Research Article

In vitro α-Amylase and α-Glucosidase Inhibitory Activity of Dioscorin from *Dioscorea alata*

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Abstract
Dioscorea alata is a species of yam found in many countries in Southeast Asia. It contains many bioactive compounds consisting of steroidal saponins, phenolic compounds flavonoids, polysaccharides, and proteins. Eighty to eighty-five of the soluble proteins in the tuber of *D. alata* are dioscorin. Dioscorin (31 kDa) is the major storage protein of yam tuber, exhibiting biochemical and immunomodulatory properties, such as antioxidant activity, reducing blood pressure and angiotensin I converting enzyme (ACE) inhibitors activity. The aims of this research focused on extraction of the total soluble protein from *D. alata* tuber, purification of dioscorin, and study of *in vitro* inhibitory activity of α-amylase and α-glucosidase of dioscorin extracts. The results showed that a maximum yield of total soluble protein was obtained from the buffers extraction using Tris-HCl buffer at pH 9.0 resulting in the protein concentration of 15.10 mg/g with a protein extraction yield of 31.56 ± 2.36%. The crude protein was further recovered using ammonium sulfate precipitation and dialysis. SDS-PAGE analysis showed that dioscorin from crude extracts was precipitated at the concentration of ammonium sulfate of 60% saturation. The purified dioscorin was obtained from anion exchange chromatography (DEAE-Sephacel) and its molecular weight of 31 kDa was estimated on SDS-PAGE. Furthermore, the α-amylase inhibitory activities of crude extract (1 mg/mL), purified dioscorin obtain from gastric digestion and purified dioscorin obtained from intestinal digestion were at 14.44% 2.21% and 78.13%, respectively. Moreover, the results showed that the α-glucosidase inhibitory activity of the crude extract, and purified dioscorin obtained from gastric digestion were at 10.56% and 2.61%, respectively.

Keywords: α-amylase, α-glucosidase, *Dioscorea alata*, Dioscorin, *In vitro* inhibitory activity, Protein extraction

1 Introduction

Diabetes is a chronic and serious illness that arises [1] when the pancreas fails to produce adequate insulin, which is responsible for regulating blood sugar levels. Alternatively, it can occur when the body is unable to efficiently utilize the insulin it generates. Considered as a significant public health concern, [2] diabetes is one of the four primary noncommunicable diseases (NCDs) targeted for global action by world leaders. Over the past few decades, there has been a consistent rise in both the numbers of diabetes cases and their prevalence [3]. In 1980, approximately 108 million adults worldwide had diabetes, whereas this number surged to an estimated 422 million in 2014. The prevalence of diabetes has almost doubled since 1980, reaching 8.5% in the adult population after starting at 4.7%. This increase can be attributed to heightened risk factors like obesity and overweight conditions. In 2012, diabetes was responsible for 1.5 million deaths, while elevated blood glucose levels beyond the optimal range contributed to an additional 2.2 million deaths by escalating the risks of various cardiovascular and other diseases.

Yam, scientifically known as *Dioscorea* spp., is a tuberous crop commonly found in Asia, contributing to approximately 6% of the global tuber production [4] and [5]. It holds the status of a staple crop and serves as a valuable nutritional supplement. In terms of composition (based on dry weight), yams typically consist of 75% to 84% starch, 6% to 8% crude protein, and 1.2% to 1.8% crude fiber [6], [7]. Among the major tuber crops cultivated in India, such as potato, cassava, yam, taro, and sweet potato, yams receive relatively less research attention. The major cultivated yams are *D. alata* (greater yam) and *D. esculenta* (lesser yam) [8] and [9]. Meanwhile, in China, yams have a long-standing tradition of being used as both health food and herbal medicine [10]. The primary storage protein in yam tubers, dioscorin is a major protein in yam tubers, and it has a molecular weight of approximately 31 kDa. It accounts for a significant, around 80% to 85% of the total soluble protein content in yam tubers [11]. This isolation was achieved using alkaline buffers (Tris-HCl, pH 8.3) and anion-exchange chromatography. These proteins are assumed to aggregate and deposit within the vacuoles of storage cells. The characteristics such as high amide content and solubility, which are common among other storage proteins. The major tuber storage proteins of *D. cayenensis*. These two classes, referred to as classes A and B, exhibited 84.1% similarity in their protein coding regions and 69.6% similarity in their deduced amino acid sequences. However, there was no sequence identity found with other storage proteins such as patatin (from potato) or sporamin (from sweet potato). These findings provided more detailed information, and the name dioscorins was introduced to represent the storage proteins in yam tubers [12]. Dioscorin has been found to possess carbonic anhydrase, dehydroascorbate reductase, monodehydroascorbate reductase, and trypsin inhibitor activities [4], [13] and further details about the enzymatic activities [14]:

- Carbonic anhydrase (CA) [15] is an enzyme that plays a crucial role in maintaining acid-base balance in the body, particularly in the blood. Carbonic anhydrase facilitates the reversible conversion of carbon dioxide and water to bicarbonate ions and protons. This reaction is known as the hydration of carbon dioxide:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

By catalyzing this reaction, carbonic anhydrase helps regulate the pH of the blood. It enables the efficient transport of carbon dioxide from tissues to the lungs for elimination [15].

- Dehydroascorbate reductase (DHAR) and Monodehydroascorbate reductase (MDAR) are enzymes that participate in the metabolic process of ascorbic acid, also known as vitamin C. These enzymes play a vital role in converting the oxidized form of ascorbic acid, called dehydroascorbic acid (DHA), back into its active form. This enzymatic reaction is crucial for maintaining a sufficient level of ascorbic acid in the body. Ascorbic acid serves as a fundamental antioxidant, offering protection to cells and tissues against oxidative damage [16].
- Trypsin Inhibitor Activity: Trypsin inhibitors are proteins that inhibit the activity of trypsin, a serine protease involved in protein digestion [5],[14].

However, there are no reports available on the biological activity of purified dioscorin from *D. alata*, specifically regarding its inhibitory effects on α-amylase and α-glucosidase. The α-amylase and α-glucosidase are enzymes involved in the digestion and breakdown of carbohydrates in the body. In the

context of diabetes, these enzymes play a role in the regulation of blood sugar levels. α-glucosidase inhibitors are a class of medications commonly used in the treatment of type 2 diabetes. Their activities were to inhibit the action of α-glucosidase enzymes in the small intestine. By slowing down the digestion and absorption of carbohydrates, these inhibitors help prevent rapid spikes in blood glucose levels after meals. This can contribute to better overall blood sugar control in individuals with diabetes [17]. Similarly, α-amylase inhibitors are substances that can inhibit the activity of the α-amylase enzyme. They are often used as a dietary supplement or natural remedy for diabetes management. By inhibiting α-amylase, these inhibitors can help reduce the rate at which complex carbohydrates are broken down into simple sugars, thereby slowing the increase in blood glucose levels. Both α-glucosidase inhibitors and α-amylase inhibitors can be beneficial for individuals with diabetes by helping to regulate post-meal blood sugar levels and improve glycemic control [11].

Also, dioscorin, a storage protein found in yam, has been studied for its potential properties in hypertension control. Dioscorin has been found to possess antihypertensive properties, meaning it may help reduce high blood pressure levels. It is believed that certain bioactive peptides derived from dioscorin can inhibit the activity of angiotensin-converting enzyme (ACE), which plays a role in blood pressure regulation [18] and [19].

In the realm of studying the gastrointestinal behavior of food or pharmaceuticals, *in vitro* methods that replicate digestion processes are widely employed. These methods offer several advantages, including rapid results, lower costs, reduced labor requirements, and the absence of ethical constraints [20]. This study specifically aimed to extract dioscorin and to study its digestion by pepsin (to simulate gastric digestion) of crude extracts and purified dioscorin obtained from *D. alata* tuber. Certain inhibitors of α-glucosidase have been developed into clinical drugs, as their ability to inhibit this enzyme helps to lower blood glucose levels. One such example is acarbose, which was originally isolated from microorganisms [21]. Acarbose is an oral medication commonly prescribed for the management of type 2 diabetes. It works by inhibiting the activity of α-glucosidase enzymes in the small intestine, thus slowing down the digestion and absorption of carbohydrates. By reducing the rate at which complex carbohydrates are broken down into glucose, acarbose helps to control post-meal blood sugar spikes. It is typically taken with the first bite of a meal to have the greatest effect on carbohydrate digestion [11] and [22].

In the present study, we first demonstrated *in vitro* α-amylase and α-glucosidase inhibitory activity of purified dioscorin from *D. alata* were assayed compared to crude protein extract. The results from this study could be beneficial for the development of a novel functional food ingredient for antidiabetic applications.

2 Experimental

2.1 *Materials*

Fresh yam (*D. alata*) tubers were purchased from a wholesaler in northeastern Thailand. The tubers were stored at room temperature to be used in subsequent experiments. Foiln-Ciocalteu's phenol reagent was purchased from Sigma-Aldrich (St. Louis, USA). Precision Plus Protein Standards Dual color ranging from 10–250 kDa, as well as chemicals for SDS-PAGE were purchased from Bio-Rad Inc (Hercules, CA, USA). All other chemicals utilized in the study were of analytical grade and were procured from Sigma-Aldrich.

