Original Article

TIPS Trends in Pharmaceutical Sciences 2024: 10(1): 35-50.

Fractionation of *Dioscorea bulbifera* bulbil protein, its antioxidative potential and inhibitory effects on Carbohydrate-hydrolyzing enzymes

Olayemi Oludele ODEKANYIN [1*] ;PhD, Fehintoluwa Deborah AKINYEMI [1] ;$MSc_{student}$, Omodolapo Eniola BADMUS[1] ;$MSc_{student}$

[1] Biochemistry and Molecular Biology Department, Faculty of Sciences, Obafemi Awolowo University, Ilelfe, Nigeria.

Abstract

The study fractionated *Dioscorea bulbifera* bulbil proteins, determined the fractions inhibitory potential against α-amylase and α-glucosidase activity, and evaluated their antioxidative activity in-vitro. *Dioscorea bulbifera* bulbil flesh was homogenized in Phosphate Buffer Saline (pH7.2), centrifuged at 10,000 xg for 30 min and supernatant was subjected to ammonium sulphate fractionation. Three fractions (25%, 60% and 90%) were produced and their hemagglutinating activity, blood group and sugar specificity were determined. Ability of the fractions to inhibit α-amylase and α-glucosidase activity was investigated. Antioxidative properties were evaluated using DPPH, Nitric oxide and hydroxyl radicals scavenging assay. Their reducing power, total antioxidant capacity and ferrous chelating activity were also evaluated. Data obtained revealed the presence of lectin in the crude protein extract, 25% and 60% protein fractions. The lectin agglutinated rabbit blood erythrocyte better than human blood groups erythrocyte. Protein fraction (60%) has no hemagglutinating activity towards human blood erythrocyte. Mannose completely inhibited the hemagglutinating activity. Alpha-amylase and α-glucosidase activities were inhibited by 25% and 60% protein fractions with no significant difference. But their inhibitory action was better than that of acarbose. All the free radicals tested were scavenged by the fractions to various degrees. Sixty percent protein fraction could not scavenge nitric oxide but it was a better scavenger of hydroxyl radical. No activity was recorded for 90% protein fraction. The results showed the anti-diabetic potential of the protein fractions and can be harnessed for possible development of therapeutics agents for the treatment of diabetes and oxidative stress associated ailments.

Keywords: Diabetes, antioxidants, α-amylase, α-glucosidase, Fractions, *Dioscorea bulbifera*, bulbil

Please cite this article as: Odekanyin OO*, Akinyemi DF, Badmus EO. Fractionation of Dioscorea bulbifera bulbil protein, its antioxidative potential and inhibitory effects on Carbohydrate-hydrolyzing enzymes. Trends in Pharmaceutical Sciences. 2024;10(1):35-50. doi: 10.30476/TIPS.2024.101274.1225

1. Introduction

Diabetes mellitus is the most serious, chronic metabolic syndrome, characterized by high blood glucose levels (1) and caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced (2). The alarming rate at which this disorder is increasing has made World Health Organization (WHO) to project that by 2030, diabetes would become seventh leading cause of death globally (3). One therapeutic approach to lower blood glucose levels is to retard the absorption of glucose via inhibition of enzymes, such as α-glucosidase and α-amylase, in the digestive organs (4, 5). Also, insulin therapy has been shown to be another satisfactory approach in the treatment and management of *Diabetic mellitus*. Antioxidants also have the potentials to ameliorate the disturbance caused by free radicals produced during oxidative stress in diabetes. Increase in free radicals' amount is closely associated with the glucose oxidation, lipids peroxidation and non-

Corresponding Author: Olayemi Oludele ODEKANYIN, Biochemistry and Molecular Biology Department, Faculty of Sciences, Obafemi Awolowo University, Ile_Ife, Nigeria.
Email: dekanyin@oauife.edu.ng

Received: 21/10/2023; Accepted: 01/03/2024

enzymatic glycation of protein which influence the diabetes mellitus (6). Natural antioxidants are abundant in plants and due to the various complications in the existing drugs for the treatment of diabetic, natural antioxidants are being employed for diabetic management. World Health Organization (WHO) also advocates the use of herbal medicines over the drugs currently in use for treatment of diabetes (7). Recently, focus has been on proteins that have the ability to reduce the postprandial hyperglycemia though the mechanism is not yet known. Wahab *et al*., (8) concluded that proteins derived from *Pleurotus pulmonarius* basidiocarps have a significant potential as alternatives in treating type 2 diabetic mellitus based on their ability to inhibit α-glucosidase and α-amylase activities *in vitro*. Also, protein from the seed coat of *Canavalia ensiformis* showed hypoglycemic activity (9). A class of protein known to agglutinate red blood cell, lectins, have also been shown to exert hypoglycemic effects in-vivo (10, 11). In 2017, a protein was isolated from the leaf of *Moringa oleifera* and was shown to possess hypoglycemic and antioxidant potential in Alloxan-Induced Diabetic Mice (12).

Dioscorea bulbifera, the air potato, is a species of true yam native to Africa, China, Japan, Northern Australia, Southern Asia, Indonesia and India with slight differences between those found in each place. It is a large creeper, 6 meters (20ft) or more in length. It is a monocotyledonous dioecious herbaceous perennial climber with broad leaves and two types of storage organ. It has been reported that *D. bulbifera* is being used in different countries of the world as traditional medicine to treat several diseases such as parasitic infections, microbial infections, oxidative stress and degenerative diseases, diabetes and digestive problems (13). Similarly, its extracts are reported to possess antihyperlipidemic, antitumor, anorexiant, analgesic, anti- inflammatory, plasmid curing, and antihyperglycemic effects (14). Though the antidiabetic potential of *D. bulbifera* has been reported (15, 16), the activity was linked to a bioactive component named diosgenin. Diosgenin is secondary metabolite obtainable only through organic solvent extraction and reported to possess various pharmacological activities (17). Literatures search shows that there are no reports on the *D. bulbifera* bulbil protein antidiabetic activity. This study intends to investigate the *in vitro* therapeutic potential of bioactive proteins in *D. bulbifera* bulbils in controlling the blood glucose levels through inhibition of carbohydrate-hydrolyzing enzyme and free radical generation.

2. Material and Methods

2.1. Sample collection

Dioscorea bulbifera bulbils were collected in the month of August from Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The plant and the bulbils were identified at the Ife Herbarium, Botany Department, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

2.2. Preparation of Crude Extract

Dioscorea bulbifera bulbils (aerial tuber) were peeled. Then, 25.84g of peeled bulbils was homogenized in 100ml of phosphate buffer saline (PBS, pH 7.2) and stirred for 4 hours on an electric magnetic stirrer at 4ºC. The mixture was centrifuged at 10,000 xg for 30 min. The supernatant which constitutes the crude protein extract was subjected to ammonium sulphate fractionation. The first protein fraction was obtained by subjecting the crude protein extract solution to 25% ammonium sulphate precipitation. The precipitate was collected 24 hrs later and represents the first protein fraction (25% fraction) and the supernatant was further brought to 60% ammonium sulphate saturation. The precipitate was also collected and represents the second protein fraction (60% fraction) and the supernatant of the second precipitation was also brought to 90% saturation. The precipitate was collected by centrifugation and represents the third protein fraction (90% fraction). All the precipitates were dialyzed extensively and the dialysates were freeze-dried separately. The freeze-dried dialysates were used for various assays.

2.3. Protein Concentration Determination

Protein concentration of the protein fractions were determined using the method described by Lowry *et al*. (18) and Bovine Serum Albumin (BSA) served as standard protein.

