centrifuged at 4000rpm for 30min and the supernatant assayed for the extent of breakdown. It was later dried by freezing and then refrigerated with the label PSPH.

Degree of Hydrolysis (DH) Assay

This was obtained by getting the portion of dissolved protein in 10% TCA in relation to the overall amount of protein in the isolate according to the method used by Aisami *et al.* (2020). 1ml each of protein hydrolysates and 20% TCA were mixed to get 10% TCA of dissolved substance. The mixtures were allowed for half an hour to form precipitate, it was later centrifuged at 4000rpm for 20 minutes. The liquid at the top after centrifugation was assayed for protein content by Biuret method with bovine serum albumin (BSA) as serving as a reference. The following expression was used for the calculation of DH:

$$\mathrm{DH} = \frac{Soluble\ peptide\ in\ 10\%\ TCA\ (mg/mL)}{Overall\ protein\ amount\ of\ isolate\ (\frac{mg}{mL})}\ X\ 100$$

Determination of Peptide Yield of Hydrolysates

This was obtained by employing the method used by Arise *et al.,* (2016). The peptide yields (%) of PSPH was obtained as the ratio of peptide mass of lyophilized hydrolysate to that of mass of unhydrolyzed protein isolate as given by the expression below:

$$\text{Peptide Yield (\%)} = \frac{Peptide\ mass\ of\ freeze\ dried\ hydrolysate\ (mg/mL)}{Protein\ mass\ of\ lyophilised\ isolate\ (mg/mL)} \times 100\%$$

α-Amylase Inhibition Assay

This assay was undertaken as reported by Aisami *et al.* (2020). 125 µL of hydrolysate (0.2 – 1.0 mg/mL) was poured in test tubes and 125 µL of 20 mM $Na_3\ PO_4^{-3}$ buffer (pH 6.9, with 6mM NaCl)

having α-amylase solution (0.5 mg/mL) was combined with the solution in the test tubes. At intervals of 5 minutes, the content of the pre-incubated (25°C for 10 minutes) test tubes were mixed with 125 µL of 1% starch solution in 20 mM sodium phosphate buffer (pH 6.9, with 6 Mm NaCl). The reactants were kept at standard temperature for 10 min. 250 µL of the coloring reagent dinitrosalicylic acid (DNS) was added at the last. Finally, the mixture was incubated for 5minutes in 100°C H_2O and then left to cool at room temperature. The absorbance of each test tube was taken at 540nm after adding 2.5ml distilled. The same steps were followed for the control but this time around distilled H_2O was used rather than the hydrolysate. The following expression was used to obtain the α-amylase inhibitory activity.

α-glucosidase inhibition

The α-glucosidase inhibitory activity will be determined according to the method of Apostolidis *et al*. (2007) 50 µL of the hydrolysate and 100 µL of α-glucosidase solution will be incubated at 25 °C for 10 min. Thereafter, 50 µl of 5 mmol/l p-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/l phosphate buffer (pH 6.9) will be added. The reacting mixture will be incubated at 25 °C for 5 min, before reading the absorbance at 405 nm in the UV-Visible spectrophotometer. Then, the alpha glucosidase inhibitory activity will be expressed as percentage inhibition.

Acarbose was used as positive control and the inhibitory activity of alpha amylase and alpha glucosidase were calculated using the following formula.

$$\text{Percentage Inhibition} = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{sample}}{\text{Absorbance}_{control}} \times 100$$

The 50 percent inhibitory concentration (IC_{50}) of the enzymes activity by the hydrolysate and standard were obtained using a graph of percentage inhibition versus hydrolysate concentrations using GraphPad Prism. All experiments were performed three times

Determination of DPPH free radical scavenging activity of seed protein

The DPPH radical-scavenging activity of *P. guajava seed* hydrolysate was assayed by method described by Arise *et al*., 2016. 0.60 ml of seed hydrolysate (1.00 – 2.50 mg/mL) was mixed with 1.00 ml of 0.05 mM DPPH solution. The mixture was agitated and kept in the dark at room temperature for 30min. The blank consist of only distilled water while the control was made of distilled water and DPPH solution. In all cases absorbance was taken at 517nm and the percentage $DPPH^{\cdot}$ inhibition was obtained using the following expression.

$$DPPH^{\cdot}\text{-scavenging Activity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100\%$$

The 50 percent Effective concentration (EC_{50}) of the enzyme activity by the hydrolysate and standard were obtained using a graph of percentage inhibition versus hydrolysate concentrations using GraphPad Prism. All experiments were performed three times.