2.2 *Dioscorin extraction*

The yam tubers were peeled and sliced in 1 × 1 cm thickness. Approximately 30 g of yam slices were homogenized with a buffer at the ratio of 1:4 (w/v). In this research, the influence of pH on protein extraction was examined by using several buffers including 50 mM acetate buffer at pH 4.0 and 5.0, 50 mM Phosphate buffer at pH 6.0 and 7.0, and 50 mM Tris-HCl buffer at pH 8.0 and 9.0. Protein extraction was conducted at a temperature of 4 °C for 30 minutes by a mechanical overhead stirrer from IKA-Werke, Inc. (Staufen, Germany). The supernatant of the crude protein samples was collected through centrifugation at 12,000 rpm for 30 min at 4 °C, and the crude protein samples were stored in a –20 °C freezer for subsequent analysis.

To precipitate the proteins, 50 mL of the crude extract underwent sequential steps. Solid ammonium sulfate was added in increments while stirring until a

specific degree of saturation was reached. The mixture was then incubated on ice at 4 °C for a minimum of 2 h. Subsequently, centrifugation was performed at 10,000 g, 4 °C, for 15 min. The precipitation process occurred when the crude extract reached 60% saturation of ammonium sulfate. The resulting protein precipitate was dissolved in 15 mL of 50 mM Tris-HCl buffer at pH 9.0. Aliquots of the precipitated fractions were subjected to analysis for protein concentration and molecular weight using SDS-PAGE.

2.3 *Purification of dioscorin*

The DEAE Sephacel column from GE Healthcare was initially equilibrated using 50 mM Tris-HCl buffer at pH 9.0, with a flow rate of 27 mL/h utilizing the ÄKTA Protein Purification Systems (GE Healthcare, Chicago, USA). The crude extracts of yam tubers were dialyzed overnight in 50 mM Tris-HCl buffer at pH 9.0. Subsequently, the dialyzed crude extracts were directly loaded onto the DEAE Sephacel column was purchased from GE Healthcare Ltd. (Chicago, USA). Following this, the column was washed with 3-column volumes of 50 mM Tris-HCl buffer at pH 9.0. The elution buffer was then changed to 50 mM Tris-HCl buffer at pH 9.0, containing 150 mM NaCl, using the same flow rate. The purified Dioscorin protein solution was collected in 2.0 mL increments, resulting in a total of 60 fractions. Only the protein solutions that were identified from individual fractions were subjected to SDS-PAGE for determining their molecular weights.

2.4 *In vitro gastrointestinal digestion*

Standard protocols [23] were followed to prepare simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The simulated gastric digestion took place at a temperature of 37 °C. Before introducing the enzymes, the SGF was adjusted to pH 2 using 0.1 mol/L HCl, while the SIF was adjusted to pH 6.8 using 0.1 mol/L NaOH. The crude extract samples were mixed with SGF in a 1:1 ratio (mL:mL). Prepare the desired volume of the 5 mL crude extract sample and 5 mL SGF. Then add crude extract samples into SGF and 0.075 mM CaCl2 was added to the final mixture. The pH was adjusted to 2.0 by adding 1 M HCl, followed by the addition of pepsin (2000 UmL^{-1} in the final solution). The pH, which was 2.0 after the addition of pepsin, was checked and adjusted every 30 min. The simulated gastric digestion was carried out for 2 h in a shaking incubator at 37 °C. Aliquots were withdrawn into Eppendorf tubes at 0, 15, 30, 60, and 120 min.

In the intestinal phase, the gastric chyme (i.e., sample: SGF mixture) was combined with SIF in a volume ratio of 1:1 and then neutralized to reach a pH of 6.8. Porcine pancreatin and bile extract were added to the SIF at a proportion of 26.8 mg and 160 mg, respectively, per 1 mL of SIF. The simulated intestinal digestion was conducted for 2 h in a shaking incubator at 37 °C. Aliquots were collected in Eppendorf tubes at 0, 15, 30, 60, and 120 min.