2.4 Hemagglutinating Activity

2.4.1 Glutaraldehyde fixation of red blood cells

In order to fix the erythrocytes, the blood

samples were collected from human and animal into heparinized bottles. It was immediately centrifuged at 3,000 rpm using a table-top centrifuge (Hospibrand 0502-1) for 15 min to obtain the erythrocytes. The erythrocytes were washed five times with PBS and then fixed in chilled 1% glutaraldehyde-PBS solution for 1 hour at 4 °C with intermittent mixing. After the fixation, the mixture was centrifuged for 5 min at 3,000 rpm to collect fixed erythrocytes. The fixed erythrocytes were extensively washed with PBS to remove the glutaraldehyde. Two percent of the erythrocytes were prepared in PBS containing 0.02 % sodium azide. This was stored in a refrigerator for further use.

2.4.2. Hemagglutinating assay

The presence of lectin in the crude extract of *D. bulbifera* bulbil and various fractions was determined through modified hemagglutinating assay procedure of Wang *et al.* (19). The assay was done in a 96-well U-shaped microtitre plate. Phosphate Buffered Saline (100 µl) was pipetted into all the wells of the microtiter plate sequentially. After this, 100 µl of the crude extract or fractions was added to the first well in the first row and the mixture was serially diluted up to the 24th well (2 rows). Fifty microliter of the fixed erythrocyte suspension (50 µl) was added to each well. The plate was left undisturbed for two hours on the laboratory bench. It was later observed for any visible hemagglutination. The control experiment was without extract or any of the fractions. Hemagglutination titre unit was taken as the highest dilution reciprocal of the crude extract or any of the fractions producing visible hemagglutination.

2.4.3. Sugar specificity test

Sugar specificities of *D. bulbifera* bulbil crude protein and protein fractions were determined by comparing the abilities of different sugars to inhibit the hemagglutinating activity of the protein fractions. Firstly, the serial dilution of the protein fractions was carried out as described in section 2.4.2 until the last dilution where hemagglutination was observed. Sugar solution (0.2 M, 50 µl) was added into each well, while PBS substituted sugar solution in control wells. The microtitre plate was then incubated at room temperature for 2 hrs. Erythrocyte suspension (50 µl) was added into each well. The plate was left undisturbed for two hours on the laboratory bench and then observed for any visible hemagglutination. The tested sugars were: glucosamine HCl, lactose, maltose, sorbose, sucrose, mannitol, galactose, N-acetyl-D-glucosamine, arabinose, glucose, mannose and 2-deoxy-D-glucose.

2.5. Enzymes Inhibition Assays

2.5.1. Alpha-amylase inhibitory activity (In-vitro)

Ability of the protein fractions (25%, 60% and 90%) to inhibit the α-amylase activity was investigated following a modified method of Ademiluyi *et al*., (20). The reacting component includes same volume (500 µL) of phosphate buffer (0.02 M, pH 6.9), 1.5% α-amylase (prepared in the Phosphate buffer) and varying concentrations of various protein fractions (0.005 – 0.25 mg/ml). The mixture was placed in a water bath set at 25ºC for half an hour. Addition of 500 µL 1% soluble potato starch solution as substrate to the reaction mixture preceded another incubation at the same temperature but for 10 min. After this, the reaction was terminated by addition of 500 µL 3, 5-dinitrosalicylic acid (DNS) color reagent. The entire experiment was then boiled for 5 min and cooled under running tap water. Distilled water (2.5 mL) was pipetted into each tube before measuring the absorbance at 540 nm. Acarbose at the same concentration range was employed as a positive control. Control that represents 100% amylase activity was set up in a parallel experiment and it contains a buffer instead of protein fractions. The level of inhibition by the fractions was calculated in percentage as follows in equation 1.

$$\%Inhibition = \frac{Abs(control) - Abs(sample)}{Abs(control)} \times 100\% \quad \text{(Eq. 1)}$$

Abs_{sample} is the absorbance in the presence of test substance and Abscontrol is the absorbance of control.

2.5.2 In vitro alpha-glucosidase inhibitory activity

Shai *et al*. (21) earlier described the method for measuring α-glucosidase activity inhibition by various agents. The method was adopted in determining the potential of the protein fractions of *D. bulbifera* bulbil to inhibit the activity of α-glucosidase *in vitro* but it was modified. The experiment was carried out in a microtiter plate with 96 wells. Twenty microlitres of each

of the following: phosphate buffer (100 mM, pH 6.8), α-glucosidase prepared in the phosphate buffer, varying concentration of the protein fractions (0.005-0.05 mg/ml) and the substrate (2.5 mM p-nitrophenylglucopyranoside) were mixed in the microtiter plate. The plate was placed in a water bath for incubation at 37 ºC for 15 min. Addition of 80 µL 0.2 M Na_2CO_3 to the reaction mixture terminates the reaction. The microtiter plate was placed in the microtiter plate reader for absorbance reading at 405 nm. The absorbance showed the amount of p-nitrophenol released. Control experiment that shows 100% α-glucosidase activity was performed in the absence of protein fractions. In addition, positive control where a standard drug acarbose was used at the same concentration range was set up along with the experiment. The inhibition of α-glucosidase activity by the protein fractions was expressed in percentage using the equation 2.

$$\%Inhibition = \frac{Abs(control) - Abs(sample)}{Abs(control)} \times 100\% \quad \text{(Eq. 2)}$$

Where, Abs_{sample} is the absorbance in the presence of test substance and Abscontrol is the absorbance of control.

2.6. In-Vitro Antioxidant Activities

2.6.1. Assay of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

Brand-Williams *et al.* (22) procedure of DPPH radical scavenging assay was used to determine the ability of the protein fractions to change the deep violent colour of the DPPH solution to yellow. The colour change measured at 517 nm spectrophotometrically indicates the level of DPPH radical scavenging ability of the fractions. Equal volume (0.15 ml) of the protein fractions/standard (ascorbic acid) and 0.3 mM DPPH in methanol were mixed in a 96-well micro-titre plate. The plate was placed in a dark room for half an hour after which the absorbance was taken at 517 nm. DPPH solution and methanol without protein fractions/standard was used as control. The inhibition of colour change in percentage was calculated with the formula below in equation 3.

$$DPPH\ radical\ scavenging(\%) = \frac{Abs(blank) - Abs(sample)}{Abs(blank)} \times 100\% \quad \text{(Eq. 3)}$$

2.6.2. Assay of hydroxyl (OH) radical scavenging

Potential of the protein fractions to scavenge hydroxyl radical produced from the Fe3+/ascorbate/EDTA/H_2O_2 system ahead of deoxyribose was determined according to the method of Halliwell *et al.* (23). Working reagent was prepared in 10 mM phosphate buffer (pH 7.4). The constituent includes 0.1 mM ascorbic acid, 3 M deoxyribose, 0.1 mM EDTA, 2 mM H2O2 and 1 mM $FeCl_3.6H_2O$. One milliliter of various concentrations of the protein fractions (0 - 350 µg/ml) was mixed with 1 ml of working reagent, incubated for 60 min at 37 ºC. Thereafter, 1 ml each of 1% (w/v) thiobarbituric acid (TBA, prepared in 0.25N HCl) and 10% (w/v) trichloro-acetic acid was added to the mixture in the tubes. Water bath was set at 100 ºC and the tubes was dip in it for 20 mins. The absorbance of the chromogen developed and extracted into 1 ml of butan-2-ol was measured at 532 nm against blank. The inhibition percentage was evaluated with the equation 4.

$$\%(OH)Inhibition = \frac{Absofcontrol - Absofsample}{Absofcontrol} \times 100\% \quad \text{(Eq. 4)}$$