Hydrogen Peroxide Scavenging Capacity

This was carried out as described by Arise *et al.*, 2016. 4mM of H_2O_2 was made in PO_4^{-3} buffer (pH 7.4). 1.00mL Seed hydrolysate (0.20 – 0.80 mg/mL) in distilled H_2O was mixed with 0.15mL of H_2O_2 solution. Phosphate buffer only was used for the blank without H_2O_2. After 10 minutes, the absorbance was taken at 230nm. Absorbance of H_2O_2 was taken as control. The H_2O_2 scavenging ability in percentage was obtained using the following expression:

$$H_2O_2\text{-scavenging Capacity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

The 50 percent Effective concentration (EC_{50}) of the enzyme activity by the hydrolysate and standard were obtained using a graph of percentage inhibition versus hydrolysate concentrations using GraphPad Prism. All experiments were performed three times.

Ferric reducing power activity of P. quajava protein hydrolysate

The power of protein hydrolysate to reducing Fe3+ was carried out as reported by Chen et al. (2009). 100 µL of hydrolysates with different concentration (0.20 - 1.0 g/L) was added to 0.7 mL of 0.2mol/L sodium phosphate buffer and 2mL of 30 mmol/L potassium ferricyanide solution. The mixture was kept in the incubator at 50oC for 20 min and then 2mL of 10 percent TCA added. The mixture was then centrifuged at 3000rpm for 10min. lastly, 1ml of the supernatant was added to 3mL of 1.7 mmol/L aqueous FeCl3, and the absorbance read at 700 nm. Reducing power was related to the absorbance of the mixture.

Statistical Analysis

Values were given in triplicate as mean ± standard deviation (SD), then analysis of variance and Tukey's multiple range tests were undertaken using Graph Pad Prism version 6.0 (Graph Pad Software, San Diego, CA, USA). Differences were taken as significant at $p < 0.05$.

RESULTS and DISCUSSION

Percentage protein yield shows how much of a protein is extracted from a given sample. Table 1 showed that the protein yield is 4.8% which is relatively low, this low value might be as a result of the extraction procedure used as pointed out by Arise *et al.*, (2016), that the protein yield is proportional to the procedure used for extraction. The amount in percentage of protein output found in this work was

less than 18.9% protein output found for watermelon seed by Arise *et al.* (2016). The same method of extraction as used in this study was used by Tejano *et al.* (2019) and the protein yield obtained was slightly lower (4.4%) than the one obtained in this study. The total protein content of *P. guajava* seed protein isolated termed as PSPI was 58.32% which is lower than 65.08% obtained for *Chlorella sorokiniana* protein isolate (Tejano *et al.,* 2019).

The degree of hydrolysis is use to determine how much of the peptide bonds are broken. The high value of DH ($p < 0.05$) obtained by trypsin hydrolysis indicates that trypsin is the best in breaking down *P. guajava* seed protein. This could mean that a lot of positively charged residues of amino acid might be present which is specific to trypsin (Naik, 2012). The DH values can be applied to foretell peptide chain size as higher DH values indicates that the peptides have shorter chain length and vice versa (Arise *et al.,* 2019)[a]. So, pepsin with the lower DH (31.85%) may consist of long peptides and trypsin hydrolysates will have a shorter length of peptides because of the higher value (42.32%) and probably due to the presence of more positively charged amino acid residues in the peptide.

The degree of peptic hydrolysis gotten in this work was more than earlier reported values for *Chlorella sorokiniana* protein hydrolysate (Tejano *et al.,* 2019) and *Azadirachta indica* seed peptic protein hydrolysis (Arise *et al.,* 2019). The extent of tryptic hydrolysis ($42.32 \pm 0.44\%$) obtained in this work is more than earlier obtained value $21.79 \pm 0.77\%$ for whey protein where tryptic breakdown also showed the best extent of hydrolysis (Kamau and Lu 2010). Nevertheless, the DH ($31.85 \pm 0.32\%$) found for pepsin is less than the earlier showed by hemp seed peptic hydrolysate 39.1% (Girgih *et al.,* 2011).