To determine the total protein concentration, Lowry's method was employed. Bovine serum albumin (BSA) served as the protein standard. Various dilutions of BSA solutions were prepared by mixing the stock BSA solution (1 mg/mL) with distilled water. The BSA concentrations ranged from 0.001 to 1 mg/mL. A volume of 0.04 mL of BSA and the sample were pipetted, followed by the addition of 0.20 mL of alkaline copper reagent. The solutions were thoroughly mixed and incubated at room temperature for 10 min. Next, 0.02 mL of Folin Ciocalteau solution was added, and the solution was incubated for 30 min. The relative protein concentration in the chromatographic fractions was determined by measuring the absorbance at 750 nm using a Multiplate Reader Synergy™ HTX from BioTek Inc. (Winooski, Vermont, USA).

2.5 *Protein staining on SDS-PAGE gels*

The gastric digestion of the samples was assessed using the reduced SDS-PAGE technique. The protein samples were boiled and kept on ice before being loaded onto the gel. To prepare the protein samples, 20 microliters were thoroughly mixed with 5 µL of loading dye. The resolving gel (12%) was composed of 1.5 M Tris-HCl (pH 8.8), 10% (w/v) SDS, 30% acrylamide, 10% ammonium persulfate, and TEMED were purchased from Bio-Rad Inc. (Hercules, CA, USA). The stacking gel (4%) was prepared using 0.5 M Tris-HCl (pH 6.8), 20% (w/v) SDS, 10% ammonium persulfate, and TEMED. Electrophoresis was conducted at 100 V using a running buffer of Tris-glycine at pH 8.0. Subsequently, the gel was stained with 0.1% Coomassie Brilliant Blue R-250 purchased

from Bio-Rad Inc. (Hercules, CA, USA) in 50% (v/v) methanol and 10% (v/v) acetic acid for 30 min. After staining, the gel was destained for 2 h in a solution of 40% (v/v) methanol and 10% (v/v) acetic acid. Before analysis, the gel was washed with distilled water for 1 h until the background was clear. Finally, the gel was observed and photographed using a gel scanner densitometer (Epson Expression 1680 Color Flatbed Scanner, Beaumont, TX, USA).

2.6 *Enzyme inhibitory assay*

The α-amylase from porcine pancreas was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) inhibitory activity of the crude extract was conducted following a standard method with minor modifications [24]. In a 96-well plate, a reaction mixture consisting of 125 µL α-amylase (2 U/mL) and 125 µL of the crude extract, pure dioscorin, pure dioscorin in gastric digestion at 120 min, and pure dioscorin in intestinal digestion at 120 min was preincubated at 37 °C for 5 min. Subsequently, 250 µL of 1% soluble starch was added as a substrate and incubated for an additional 5 min at 37 °C. Then, 250 µL of the DNS color reagent was added, and the mixture was boiled for 5 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader Synergy™ HTX from BioTek Inc. (Winooski, Vermont, USA). Acarbose at various concentrations (0.1–0.5 mg/mL) was employed as a standard. A control setup without the test substance (extract and fractions) was run in parallel, and each experiment was performed in triplicate. The results were expressed as percentage inhibition, calculated using Equation (1).

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100 \quad (1)$$

Where, *As* represents the absorbance in the presence of the test substance, and *Ac* is the absorbance of the control.

The α-glucosidase was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) inhibitory activity of the crude extract was carried out following a standard method with minor modifications [25]. In a 96-well plate, a reaction mixture consisting of 460 µL potassium phosphate buffer (100 mM, pH 6.8), 20 µL α-glucosidase (1 U/mL), and 20 µL of the crude extract, pure dioscorin, pure dioscorin in gastric digestion at 120 min, and pure dioscorin in intestinal digestion at 120 min was preincubated at 37 °C for 20 min. Then, 200 µL of p-nitrophenyl-α-D-glucopyranoside (PNPG) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), PNPG (1 mM) was added as a substrate and incubated for an additional 15 min at 37 °C. The reaction was stopped by adding 500 µL of Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a Multiplate Reader Synergy™ HTX from BioTek Inc. (Winooski, Vermont, USA). Acarbose at various concentrations (0.1–0.5 mg/mL) was included as a standard. A control setup without the test substance was run in parallel, and each experiment was performed in triplicate. The results were expressed as percentage inhibition, calculated using Equation (1).

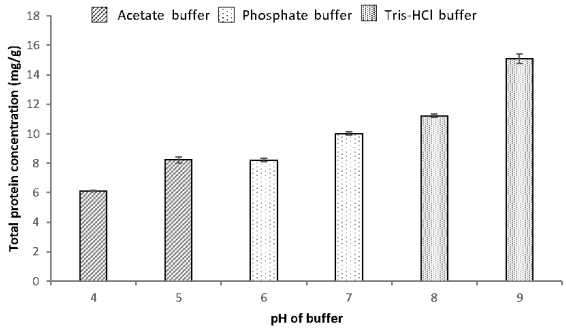


Figure 1: Effects of pH of extraction buffers on yield of total soluble protein extraction from *D. alata* tuber.