2.6.3. Nitric oxide (NO) radical scavenging assay

Amended assay protocol of Green *et al.* (24) was used to determine the protein fraction's ability to inhibit nitric oxide radical generation. Sodium nitroprusside (2.5 mM) prepared in phosphate buffer saline and different concentration (0.0 – 5.0 mg/ml) of the protein fractions were mixed in test tubes in ratio 9:1, respectively. The mixture was incubated under illumination for two and half hours. Thereafter, 0.5 ml each of 1% sulphanilamide prepared in 5% phosphoric acid and 0.1% N-1- napthylethylenediamine dihydrochloride (NEI) was added to the mixture one after the other with 10 mins incubation in the dark room interval between them. Absorbance measurement of the deepness of the chromophore formed was done at wavelength of 546 nm. Nitric oxide radical generation inhibition percentage was evaluated with the formula 5.

$$\%NoInhibition = \frac{Absofcontrol - Absofsample}{Absofcontrol} \times 100\% \quad \text{(Eq. 5)}$$

2.6.4. Total Antioxidant Capacity (TAC) assay

Reduction of molybdate (VI) to molybdate (V) by the protein fractions was measure using Prieto *et al*, (25) procedure. Reagent solution (containing 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid) was mixed with protein fractions/standard solution (ascorbic acid) in ratio 10:1 in test tubes. The mixture in the test-tubes was later incubated at 95ºC for one and half hour in a regulated water-bath. The absorbance was measured at wavelength 695 nm after the reacting mixture has cooled to room temperature. Distilled water replaced the protein fractions in the reacting mixture that served as control. The antioxidant activities of the protein fractions were expressed as an ascorbic acid equivalent per gram of the protein fractions

2.6.5. Ferric Reducing Antioxidant Power (FRAP) assay

To measure the reducing antioxidant power of the protein fractions, Benzie and Strain (26) procedure was employed with little amendment. Three hundred millimolar of acetate buffer (pH 3.6) was prepared. Also, 10 mM 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ were separately prepared. The solutions were mixed at point of use in ratio 10:1:1 respectively, to obtain the FRAP working reagent. The reacting mixture for the assay contain 50 µl of protein fractions/standard solution (ascorbic acid) in concentration range of 0-100 µg/ml and 1 ml of FRAP working reagent. The reaction was allowed to proceed for 10 min, thereafter absorbance reading was measured at 593 nm against reagent blank. The reacting mixture was shaded from direct sunlight when taken the reading. The ferric reducing antioxidant power of the protein fractions was expressed as ascorbic acid equivalent.

2.6.6. Ferrous-ion chelating activity assay

Evaluation of the ability of the protein fractions to chelate ferrous ion was carried out according to the amended assay procedure of Singh and Rajini (27). Reagents used are 2 mM $FeCl_3.4H_2O$ and 5 mM Ferrozine which was diluted 20 folds. The reaction started with mixing of 1 ml of different concentration of protein fractions with 1 ml $FeCl_3.4H_2O$. The reaction proceeded for 5 mins at ambient temperature followed by the addition 1 ml ferrozine solution.

The reacting mixture was vortexed and incubated for 10 mins. Absorbance of the mixture was taken at 562 nm. Chelating effect percentage was expressed as equation 6,

$$\%NoInhibition = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100\% \quad \text{(Eq. 6)}$$

2.7. Data analysis

Results were subjected to statistical analysis performed with GraphPad Prism Version 6.0 (Graphpad Software Inc., San Diego, California). The experimental values were shown as Mean ± Standard Error of Mean where n=3. One-way ANOVA was used to examine multiple comparisons. The level of significance was $p<0.05$.

3. Result

3.1. Protein concentration

The soluble protein of D. bulbifera bulbil was extracted using PBS (pH 7.2) and further fractionated with ammonium sulphate. The amount of soluble protein present in crude extract is 12.53 mg protein per milliliter of crude extract solution. The ammonium sulphate fractionation yielded 6.1 mg protein for 25% fraction, 4.21 mg protein for 60% fraction and 0.98 mg protein for 90% fraction. Protein in 25% fraction is about 50% of total crude protein extract. Sixty percent fraction con-

Table 1. Hemagglutinating activity and Blood Group Specificity of *D. bulbifera* bulbils lectin

Source of Erythrocyte	Hemagglutinating Activity		
	Crude extract	25% Fraction	60% Fraction
Human A	2^3	2^2	2^0
Human B	2^2	2^3	2^0
Human O	2^3	2^3	2^0
Human AB	2^2	2^2	2^0
Rabbit	2^7	2^6	2^3

Table 2. Sugar specificity for *Dioscorea bulbifera* bulbil crude protein extract and 25% fraction lectin

Sugars	Crude extract	25% Fraction
Negative	2^0	2^0
Positive	2^7	2^7
D galactose	2^4	2^5
Arabinose	2^5	2^5
Glucose	2^5	2^5
N-acetyl-D-Glucosamine	2^3	2^3
Mannose	2^0	2^0
Glucosamine HCl	2^7	2^7
Maltose	2^6	2^4
D-mannitol	2^6	2^5
D-deoxy-D-glucose	2^7	2^5
Lactose	2^6	2^5
Sucrose	2^5	2^5
Sorbose	2^3	2^4

tained about 34% while 90% have 8%.

3.2. Hemagglutinating Activity

Presence of lectin was detected in the crude protein extract and also in the protein fractions. Crude protein extract and 25% fraction had better preference for rabbit erythrocytes agglutination when compare to ABO blood grouping of human. Sixty percent (60%) protein fraction only agglutinated rabbit erythrocyte with low hemagglutination titre (23) (Table 1). It was established that the lectin present in the 25% fraction is mannose specific (Table 2).

3.3. Carbohydrate –hydrolyzing enzyme activity inhibition

The results obtained revealed the efficacy of the protein fractions in inhibiting the activity of two carbohydrates-hydrolyzing enzymes assayed. Protein fractions (25% and 60%) inhibited the enzymes better than acarbose, a standard inhibitor. The results also showed that α-glucosidase was better inhibited by the fractions (Table 3). Inhibition of α-amylase by 25% and 60% protein fractions were not significantly different. The same occurrence was noticed for α-glucosidase. Figure 1, Figure 2, and Table 3 showed the enzymes inhibition results and their Half-maximal inhibitory concentration (IC_{50}). The α-amylase and α-glucosidase inhibition was directly proportional to the concentration of the protein fractions (Figure 1 and 2). The third protein fraction (90%) exhibited/showed no enzyme inhibitory activity.

3.4. Antioxidant activity

Table 4-7 and Figure 3 depicted the antioxidant activity assay results of the various methods used. Table 4 revealed that the protein fractions,

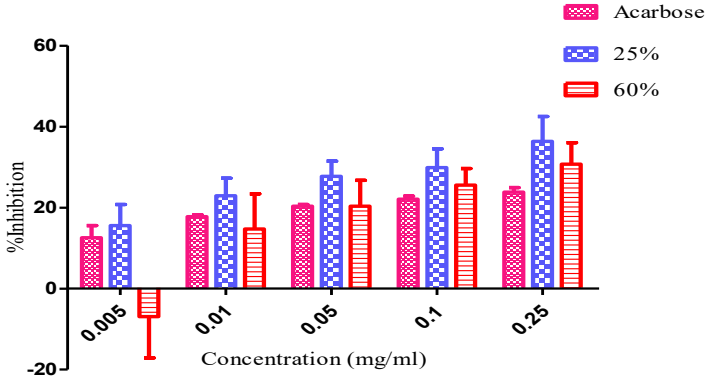

Figure 1. α- Amylase Inhibitory Activity of *D. bulbifera* bulbil Protein Fractions.

Table 3. IC_{50} values of Protein Fractions of *D. bulbifera* bulbil and Acarbose on Alpha- amylase and Alpha- glucosidase.