The lower peptide yield by pepsin (4.80%) when matched with the yield of trypsin (7.73%) suggests that alot of bioactive peptides possibly will have been obtained by trypsin hydrolysis than by pepsin hydrolysis. The large figure for trypsin may perhaps be as a result of the amide and the esters of amino acids broken down and also the specificity of the enzyme for non-polar amino acids (Naik, 2012). The 4.80% and 7.73% peptide yield gotten from the peptic and tryptic digestion of PSPH was significantly less than that found for *Luffa cylindrica* seed peptic and tryptic hydrolysis by Arise *et al.,* (2019)[a] with a yield of 16.93% and 34.04% respectively.

Table 1. Peptide yields, protein isolate yield, and extent of hydrolysis

Parameter/Enzyme		**Degree of Hydrolysis (%)**	**Peptide Yield (%)**
Protein Yield of Isolate (%)	4.8	—	—
Protein content of isolate (%)	58.32±0.35	—	—
Pepsin	—	31.85±0.32[a]	4.80 ± 0.58[a]
Trypsin	—	42.32±0.44[b]	7.73 ± 0.59[b]

Values represent the mean of triplicate determinations ± standard deviation (SD). Figures with dissimilar alphabets in one column are considerably dissimilar at $p < 0.05$

α-amylase inhibitory activity

Tryptic PSPH showed incredible inhibitory property against α-amylase function (Figure 1). This is obvious in the portion of inhibition achieved by tryptic (64.06%) PSPH. It showed inhibitory effect that increases as the concentration of the hydrolysate increase. Peptic hydrolysate inhibitory effect against α-amylase also displayed an increasing trend as the concentration increases. However, tryptic PSPH had the most inhibition against α-amylase function, as it was also revealed in its small IC_{50} (0.65± 0.01 mg/mL).

Trypsin breaks down protein to release amino acids with cationic amino acid residues and it has been mentioned that α-amylase likes binding to peptides with positively charged ion and branched chain residues (Aisami *et al.,* 2020). The 64.06% α-amylase-inhibitory effect showed at 1.0 mg/mL by tryptic PSPH in the present work is lower to the earlier disclosed values for Guava seeds Alcohol Extract with 71.02% inhibitory activity (Balasubramanian *et al.,* 2015).

The IC_{50} value decides the concentration of hydrolysates wanted, to stop the activity 50% of the amylase, (Razali *et al.,* 2015). Low IC_{50}value is needed as smaller values show higher activity. The IC_{50} value (0.52± 0.02mg/mL) of acarbose is significantly less ($p < 0.05$) than the values obtained by the hydrolysates (Figure 2). Conversely, the IC_{50} value of peptic and tryptic hydrolysates were significantly dissimilar ($p > 0.05$) from one another (0.78± 0.03 and 0.65± 0.01 respectively).

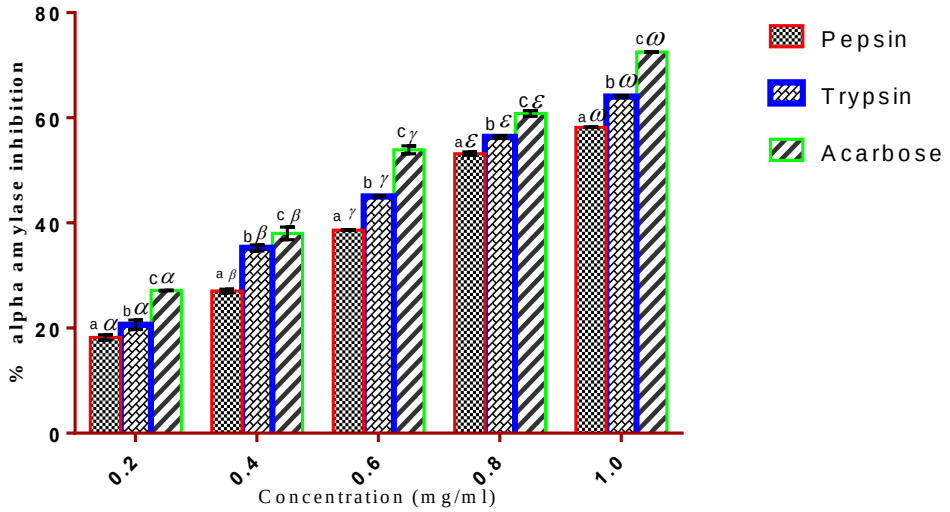

Figure 1. α- amylase-inhibitory effect of *P. guajava* protein hydrolysates (Pepsin and Trypsin) and Acarbose (standard).