3 Results and Discussion

3.1 *Effect of pH of extraction buffer on total soluble protein extraction*.

Dioscorin was extracted from *D. alata* tuber following the method described by Hou [26], with precipitation performed using 60% ammonium sulfate. The concentration of the crude extract was determined using the Folin Lowry method and measured using a microplate reader, as shown in Figure 1. The results indicated that the total protein concentration increased as the pH increased. Notably, protein extraction using a 50 mM Tris-HCl buffer at pH 9.0 resulted in the highest total soluble protein content in the crude extract, reaching a concentration of 15.10 mg/g.

According to the results of Yong *et al.*, protein

extraction methods (homogenate method, acetone precipitation method, TCA-acetone precipitation method, and phenol method) were compared. Then evaluated these methods based on various parameters including protein yield, SDS-PAGE, and 2-DE techniques. In their experiment, 0.5 g of fresh tuber was subjected to each of the four extraction methods, resulting in yields of 1.6, 2.4, 1.2, and 1.9 mg of soluble protein, respectively [27].

From the study by Xue *et al.*, the effect of extraction pH (7, 8, 9, 10, and 11) on the yield of yam soluble protein was investigated when the extraction time and liquid-to-solid ratio were fixed at 20 min and 3:1 mL/g, respectively. The yield increased to its maximum of 49.7% at pH 9 and then decreased [28]. The pH value of 9 obtaining the highest yield of yam soluble protein can be attributed to several factors. At pH 9, the protein molecules in the yam sample acquire a greater number of negative charges. This increased negative charge leads to greater electrostatic repulsion between protein molecules, preventing their aggregation and promoting their solubility. Consequently, more proteins are extracted from the yam sample, resulting in a higher yield of soluble protein. The finding that pH 9 yielded the highest protein extraction yield is consistent with previous studies in the field of protein extraction. Proteins have been reported to exhibit increased solubility and extraction efficiency at alkaline pH values. This is due to the disruption of non-covalent interactions and the increased repulsion between protein molecules under alkaline conditions, leading to improve solubility [4], [5], [12], [28].

Figure 2 showed crude yam extracts of acetate buffer pH 5 (lane 1), phosphate buffer pH 7.0 (lane 2) and Tris-HCl buffer pH 9.0 (lane 3) on a 12% SDS-PAGE gel. The results of the SDS-PAGE analysis showed that dioscorin was detected in the crude protein extracts from *D. alata* at the molecular weight of about 31 kDa, which was the same as explained in [29] and [30].

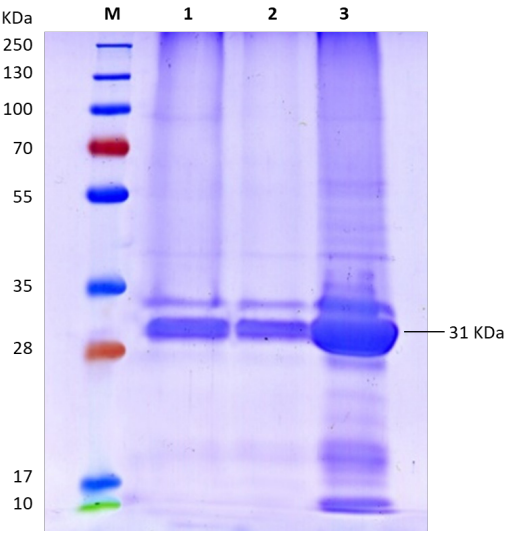


Figure 2: SDS-PAGE analysis of crude protein extracts from *D. alata* tuber extraction using 12% SDS-PAGE gel. Lane M, Protein marker; Lane 1, crude extracts of *D. alata* by acetate buffer pH 5; Lane 2, crude extracts of *D. alata* by phosphate buffer pH 7.0 and Lane 3, crude extracts of *D. alata* by Tris-HCl buffer pH 9.0.

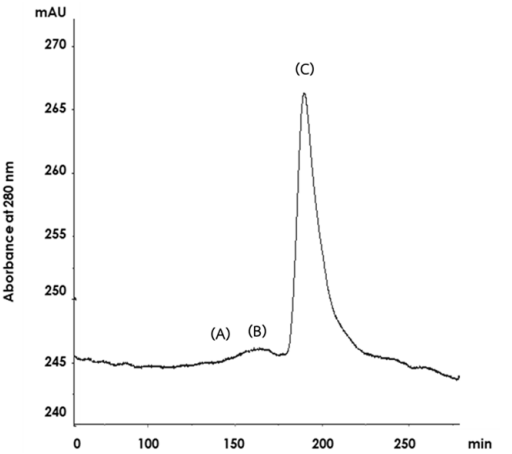


Figure 3: Chromatogram of purified dioscorin by DEAE-Sephacel ion exchange column using AKTA purifier system at 280 nm at the flow rate of 0.75 mL/min and buffer gradient of 120 min.