Sample	IC_{50} values of α- amylase (mg/ml)	IC_{50} values of α-glucosidase (mg/ml)
Acarbose	1.460±0.215	0.028±0.002
25%	0.470±0.142	0.005±0.001
60%	0.424±0.076	0.006±0.001

25% and 60%, were able to scavenged DPPH radical in a dose dependent way, though their ability is significantly different from the standard radical scavenging agent (ascorbic acid). The IC_{50} for the ascorbic acid and fractions are shown in Table 4. Aside the ability to scavenge DPPH, the protein fractions also scavenged hydroxyl radical but 60% fraction was a better scavenger of OH radical. Its ability was also higher than one display by ascorbic acid (Figure 3).

Nitric oxide (NO) radical formation was inhibited by 25 % protein fraction with IC_{50} of 1.3 ± 0.06 mg/ml; result that is significantly different from ascorbic acid used as standard (Table 5). 60% fraction concentration below 2.0 mg/ml could not inhibit the NO formation while 90% fraction showed no inhibition even at high concentration of 10 mg/ml.

Table 6 showed that protein fractions possessed considerable potential to reduce molybdenum (IV) to molybdenum (V) leading to the molybdenum (V) complex formation with 25% fraction having higher value. They also exhibited ability to reduce Fe^{3+} to Fe^{2+} as shown in Table 7. It was deduced that the protein fractions were found to inhibit ferrozine-Fe^{2+} complex formation. The ability increases as the concentration of fractions goes up. 25% exhibited a better inhibitory action, though below the value of ascorbic acid.

4. Discussion

The protein fractions of the *D. bulbifera* bulbils were obtained by ammonium sulphate fractionation. This is usually done by modifying the solvent conditions and taking advantage of the variations in solubility of the target protein relative to other proteins and macromolecules present in a crude extract. Researchers commonly employ differential precipitation of protein with ammonium sulphate at the initial stages of protein purification either from animals or plants sources (28). It is also used to concentrate protein towards the last stages of purification. The amount of protein obtained from the crude extract of *D. bulbifera* bulbils was found to be in accordance with the report of (29) and (30). The same methods used in this study have been employed by Saha et al., (28), Manukumar *et al*., (31), and Odekanyin and Akeredolu (32) to extract some bioactive proteins from *Cajanus cajan* seed coat, *Tinospora tomentosa* stem and *Pterocarpus osun* and *Bosquiea angolensis* seeds, respectively for the purpose of biological characterization.

Lectins are biological molecules present in almost all living organisms. Lectins are protein/glycoprotein that possess the ability to bind sugar moiety and agglutinate cells. Lectin plays significant roles in biological systems because of their

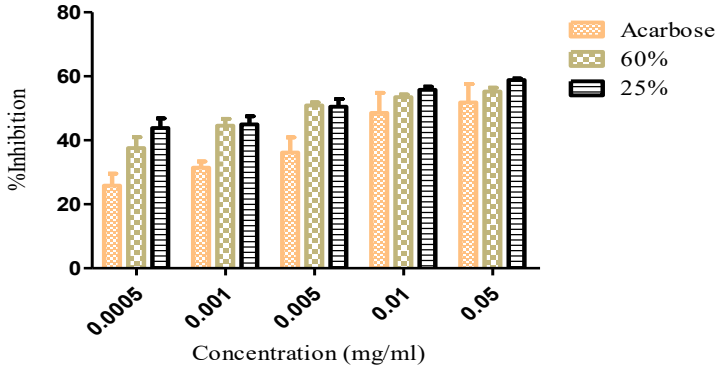

Figure 2. α- glucosidase Inhibitory Activity of *D. bulbifera* bulbil Protein Fractions.

Table 4. DPPH Radical Scavenging Activity of *D. bulbifera* Bulbil Protein Fractions.

Sample	Concentration (µg/ml)	% Inhibition ± SEM	IC_{50} ± SEM
Ascorbic acid	0.625	-15.68±1.52	0.013±0.0002
	1.250	-10.30±0.94	
	2.500	5.563±1.52	
	5.000	12.832±1.47	
	10.00	39.381±1.92	
	20.00	83.186±0.54	
60%	156.25	11.44±1.83	3.404±0.01
	312.50	14.54±0.98	
	625.00	22.31±1.92	
	1250.0	26.87±1.21	
	2500.0	38.88±0.49	
	5000.0	39.57±0.81	
25%	62.50	13.34±0.76	2.526±0.30
	125.0	14.48±0.76	
	250.0	18.52±1.74	
	500.0	24.65±1.43	
	1000	31.42±1.83	
	2000	41.21±2.06	

therapeutic functions. Lectin activity was detected in the crude protein extract so also in the two fractions (25% and 60%). Crude protein extract and 25% fraction are not blood group specific but prefer rabbit blood erythrocytes while 60% fraction is rabbit blood specific. The lectin was also established to be mannose-binding lectin. Previously, mannose-binding lectins have been isolated and purified from *D. batatas* tuber (33) and *D. bulbifera* bulbil (34). In addition, lectin isolation, purification and biological characterization have been announced for other Dioscorea spp which include *D. preussii* (35), *D. mangenotiana* (36), *D. opposita* (37), *D. alata* (38), *D. japonica* (39) among others. Different biological and therapeutic activities such as antioxidant, insecticidal, anti-tumor, anti-proliferative and antiviral were established for the above-mentioned purified lectins. None of the lectins from Dioscorea spp purified up-to-date have been implicated in the inhibition of α-amylase and α-glucosidase or in any mechanism of diabetes mellitus management. Numerous reports on the lectins from other sources with anti-diabetic activity have been published (40 - 42).

Plant extracts have been used since time immemorial for the treatment and control of diseases. Based on the World Health Organization report of 2002, a higher percentage of the world

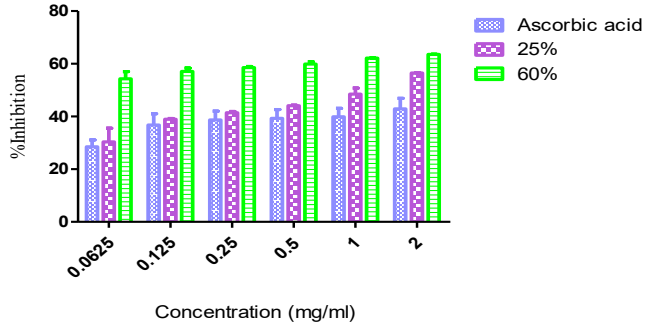

Figure 3. Hydroxyl Radical Scavenging Activity of *D.bulbifera* bulbil Protein Fractions.

Table 5. Inhibition of Nitric Oxide Radical by *D. bulbifera* bulbil Protein Fractions.

Sample	Concentration (µg/ml)	% Inhibition ± SEM	IC_{50} ± SEM
Ascorbic acid	7.81	8.07±2.50	0.06±0.002
	15.63	16.61±2.63	
	31.25	34.98±1.14	
	62.50	52.86±0.75	
	125.0	86.86±0.58	
60%	31.25	-17.45±2.37	13.93±4.88
	62.50	-10.77±5.35	
	125.0	-7.74±4.24	
	250.0	-5.17±4.81	
	500.0	-4.54±4.79	
	1000	-1.21±2.78	
	2000	4.66±0.81	
25%	31.25	10.34±6.03	1.30±0.06
	62.50	9.56±5.35	
	125.0	14.97±7.85	
	250.0	22.11±6.70	
	500.0	30.61±5.09	
	1000	47.70±1.43	
	2000	68.26±0.79	

population is using traditional means to treat various diseases. This is because the tradomedicinal care is very cheap with rather no negative side effects when put side-by-side with the conventional medicine. Researchers have therefore focused on getting more scientific-based facts on the usage of plants and its products for the benefit of human health. Management of diabetes mellitus, a condition characterized by an increase in blood glucose level, is currently being treated with herbal extract, which is more effective, affordable, and with no or little adverse effects. At chronic stage, diabetes is associated with other diseases such as heart damage, eye blurring, kidney failure, stroke and other health challenges.