Each bar portrays the mean of triple calculations ±SD, bars at a particular concentration but with dissimilar letters (abc) are considerably different at $p<0.05$.Bars of a particular sample at varying concentrations with dissimilar Greek letters (αβγεώ) indicate significant variation at $p<0.05$.

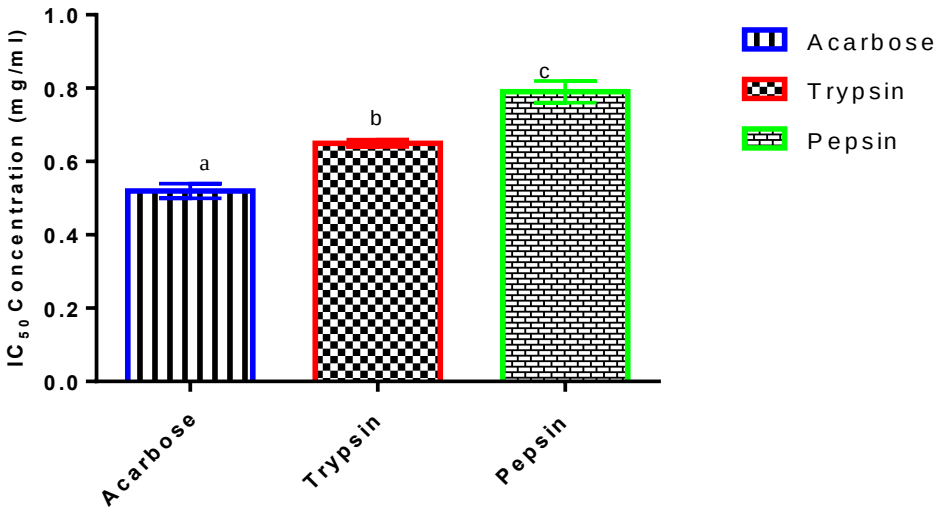

Figure 2. 50% Inhibition concentration (IC50) values of *P. guajava* seed protein hydrolysates in inhibition of alpha amylase.

Each bar denotes mean of three calculations of IC_{50} ± SD. Bars with varying letters are considerably dissimilar at $p < 0.05$.

α-glucosidase inhibitory activity

α-glucosidase is a vital enzyme that breaks down the product of α-amylase into glucose as such, inhibiting its activity will to a great extent reduce the amount of glucose produced. Trypsin hydrolysate showed significant inhibitory activity against α-glucosidase more than the other hydrolysate, but all the hydrolysates have significantly lower α-glucosidase inhibitory activity when compared with the standard at the highest concentration used (Figure 3). The inhibition was concentration dependent as reported by Arise *et al*. (2019)[a]. The result obtained in this study for tryptic hydrolysate at 1.0mg/mL (68.79%) is higher than 54.54% reported for *Luffa cylindrica* at the same concentration (Arise *et al.*, 2019)[a].

However, the IC50 values of guava seed protein hydrolysates against α-glucosidase inhibitory activity shows that peptic and tryptic hydrolysates had IC50 value of 0.68 mg/mL and 0.49mg/mL respectively. When the two values are compared, tryptic hydrolysate is a better inhibitor of α-glucosidase as the lower the IC50 value the better the hydrolysate (Aisami *et al.*, 2020).