3.2 *Protein purification using ion exchange chromatography*

Dioscorin was purified from tubers of *D. alata* by a modified method based on Hsu (2002) using DEAE Sephacel ion exchange chromatography by AKTA purifier. The results of the chromatograms were shown in Figure 3. All collected fractions were analyzed using SDS–PAGE for possible protein existence. A chromatogram was obtained from protein purification. Samples, labeled as A, B, and C, were collected for protein analysis using SDS-PAGE. As Figure 3, C

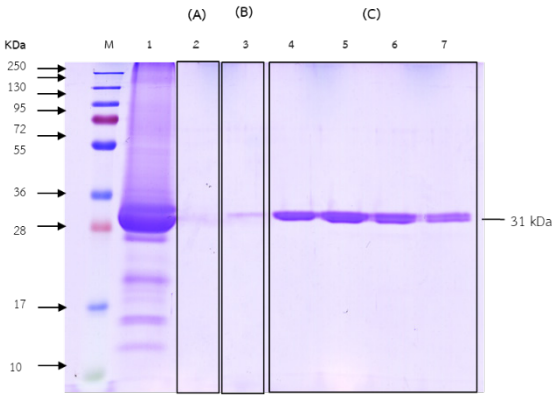


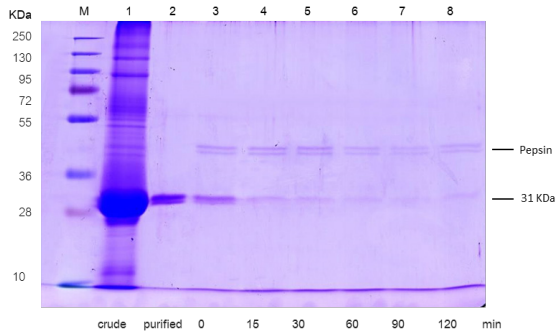
Figure 4: SDS-PAGE analysis of purification of dioscorin using DEAE-Sephacel ion exchange column. Lane M is the protein marker; Lane 1 is crude extracts; Lane 2–7 are sample fractions from chromatography.

was the highest peak in the chromatogram, samples A and B were also collected to compare the presence of dioscorin. The results were found in SDS-PAGE gel (Figure 4). A single band with a molecular weight of approximately 31 kDa was observed in lanes 4–7. This band corresponds to dioscorin, which is a storage protein found in yam tubers. Previous studies have also reported the presence of a protein band with a molecular weight of 31 kDa on SDS-PAGE gels, confirming its identification as dioscorin [31] and [32].

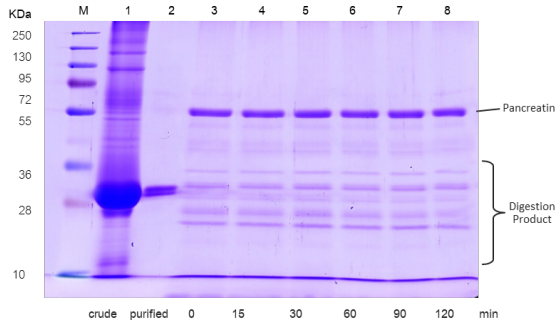
3.3 *In vitro gastrointestinal digestion of dioscorin*

SDS-PAGE was performed to analyze the crude extracts obtained from *D. alata* tuber during simulated gastric digestion to monitor the behavior of proteins, such as dioscorin, under conditions that simulate the gastric environment. The reason for conducting this analysis is to gain insights into the stability and behavior of the protein during gastric digestion. The aim was to study the *in vitro* gastric digestion of dioscorin from *D. alata* tuber. Before adding pepsin, the crude extracts were readjusted to pH 2 using an SGF solution and incubated for 2 h under acidic conditions. The samples were then subjected to SDS-PAGE to examine the *in vitro* gastric digestion of dioscorin at 37 °C for different time intervals, including 0, 15, 30, 60, 90, and 120 min.