Among the mechanisms through which the plant-based drugs work in the management of diabetes are controlling hyperlipidemia, hyperglycemia and putting oxidative stress under control. Inhibition of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) is currently employed as the main therapeutic means of managing diabetes mellitus conditions. These enzyme inhibitors are abundantly present in various parts of the plant. So far, many of the isolated, purified and tested inhibitors are obtained through extraction with organic solvent (15, 16, 43). Several reports on the efficacy of a variety of secondary metabolites as inhibitors of these enzymes are available (43, 44). Protein has also been reported to have inhibitory activity against α-amylase and α-glucosidase (45, 46). Studies conducted have also shown that protein extracted from plants plays a beneficial role in combating chronic diseases like

Table 6. Ferric Reducing potential and Total Antioxidant Capacity of *D. bulbifera* bulbil Protein Fractions.

Assays	Milligram ascorbic acid equivalent per gram of sample (mgAAE/g)	
	25%	60%
Ferric Reducing potential (FRAP)	1.935 ± 0.077	2.296 ± 0.111
Total Antioxidant Capacity (TAC)	17.167 ± 0.785	10.867 ± 0.640

Table 7. Ferrous Ion-chelating Ability of *D. bulbifera* bulbil Protein Fractions.

Sample	Concentration (µg/ml)	% Inhibition ± SEM	IC_{50} ± SEM
Ascorbic acid	1.563	-0.08±2.05	0.022±0.0001
	3.125	8.10±1.01	
	6.250	17.71±2.14	
	12.50	41.94±0.48	
	25.00	82.37±0.30	
	50.00	84.63±0.38	
60%	62.50	37.11±1.50	1.036±0.0117
	125.0	56.52±1.26	
	250.0	70.71±1.21	
	500.0	76.75±0.92	
	1000	79.22±0.53	
	2000	81.89±0.87	
25%	62.50	-42.73±0.82	0.247±0.1151
	125.0	42.87±3.73	
	250.0	46.43±4.80	
	500.0	63.92±1.07	
	1000	65.36±0.05	
	2000	71.06±0.87	

cancer and diabetes particularly in the control of type 2 diabetes mellitus (15). These plant proteins are able to play the beneficial role as a result of the important bioactive compounds they contain, these bioactive components possess various biological properties such as antioxidant, analgesic and anti-inflammatory (47), anti-tumor (48), antidiabetic and anti-microbial properties (49). Two-ammonium sulphate protein fractions obtained from *D. bulbifera* bulbil were effective in inhibiting α-amylase and α-glucosidase in-vitro. In vitro α-amylase and glucosidase inhibitory studies conducted by (15) revealed that *G. glauca* and *D. bulbifera* extracts had inhibitory activity against these enzymes. The percentage of inhibition depend on the type of organic solvent used for extraction and source of the enzyme employed for the assay and it ranges between 61.65% - 73.39% for α-amylase and 18.13% - 99.6% for α-glucosidase (15). In this study, 25% and 60% protein fractions of *D. bulbifera* bulbil were found to also display significant inhibition against α-amylase and α-glucosidase. Protein fractions of *D. bulbifera* bulbil (25% and 60%) show IC_{50} values of 5 µg/ml and 6 µg/ml respectively, which makes it a better inhibitor of α-glucosidase than acarbose (IC_{50}= 28.11±2.7 µg/ml). Present study also revealed that 25% and 60% protein fractions of *D. bulbifera* bulbil are more potent inhibitors of α-amylase with IC50 values of 47.0 and 42.4 µg/ml respectively than acarbose. Storage protein of Dioscorea spp (Dioscorin) has not been reported to exhibit antidiabetic activity. Antidiabetic potentials of extracts obtained through organic solvent extraction from *D. alata*, *D. batatas* and *D. opposite* tubers have been reported (50-52).

Excessive production of reactive oxygenated species or total failure of the endogenous antioxidants leads to oxidative stress. Oxidative stress has been reported as the main cause of numerous diseases such as ageing, cancer, neurodegenerative disorders, chronic inflammation, diabetes mellitus and atherosclerosis. Discovery of more potent antioxidant agents from plant sources that possess other therapeutic potentials will provide relief to the radical imbalance-mediated oxidative stress and associated diseases. This is a sequel to the various limitations of conventional drugs.

It is well established that the Dioscorea spp possess huge antioxidant potential confined in

the phytochemicals present in the plants (53). Only few reports have mentioned the antioxidant activity of Dioscorea spp proteins or its hydrolysates. The in-vitro antioxidant activity of protein fractions of *D. bulbifera* bulbil was determined using assay methods with different mechanisms, which include DPPH radical scavenging activity, Nitric oxide scavenging assay, hydroxyl radical scavenging assay, Total antioxidant capacity, Ferrous ion-chelating ability assay, and Ferric reducing antioxidant power.

DPPH radical scavenging assay is the simplest, most reliable, highly sensitive and acceptable antioxidant assay method coupled with its good reproducible results. DPPH is a nitrogen-containing compound that accepts electron or hydrogen atoms to become stable from antioxidants. The ability of antioxidants in the extract to scavenge the DPPH radical is the basis for the assay. This is noticed in the colour change from purple to yellow upon the formation of stable DPPH. Decrease in spectrophotometric reading at 517 nm allowed the evaluation of the antioxidant effect. The extract donates an electron or hydrogen atom to DPPH for it to become stable. Two protein fractions produced from *D. bulbifera* bulbil crude protein were able to bleach the DPPH colour that shows the capacity of the fractions to scavenge the free DPPH radical. Our findings compare favourably well with the available results of antioxidant capacity of a variety of Dioscorea spp (53). In the comprehensive review of the Dioscorea spp antioxidant potentials, various in-vitro chemical methods, in-vivo assays using rat and mouse model and cell cultures were employed. Different parts of the plants and a variety of organic solvent were reportedly used.

Lipid peroxidation and *in vivo* damage to biological molecules are due to hydroxyl radicals, an oxygen species with high reactivity produced by some metabolic pathways. Hydroxyl radical scavenging activity is used as a measure of antioxidant activity of biological extract or pure isolates that may function in food and health products. In-vitro generation of hydroxyl radical is through Fenton reaction using Fe^{2+}/ascobate// EDTA/H_2O_2 system. In the presence of a catalyst – transition metal ion (Fe^{2+}) and hydroxyl radical at an acidic pH, malondialdehyde, which produces a coloured chromogen in the presence of thiobarbituric acid, is generated from the degradation of deoxyribose. Both 25% and 60% fractions exhibited better hydroxyl radical scavenging than ascorbic acid employed as standard. Sixty per cent (60%) protein fraction showed excellent scavenging activity, which was significantly different from 25% protein fraction and ascorbic acid. The result is in agreement with reports of (54) and (55). In their studies, Ghosh *et al*, (54) reported on the antioxidant activity of *D. bulbifera* bulb while Liu et al, (55) study centered on *D. opposite* rhizome peel and flesh. From the data presented, it could be suggested that the fractions render the process of Fe2+-dependent hydroxyl radical production inactive or poorly active, thereby reducing the effect of hydroxyl radical on biological systems.

Among the physiological roles of Nitric Oxide (NO) are acting as a vasodilator in the cardiovascular system, as an intracellular signal in several cellular pathways and playing a protective role against oxidative stress (56). Its plausible involvement in cancer treatment and SARS-COV-ID-19 has been reported (57). Also NO produced by macrophages protects the body against infection as an antimicrobial agent (58). Despite these significant roles, excessive increase in NO generation in intracellular space can lead to cytotoxic effects. Nitric oxide has therefore been linked as the root cause of many degenerative diseases. The clearance or removal of excess NO will definitely play high impact roles in management and treatment of such diseases. The Nitric Oxide scavenging ability of 25% protein fraction was evaluated by the decline in the absorbance reading at 546 nm, resulting from a nitric oxide generation decrease. The present study established the ability of 25% protein fraction to scavenge NO radical. This potential will assist in stopping further synthesis of a more reactive oxygen radical species that can increase the development of oxidative stress. Based on this 25% protein fraction may be suitable for the treatment and management of oxidative stress related ailments.