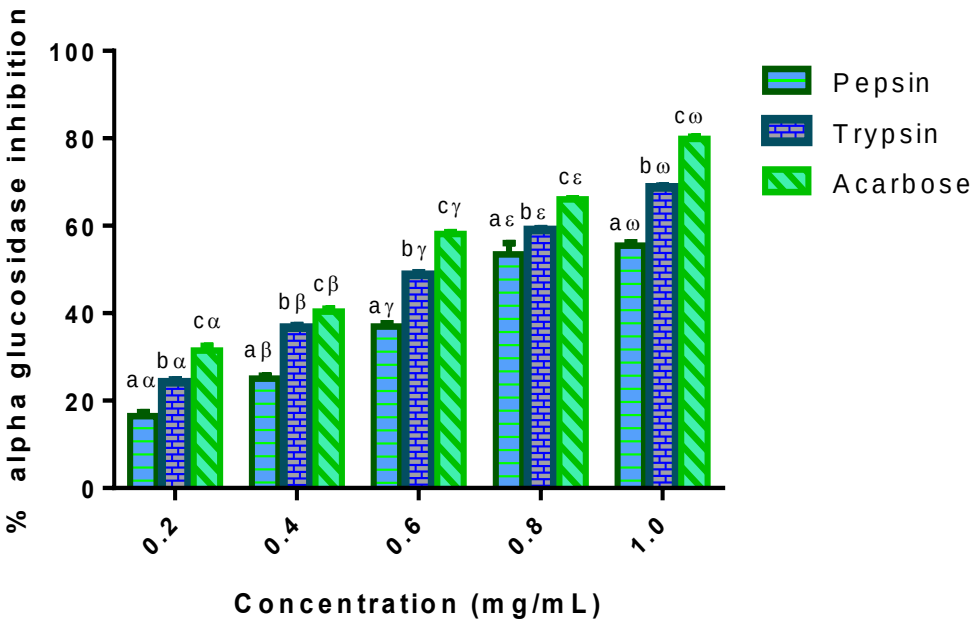

Figure 3. α- glucosidase-inhibitory effect of *P. guajava* protein hydrolysates (Pepsin and Trypsin) and Acarbose (standard).

Each bar portrays the mean of triple calculations ±SD, bars at a particular concentration but with dissimilar letters (abc) are considerably different at $p<0.05$.Bars of a particular sample at varying concentrations with dissimilar Greek letters (αβγεώ) indicate significant variation at $p<0.05$.

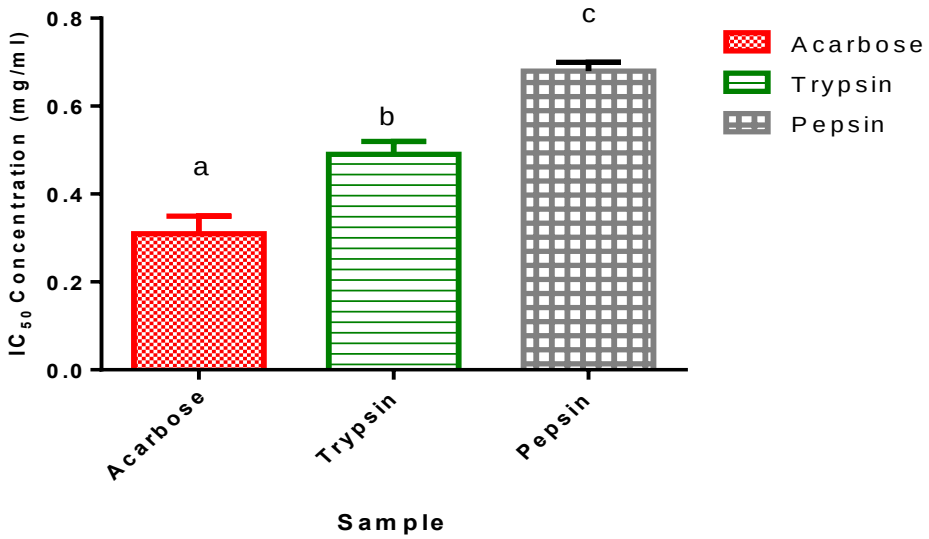

Figure 4. 50% Inhibition concentration (IC50) values of *P. guajava* seed protein hydrolysates in inhibition of alpha glucosidase.

Each bar denotes mean of three calculations of IC_{50} ± SD. Bars with varying letters are considerably dissimilar at $p < 0.05$.

Antioxidant studies

The percentage of DPPH scavenging potential of ascorbate as a standard was considerably more ($p < 0.05$) than of all the hydrolysates at every concentration of the study (Figure 5). The tryptic hydrolysate however showed the least inhibition having the lowest percentage of 39.90±0.10% at 0.2mg/mL and peptic hydrolysates at 0.2mg/mL had percentage 43.76±0.15%, which are considerably different from one another ($p >0.05$).