Figure 5(a) and (b) displayed SDS-PAGE gels revealing the protein profiles before (0 min) and after



(a)



(b)

Figure 5: SDS-PAGE analysis of *in vitro* gastrointestinal digestion of purified dioscorin. (a) gastric digestion and (b) intestinal digestion. Lane M; Protein marker, Lane 1; crude protein extract, Lane 2; purified, Lane 3–7; samples from digestion at 15, 30, 60, 90, and 120 min.

the addition of pepsin (15, 30, 60, 90, and 120 min) during gastric and intestinal digestion, respectively. A comparison of the protein profiles between pure dioscorin (Figure 5) and crude extract (Figure 6) reveals notable differences. In the gastric digestion results, dioscorin and pepsin bands could be observed at 31 kDa and 38.9 kDa, respectively. The intensity of the dioscorin band at 31 kDa showed a decrease in intensity after 0 min of *in vitro* pepsin digestion, which remained relatively constant throughout the digestion process [Figures 5(a) and 6(a)]. In the case of intestinal digestion, the protein band of dioscorin (31 kDa) was degraded after 0 min of *in vitro* pancreatin digestion [Figures 5(b) and 6(b)]. Figure 5 showed the purified dioscorin during gastrointestinal digestion and Figure 6 showed gastrointestinal digestion of crude protein extract from *Dioscorea alata*.

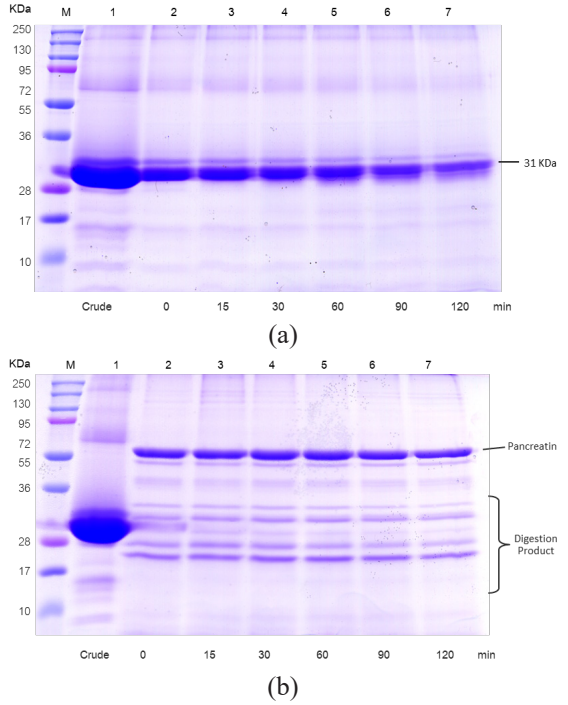


Figure 6: SDS-PAGE analysis of *in vitro* gastrointestinal digestion of crude protein extract from *D. alata* (a) samples from SGF and (b) samples from simulated intestinal digestion. Lane M; Protein marker, Lane 1; crude protein extract, Lane 2; purified, Lane 3–7; samples from digestion at 15, 30, 60, 90, and 120 min.

The decrease in intensity of the dioscorin band at 31 kDa observed during SDS-PAGE analysis of *in vitro* gastrointestinal digestion can be attributed to several possible mechanisms:

- Proteolytic degradation: Gastrointestinal digestion involves the action of proteolytic enzymes, such as pepsin in the stomach and pancreatic enzymes (trypsin, chymotrypsin) in the small intestine. These enzymes break down proteins into smaller peptides and amino acids. It is possible that dioscorin undergoes proteolytic degradation during digestion, resulting in a decrease in its intensity on SDS-PAGE.
- Denaturation: The harsh conditions of the gastrointestinal tract, including low pH in the stomach and exposure to bile salts and enzymes in the small intestine, can cause protein denaturation. Denaturation can lead to conformational changes and loss of protein structure, potentially affecting the binding of SDS and resulting in the reduced intensity of the dioscorin band on SDS-PAGE.

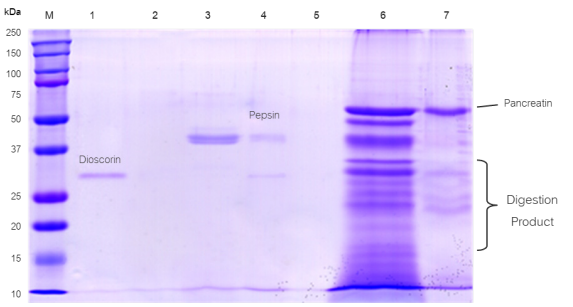


Figure 7: SDS-PAGE analysis of gastrointestinal digestion. Lanes show the protein bands during gastrointestinal digestion. Lane M; Protein marker, Lane 1; Purified dioscorin, Lane 2; SGF, Lane 3; SGF and pepsin, Lane Lane 4; Purified dioscorin during gastric digestion at 120 min 5; SIF, Lane 6; SIF and pancreatin, Lane 7; Intestinal digestion at 120 min.