Ferric reducing antioxidant power assay permits the measurement of reduction of Fe3+-TPTZ to Fe^{2+}-TPTZ by antioxidant compounds present in an extract under acidic condition. The

intense blue colour formed was absorbed maximally at 593 nm wavelength. The advantage of this antioxidant assay method is that it is fast, simple and gives reproducible results. It was first used to quantify ascorbic acid in serum and plasma (26, 58). Spectrophotometrically, the production of Fe^{2+}-TPTZ complex was monitored which indicates the reducing ability of protein fractions. 25% protein fraction has a significant potential to reduce Fe^{3+} to Fe^{2+} though lower than the standard used. Ghosh et al., (59) applied FRAP to determine the antioxidant activity of *D. bulbifera* bulb using Gallic acid equivalent antioxidant capacity to express their results. Moon and Shibamoto (60) suggested that the standard antioxidant for this assay must be water-soluble. Ascorbic acid equivalent used in the current study revealed that the results for 25% protein fraction is within the range reported in the literature for those that also used ascorbic acid equivalent (61, 62). A number of studies have stated that the reducing power of an extract or isolate may serve as an important pointer of potential antioxidant activity. Antioxidative activity has been suggested to be associated with reducing power.

The importance of heavy metals in the biological system cannot be over-emphasized. Among the events in the living system in which metals are involved are aiding enzyme catalysis, cell growth, biosynthesis of molecules, body immune system and cell proliferation and development (63). Presence of excess metal ions in different pathways is injurious to the biological systems. Transition metal ions, putting aside their goods, are also liable to cause peroxidation of unsaturated fatty acid and also contribute to the oxidative damage in some diseases such as Alzheimer's and Parkinson's diseases. The condition referred to as metal toxicity or poisoning can only be treated using Metal chelating therapy in which drugs like dimercaptosuccinic acid, EDTA, deferoxamine and dimercaprol are prescribed for the patient. Metal chelating activity of the protein fractions was tested using ferrozine as a chelating agent. In this assay, decolorization of Fe^{2+}-ferrozine complex takes place due to the presence of chelating agents in the protein fractions of *D. bulbifera* bulbils. Colour reduction observed in the assay showed that the metal ion chelating activity of the protein fractions of *D. bulbifera* increases. The results revealed that the 25% protein fraction is five times better in chelating ferrous ions than 60% protein fraction. The result is in agreement with the study conducted by Narkhede and Jagtap, (64), it was observed that methanolic extracts of *D. bulbifera* bulbils and *D. bulbifera* root tuber showed a higher chelating activity than the other six different tubers tested.

Total antioxidant capacity (TAC) is based on the potential of an oxidative agent to reduce molybdenum (VI) to molybdenum (V). The assay is quantitative spectrophotometric techniques that measure both fat-soluble and water-soluble antioxidants present in a sample. There is formation of green phosphomolybdenum (V) complex without induction of free metal ions solution. It also gives a direct estimation of reducing power of antioxidants. The results revealed that 25% protein fraction has higher TAC expressed in ascorbic acid equivalent per gram of sample than 60% protein fraction. This showed that the 25% fraction contains molecules that can compete with ferrozine for ferrous ions thereby preventing the formation of Fe^{2+}-ferrozine complex. This fraction can be of great therapeutic importance especially in treatment of disease conditions called Thalassemia or other anemia ailments. The excess iron in the body is neutralized using metal chelating therapy in which the 25 % fraction can replace the expensive synthetic drugs that also have some side effects. Prieto *et al*, (25) stated that this method aside its usage to analyze the antioxidant contribution of specific food components, it can also be employed in analyzing changes in plasma antioxidant activity related to oxidative stress and in understanding the structure activity relationship of pure antioxidant molecules.

5. Conclusion

The data presented clearly showed and established the inhibitory potential of 25% and 60% protein fractions against the activity of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase). Their inhibitory action was better than that of standard drug (acarbose) used in this study. The fractions were also found to display antioxidative prowess by clearing the free radicals

from solution. Ability to chelate metal ions was also established coupled with potentials to reduce ferric ions to ferrous ions. These properties could help in mitigating the development of oxidative stress, the sole cause of several biological damages that lead to numerous ailments. The fractions can be considered for possible development as a promising therapeutics agent for the treatment of diabetes and other oxidative stress-related diseases. Therefore, the ineffectiveness of 90% protein fraction in the manifestation of anti-amylase and anti-glucosidase activities may be due to the presence of high molecular weight proteins that could possibly block the antioxidant activity of lower molecular weight proteins. The fractions can therefore be subjected to further studies like in-vivo animal model experiment and purification of the lectin and other possible active components in the fractions.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Kumar S, Narwal S, Kumar V, Prakash O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn Rev.* 2011 Jan;5(9):19-29. doi: 10.4103/0973-7847.79096. PMID: 22096315; PMCID: PMC3210010.
2. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci*. 2020 Aug 30;21(17):6275. doi: 10.3390/ijms21176275. PMID: 32872570; PMCID: PMC7503727.
3. Alwan AD, Galea G, Stuckler D. Development at risk: addressing noncommunicable diseases at the United Nations high-level meeting. *Bull World Health Organ.* 2011 Aug 1;89(8):546-546A. doi: 10.2471/BLT.11.091074. PMID: 21836748; PMCID: PMC3150770.
4. Kim KY, Nam KA, Kurihara H, Kim SM. Potent alpha-glucosidase inhibitors purified from the red alga Grateloupia elliptica. *Phytochemistry.* 2008 Nov;69(16):2820-5. doi: 10.1016/j.phytochem.2008.09.007. Epub 2008 Oct 23. PMID: 18951591.
5. Holman RR, Cull CA, Turner RC. A randomized double-blind trial of acarbose in type 2 diabetes shows improved glycemic control over 3 years (U.K. Prospective Diabetes Study 44). *Diabetes Care.* 1999 Jun;22(6):960-4. doi: 10.2337/diacare.22.6.960. Erratum in: Diabetes Care 1999 Nov;22(11):1922. PMID: 10372249.
6. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. *Saudi Pharm J.* 2016 Sep;24(5):547-553. doi: 10.1016/j.jsps.2015.03.013. Epub 2015 Mar 21. PMID: 27752226; PMCID: PMC5059829.
7. World Health Organization. Global reports on diabetes. 2016. Available from: https://www.who.int/publications/i/item/9789241565257.
8. Wahab NA, Abdullah N, Aminudin N. Characterisation of potential antidiabetic-related proteins from Pleurotus pulmonarius (Fr.) Quél. (grey oyster mushroom) by MALDI-TOF/TOF mass spectrometry. *Biomed Res Int.* 2014;2014:131607. doi: 10.1155/2014/131607. Epub 2014 Aug 28. PMID: 25243114; PMCID: PMC4163432.
9. Oliveira AEA, Machado OLT, Gomes VM, Neto JX, Pereira AC, Vieira JGH, Fernandes KV, Xavier-Filho J. Jack bean seed coat contains a protein with complete sequence homology to bovine insulin. *Protein and Peptide Letters*. 1999 Feb 1;6(1): 15-22
10. Li Q, Ye XL, Zeng H, Chen X, Li XG. [Study on the extraction technology and hypoglycemic activity of lectin from Trichosanthes kirilowi]. *Zhong Yao Cai.* 2012 Mar;35(3):475-9. Chinese. PMID: 22876690.
11. da Rocha AA, Araújo TF, da Fonseca CS, da Mota DL, de Medeiros PL, Paiva PM, Coelho LC, Correia MT, Lima VL. Lectin from Crataeva tapia Bark Improves Tissue Damages and Plasma Hyperglycemia in Alloxan-Induced Diabetic Mice. *Evid Based Complement Alternat Med.* 2013;2013:869305. doi: 10.1155/2013/869305. Epub 2013 Nov 13. PMID: 24324521; PMCID: PMC3845403.
12. Paula PC, Sousa DO, Oliveira JT, Carvalho AF, Alves BG, Pereira ML, Farias DF, Viana MP, Santos FA, Morais TC, Vasconcelos IM. A Protein Isolate from Moringa oleifera Leaves Has Hypoglycemic and Antioxidant Effects in Alloxan-Induced Diabetic Mice. *Molecules*. 2017 Feb 11;22(2):271. doi: 10.3390/molecules22020271. PMID: 28208654; PMCID: PMC6155657.
13. Subasini U, Thenmozhi S, Sathyamurthy