The radical-Scavenging activities became greater considerably ($p<0.05$) for all the samples with rise in concentration (0.2-1.0 mg/ml). Among the different hydrolysates, the results in figure 5 showed that the peptic hydrolysate demonstrated the highest activity (69% at 1.0mg/ml) which was comparable to the scavenging activity of ascorbate (82.53%). At 0.6 mg/mL, there was no considerable difference ($p<0.05$) in the hunting potential of peptic and tryptic hydrolysate.

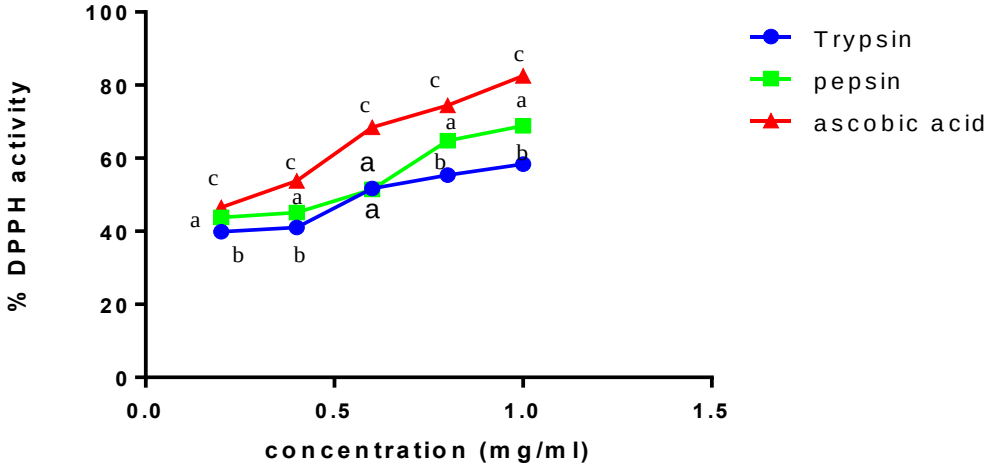

Figure 5. DPPH radical-scavenging potential of P. guajava seed protein product of hydrolysis

Each dot indicates the average of three calculations ± SD. Dots at equal concentration with dissimilar alphabets are considerably different ($P < 0.05$).

EC_{50} is the concentration that leads to the reduction in the initial DPPH concentration by 50%. EC_{50} of the standard (0.549 ± 0.009 mg/mL) was considerably less than peptic hydrolysate (figure 6). However, EC_{50} of tryptic PSPH and that of ascorbic acid were not significantly dissimilar ($P < 0.05$). This is similar to the pattern of result obtained by Adiamo *et al.*, 2016. The EC_{50} defines the concentration of the samples needed to stop 50% of the oxidant, hence commonly applied to determine antioxidant, antiradical and reduction efficacies (Razali *et al.*, 2015). Low EC_{50} is wanted as smaller figures show more effectiveness.

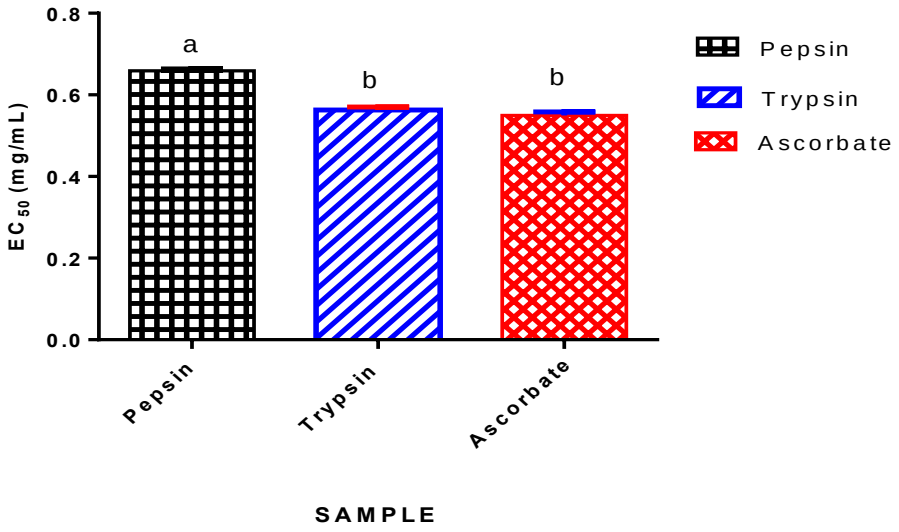

Figure 6. 50% effective concentration (EC50) values of *P. guajava* seed protein hydrolysate in scavenging DPPH radical.