To assess the impact of simulated gastrointestinal solutions on protein analysis through SDS-PAGE, the samples of SGF, SIF, pepsin, and pancreatin were compared with the samples obtained from the digestion of dioscorin in the simulated gastrointestinal study. The method used for simulating the gastrointestinal digestion of dioscorin was adapted from the reference [20]. In Figure 7, lane 4 represents the samples obtained after 120 minutes of gastric digestion. The results revealed the presence of dioscorin bands, indicating partial digestion during gastric digestion. Furthermore, in lane 7, corresponding to intestinal digestion using pancreatin, the results demonstrated that the dioscorin protein was initially fragmented at the onset of intestinal digestion (0 min), as depicted in Figure 7.

3.4 *In vitro α-amylase and α-glucosidase inhibitory activity*

The analysis of α-amylase and α-glucosidase inhibitory activity of dioscorin from *D. alata* was conducted *in vitro*. The results showed that crude protein extracts from *D. alata* exhibited inhibitory activities against α-amylase and α-glucosidase enzymes, with IC50 values of 0.81 mg/mL and 1.52 mg/mL [33]. However, purified dioscorin did not demonstrate any *in vitro* inhibitory activity against α-amylase and α-glucosidase. Interestingly, when purified dioscorin

was subjected to intestinal digestion for 120 min, it exhibited a significant α-amylase inhibitory activity of 78.13%, which was comparable to the inhibitory activity of acarbose. The positive control, acarbose, showed an α-amylase inhibitory activity of 88.76% in this study. Additionally, it was observed that crude extracts obtained from *D. alata* tuber using Tris-HCl buffer at pH 9.0, at a concentration of 711 micrograms, exhibited the highest α-glucosidase inhibitory activity of 10.56%. Meanwhile, acarbose demonstrated an α-glucosidase inhibitory activity of 92.11%, as shown in Table 1. The inhibition of these enzymes is crucial in reducing the absorption of glucose from the small intestine into the bloodstream, thus potentially helping to regulate hyperglycemia. The report by Adedayo *et al.*, [34] demonstrated that water extracts of raw yam possessed a good inhibitory effect on α-amylase and α-glucosidase activities *in vitro* in a dose-dependent pattern (1–4 mg/mL). The present study aimed to explore natural sources for potential alternative drugs with enhanced efficacy and reduced adverse effects compared to current medications used in the treatment of diabetes. One approach in managing diabetes involves targeting carbohydrate-digesting enzymes, specifically α-amylase and α-glucosidase, to reduce glucose absorption in the intestines and thereby lower postprandial glucose levels [35]. These findings suggest the potential use of these substances as functional ingredients or in the development of functional food in the future.

Table 1: *In vitro* inhibitory activities of α-amylase and α-glucosidase of dioscorin from *D. alata*

Sample	Inhibitory Activities of α-amylase (%)	Inhibitory Activities of α-glucosidase (%)
Crude extracts	14.44 ± 0.12	10.56 ± 0.01
Pure dioscorin	ND	ND
Pure dioscorin in gastric digestion at 120 min	2.21 ± 0.24	2.61 ± 0.02
Pure dioscorin in intestinal digestion at 120 min	78.13 ± 0.17	ND
Acarbose	88.76 ± 0.12	92.11 ± 0.15
Intestinal digestion at 120 min (without crude extract)	ND	ND

ND: Not detected of the inhibitory activity.

4 Conclusions

This study revealed that the maximum protein concentration was obtained at 15.10 mg/g fresh weight (with a moisture content of 65.50%) using 50 mM Tris-HCl buffer at pH 9.0 for 30 min of extraction time. The protein purification results indicated a single protein band with a molecular weight of approximately 31 kDa. Moreover, the pure dioscorin experienced partial digestion during gastric digestion at 120 min, while at the beginning of intestinal digestion (0 min), it was fragmented. Additionally, the inhibitory activity results demonstrated that purified dioscorin exhibited the highest α-amylase inhibitory activity of 78.13% during intestinal digestion (120 min). On the other hand, the crude extract showed the highest α-glucosidase inhibitory activity at a level of 10.56%.

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Author Contributions

A.S.: research design, investigation, data analysis, writing an original draft; D.J.: writing—reviewing, editing; S.S.: research design, conceptualization; K.R.: conceptualization, research design, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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