D, Vetriselvan S, Victor RG, Dubey GP. Pharmacognostic and phytochemical investigations of Dioscorea bulbifera L. *Int J Pharm Life Sci*. 2013; 4(5): 2693 – 2700.
14. Nguelefack TB, Dutra RC, Paszcuk AF, Andrade EL, Tapondjou LA, Calixto JB. Antinociceptive activities of the methanol extract of the bulbs of Dioscorea bulbifera L. var sativa in mice is dependent of NO-cGMP-ATP-sensitive-K(+) channel activation. *J Ethnopharmacol.* 2010 Apr 21;128(3):567-74. doi: 10.1016/j.jep.2010.01.061. Epub 2010 Feb 10. PMID: 20152893.
15. Ghosh S, Ahire M, Patil S, Jabgunde A, Bhat Dusane M, Joshi BN, Pardesi K, Jachak S, Dhavale DD, Chopade BA. Antidiabetic Activity of Gnidia glauca and Dioscorea bulbifera: Potent Amylase and Glucosidase Inhibitors. *Evid Based Complement Alternat Med*. 2012;2012:929051. doi: 10.1155/2012/929051. Epub 2011 Jul 9. PMID: 21785651; PMCID: PMC3140190.
16. Ghosh S, More P, Derle A, Patil AB, Markad P, Asok A, Kumbhar N, Shaikh ML, Ramanamurthy B, Shinde VS, Dhavale DD, Chopade BA. Diosgenin from Dioscorea bulbifera: novel hit for treatment of type II diabetes mellitus with inhibitory activity against α-amylase and α-glucosidase. *PLoS One.* 2014 Sep 12;9(9):e106039. doi: 10.1371/journal.pone.0106039. PMID: 25216353; PMCID: PMC4162539.
17. Arya P, Munshi M, Kumar P. Diosgenin: Chemistry, extraction, quantification and health benefits. *Food Chem Adv*. 2023; 2:100170.
18. Lowry OH, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951 Nov;193(1):265-75. PMID: 14907713.
19. Wang H, Gao J, Ng TB. A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom Pleurotus ostreatus. *Biochem Biophys Res Commun*. 2000 Sep 7;275(3):810-6. doi: 10.1006/bbrc.2000.3373. PMID: 10973803.
20. Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Exp Toxicol Pathol.* 2013 Mar;65(3):305-9. doi: 10.1016/j.etp.2011.09.005. Epub 2011 Oct 17. PMID: 22005499.
21. Shai LJ, Magano SR, Lebelo SL, Mogale AM. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. *J Med Plants Res.* 2011; 5(13):2863-7.
22. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol.* 1995; 28(1):25-30
23. Halliwell B, Wasil M, Grootveld M. Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by ascorbic acid. Implications for antioxidant protection in the inflamed rheumatoid joint. *FEBS Lett.* 1987 Mar 9;213(1):15-7. doi: 10.1016/0014-5793(87)81456-4. PMID: 3030805.
24. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem*. 1982 Oct;126(1):131-8. doi: 10.1016/0003-2697(82)90118-x. PMID: 7181105.
25. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999 May 1;269(2):337-41. doi: 10.1006/abio.1999.4019. PMID: 10222007.
26. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol*. 1999;299:15-27. doi: 10.1016/s0076-6879(99)99005-5. PMID: 9916193.
27. Singh N, Rajini PS. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem*. 2004; 85(4):611-6.
28. Saha RK, Acharya S, Jamiruddin M, Roy P, Islam MM, Shovon SS. Antimicrobial effects of a crude plant lectin isolated from the stem of Tinospora tomentosa. *J Phytopharmacol.* 2014; 3:44-51
29. Celestine AA, David OI. Comparative nutritional and phytochemical evaluation of the aerial and underground tubers of air potato (Dioscorea bulbifera). Available in Abakaliki, Ebonyi State, Nigeria. *Br J Appl Sci Technol.* 2015; 11(4): 1-7.
30. Libra MA, Gonnety JT, Ahi AP, Dabonne S, Ahipo ED, Kouame LP. Physicochemical changes in bulbils of two cultivars of Dioscorea