Every bar denotes the mean of three calculations of EC_{50} ± standard deviation. Bars with dissimilar alphabets are considerably dissimilar ($P < 0.05$).

All the hydrolysates demonstrated significantly lower H_2O_2-scavenging capacity ($p < 0.05$) than the standard with scavenging-activity of pepsin protein hydrolysate being considerably more ($p < 0.05$) than trypsin protein hydrolysates at the entire concentrations in this study (Figure 7). Among the hydrolysates, the peptic hydrolysate revealed the best H_2O_2-scavenging potential with 24.77% whereas trypsin hydrolysate had the least (23.15%) at 1.0mg/ml. The result found is probably as a result of the existence of some amino acid residues that exist in pepsin hydrolysate which might scavenge H_2O_2, and not displayed for some other oxidants.

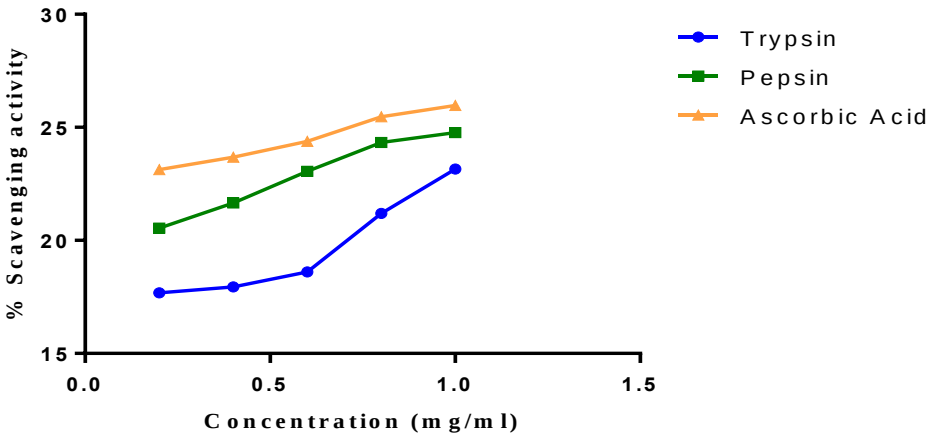

Figure 7. H_2O_2-Scavenging activities of *P. guajava* seeds protein hydrolysates.

Each dot indicates the mean of three calculations ± SD. Dots at equal concentration with dissimilar alphabets are considerably dissimilar ($P < 0.05$).

Trypsin with the EC_{50} of 0.74 ± 0.01 is considerably higher ($p < 0.05$) than pepsin 0.52 ± 0.02 and ascorbic acid (Figure 8). The standard antioxidant has the lowest of EC_{50} value of 0.60 ± 0.02. Tryptic hydrolysate obtains higher EC_{50} for hydrogen peroxide scavenging activities with (0.84 ± 0.02 mg/mL) while the peptic hydrolysate (0.43 ± 0.02 mg/mL) had the least EC_{50} value. The standard ascorbic acid obtains the EC_{50} of 0.54 ± 0.02 mg/mL which is slightly higher than the peptic hydrolysate. Therefore, peptic hydrolysate is more effective than tryptic hydrolysate in inhibiting 50% of the oxidant as compared with the standard.

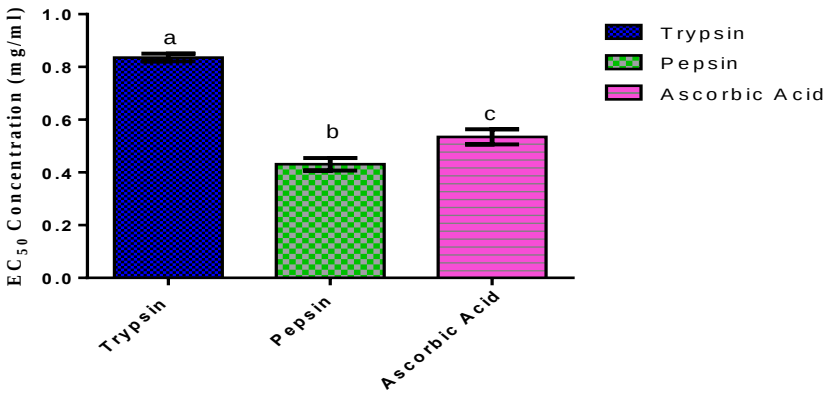

Figure 8. 50% effective concentration (EC_{50}) values of P. guajava Seeds Protein Hydrolysates in Scavenging H_2O_2

Every bar denotes the mean of three calculations of EC_{50} ± standard deviation. Bars with nonidentical alphabets are considerably dissimilar ($P < 0.05$).

The Fe^{3+}-reducing strength of standard was significantly more ($p < 0.05$) than that of pepsin hydrolysate but lower than that of trypsin hydrolysate at all study concentrations (Figure 9). The essence of the ferric-reducing antioxidant activity assay is to assess the capacity of an antioxidant to offer an electron, and some studies have revealed the existence of a link between antioxidant property and reducing ability of peptides (Arise *et al.,* 2016). In a concentration-dependent manner, PSPH reduced the Fe^{3+} to the Fe^{2+} form which is similar with the studies of *azadirachta indica* (*Arise et al.,* 2019)[b]. The results showed that tryptic hydrolysate possesses the highest Fe3+-reducing power at high concentrations. This may be due to the high degree of hydrolysis obtained with tryptic hydrolysis. Several studies have reported that there may be a direct correlation between degree of hydrolysis and ferric-reducing power, indicating that smaller peptides often exhibit higher reducing power (Vastag *et al*. 2011)

Pepsin hydrolysate had considerably low ($p<0.05$) reducing ability the entire concentrations used, demonstrating a feeble response to higher hydrolysate concentration. Meanwhile trypsin hydrolysate exhibited great ($p < 0.05$) Fe^{3+}--reducing power than pepsin and the standard. This similar was reported in the studies of *azadirachta indica* (*Arise et al.,* 2019)[b]. However, in another study, the Fe^{3+}- reducing

strength of *Luffa cylindrica* seed hydrolysates were significantly lower when compared with the standard antioxidant (ascorbate). (Arise et al., 2019)a

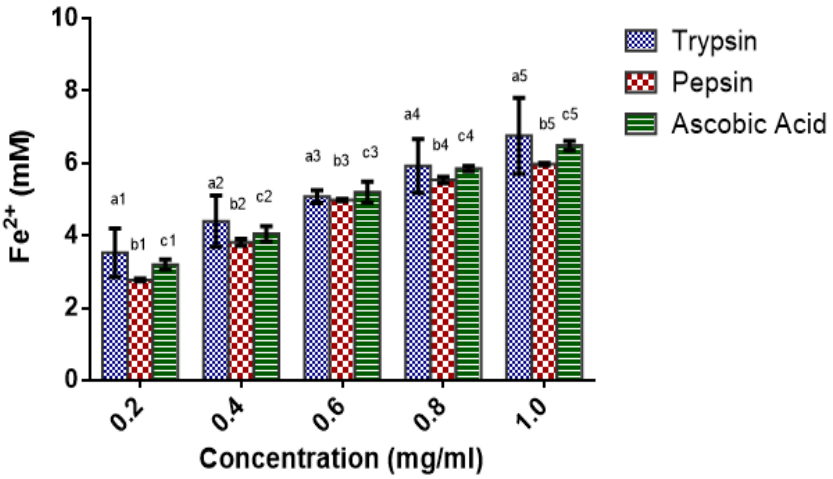

Figure 9. Ferric-reducing antioxidant ability of ascorbate and *p. guajava* seed protein hydrolysate

Every bar depicts the average of three calculations ± SD. Bars at equal concentration with dissimilar Alphabets (abc) are considerably different at $p < 0.05$. Bars of a particular sample with dissimilar numbers (12345) at dissimilar concentrations are considerably dissimilar at $p < 0.05$.

Conclusion

Guava seed protein hydrolysate due to its multidirectional bioactivities may serve as an alternative treatment for diabetes if further in-vivo studies and amino acid profile of the peptides are well known and characterized.

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