bulbifera during the ripening period. *Adv J Food Sci Technol*. 2011; 3(5):327-31
31. Manukumar HM, Madhu CS. Comparative evaluation of fractional efficiency on antioxidant activity of red gram (Cajanus cajan) seed coat crude protein extracts. *Int J Recent Sci Res.* 2013; 4(9): 1395-1399.
32. Odekanyin OO, Akeredolu EO. Comparative Investigation of the Biological Activity of the Pterocarpus osun and Bosqueia angolensis Seeds Protein Extracts. *Int J Curr Res Biosci Plant Biol.* 2016; 3(9):16-26.
33. Ohizumi Y, Gaidamashvili M, Ohwada S, Matsuda K, Kominami J, Nakamura-Tsuruta S, Hirabayashi J, Naganuma T, Ogawa T, Muramoto K. Mannose-binding lectin from yam (Dioscorea batatas) tubers with insecticidal properties against Helicoverpa armigera (Lepidoptera: Noctuidae). *J Agric Food Chem.* 2009 Apr 8;57(7):2896-902. doi: 10.1021/jf8040269. PMID: 19271711.
34. Sharma M, Hotpet V, B R S, A S K, Swamy BM, Inamdar SR. Purification, characterization and biological significance of mannose binding lectin from Dioscorea bulbifera bulbils. *Int J Biol Macromol.* 2017 Sep;102:1146-1155. doi: 10.1016/j.ijbiomac.2017.04.085. Epub 2017 May 1. PMID: 28472687.
35. Odekanyin O. O. and Akanni SI. Hemagglutinin from the Root Tuber of Dioscorea preussii Pax Exhibit Antioxidative Prowess and Haemolysis Inhibition. *Turk J Pharma. Sci.* 2023 DOI:10.4274/tjps.galenos.2023.62293(Article in Press)
36. Akinyoola KA, Odekanyin OO, Kuku A, Sosan MB. Anti-insect potential of a lectin from the tuber, Dioscorea mangenotiana towards Eldana saccharina (Lepidoptera: Pyralidae). *J Agric Biotechnol Sustain Dev.* 2016; 8(3):16-26. DOI:10.5897/JABSD2015.0249
37. Chan YS, Ng TB. A lectin with highly potent inhibitory activity toward breast cancer cells from edible tubers of Dioscorea opposita cv. nagaimo. *PLoS One.* 2013;8(1):e54212. doi: 10.1371/journal.pone.0054212. Epub 2013 Jan 21. PMID: 23349827; PMCID: PMC3549954.
38. Harijono H, Estiasih T, Apriliyanti MW, Afriliana A, Kusnadi J. Physicochemical and Bioactives Characteristics of Purple and Yellow Water Yam (Dioscorea alata) Tubers. *Inter J PharmTech Res*. 2013; 5(4):1691-701.
39. Araki T, Funatsu J, Kuramoto M, Konno H, Torikata T. The complete amino acid sequence of yam (Dioscorea japonica) chitinase. A newly identified acidic class I chitinase. *J Biol Chem*. 1992 Oct 5;267(28):19944-7. PMID: 1400311.
40. Govindappa M, Sadananda T, Ramachandra YL, Chandrappa CP, Rai PS, Prasad SK. In vitro and in vivo antidiabetic activity of lectin (N-acetyl-galactosamine, 64 kDa) isolated from endophytic fungi, Alternaria species from Viscum album on alloxan induced diabetic rats. *Integr Diabetes Obesity*. 2015; 1(1):11-9.
41. Olodude SO, Odekanyin OO, Fatokun AB. Evaluation of antioxidant and carbohydrate-hydrolysing enzymes inhibitory activities of Trilepisium madagascariense Ficalho seeds hemagglutinin. *IOSR J Pharm Biol Sci.* 2017; 12(6):29-41.
42. Bhagyawant SS, Narvekar DT, Gupta N, Bhadkaria A, Gautam AK, Srivastava N. Chickpea (Cicer arietinum L.) Lectin Exhibit Inhibition of ACE-I, α-amylase and α-glucosidase Activity. *Protein Pept Lett.* 2019;26(7):494-501. doi: 10.2174/0929866526666190327130037. PMID: 30919768.
43. Tran N, Pham B, Le L. Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. *Biology (Basel)*. 2020 Aug 28;9(9):252. doi: 10.3390/biology9090252. PMID: 32872226; PMCID: PMC7563488.
44. Chan CH, Ngoh GC, Yusoff R. A brief review on anti diabetic plants: Global distribution, active ingredients, extraction techniques and acting mechanisms. *Pharmacogn Rev.* 2012 Jan;6(11):22-8. doi: 10.4103/0973-7847.95854. PMID: 22654401; PMCID: PMC3358964.
45. Svensson B, Fukuda K, Nielsen PK, Bønsager BC. Proteinaceous alpha-amylase inhibitors. *Biochim Biophys Acta.* 2004 Feb 12;1696(2):145-56. doi: 10.1016/j.bbapap.2003.07.004. PMID: 14871655.
46. Poovitha S, Sai MS, Parani M. Protein extract from the fruit pulp of Momordica dioica shows anti-diabetic, anti-lipidemic and antioxidant activity in diabetic rats. *J Funct Foods*. 2017; 33:181-7.
47. Mbiantcha M, Kamanyi A, Teponno RB, Tapondjou AL, Watcho P, Nguelefack TB. Analgesic and Anti-Inflammatory Properties of Extracts from the Bulbils of Dioscorea bulbifera L. var sa-

tiva (Dioscoreaceae) in Mice and Rats. *Evid Based Complement Alternat Med.* 2011;2011:912935. doi: 10.1155/2011/912935. Epub 2010 Sep 8. PMID: 20953397; PMCID: PMC2952333.
48. Wang JM, Ji LL, Branford-White CJ, Wang ZY, Shen KK, Liu H, Wang ZT. Antitumor activity of Dioscorea bulbifera L. rhizome in vivo. *Fitoterapia.* 2012 Mar;83(2):388-94. doi: 10.1016/j.fitote.2011.12.001. Epub 2011 Dec 9. PMID: 22178682.
49. Kuete V, Betrandteponno R, Mbaveng AT, Tapondjou LA, Meyer JJ, Barboni L, Lall N. Antibacterial activities of the extracts, fractions and compounds from Dioscorea bulbifera. *BMC Complement Altern Med*. 2012 Nov 23;12:228. doi: 10.1186/1472-6882-12-228. PMID: 23176193; PMCID: PMC3528471.
50. Maithili V, Dhanabal SP, Mahendran S, Vadivelan R. Antidiabetic activity of ethanolic extract of tubers of Dioscorea alata in alloxan induced diabetic rats. I*ndian J Pharmacol*. 2011 Jul;43(4):455-9. doi: 10.4103/0253-7613.83121. PMID: 21845005; PMCID: PMC3153713.
51. Go HK, Rahman MM, Kim GB, Na CS, Song CH, Kim JS, Kim SJ, Kang HS. Antidiabetic Effects of Yam (Dioscorea batatas) and Its Active Constituent, Allantoin, in a Rat Model of Streptozotocin-Induced Diabetes. *Nutrients.* 2015 Oct 15;7(10):8532-44. doi: 10.3390/nu7105411. PMID: 26501316; PMCID: PMC4632431.
52. Zong A, Guan Q, Ma W, Liu L, Jia M, Du F, Xu T. Antidiabetic Effect of Chinese yam (Dioscorea opposita Thunb.) in High-Fat Diet/Streptozotocin-Induced Diabetic Rats. *Starch-Stärke*. 2023; 75(7-8):2200281. https://doi.org/10.1002/star.202200281
53. Adomėnienė A, Venskutonis PR. Dioscorea spp.: Comprehensive Review of Antioxidant Properties and Their Relation to Phytochemicals and Health Benefits. *Molecules*. 2022 Apr 17;27(8):2530. doi: 10.3390/molecules27082530. PMID: 35458730; PMCID: PMC9026138.
54. Ghosh S, Derle A, Ahire M, More P, Jagtap S, Phadatare SD, et al. Phytochemical analysis and free radical scavenging activity of medicinal plants Gnidia glauca and Dioscorea bulbifera. *PLoS One.* 2013 Dec 18;8(12):e82529. doi: 10.1371/journal.pone.0082529. PMID: 24367520; PMCID: PMC3867356.
55. Liu Y, Li H, Fan Y, Man S, Liu Z, Gao W, Wang T. Antioxidant and Antitumor Activities of the Extracts from Chinese Yam (Dioscorea opposite Thunb.) Flesh and Peel and the Effective Compounds. *J Food Sci.* 2016 Jun;81(6):H1553-64. doi: 10.1111/1750-3841.13322. Epub 2016 Apr 27. PMID: 27122252.
56. Adebayo SA, Ondua M, Shai LJ, Lebelo SL. Inhibition of nitric oxide production and free radical scavenging activities of four South African medicinal plants. *J Inflamm Res*. 2019 Aug 8;12:195-203. doi: 10.2147/JIR.S199377. PMID: 31496781; PMCID: PMC6691489.
57. Xiao S, Yuan Z, Huang Y. The Potential Role of Nitric Oxide as a Therapeutic Agent against SARS-CoV-2 Infection. *Int J Mol Sci*. 2023 Dec 5;24(24):17162. doi: 10.3390/ijms242417162. PMID: 38138990; PMCID: PMC10742813.
58. Flannagan RS, Heit B, Heinrichs DE. Antimicrobial Mechanisms of Macrophages and the Immune Evasion Strategies of Staphylococcus aureus. *Pathogens.* 2015 Nov 27;4(4):826-68. doi: 10.3390/pathogens4040826. PMID: 26633519; PMCID: PMC4693167.
59. Liu TZ, Chin N, Kiser MD, Bigler WN. Specific spectrophotometry of ascorbic acid in serum or plasma by use of ascorbate oxidase. *Clin Chem*. 1982 Nov;28(11):2225-8. PMID: 7127767.
60. Moon JK, Shibamoto T. Antioxidant assays for plant and food components. *J Agric Food Chem*. 2009 Mar 11;57(5):1655-66. doi: 10.1021/jf803537k. PMID: 19182948.
61. Zhao C, Zhou B, Miao J, Li X, Jing S, Zhang D, Yijia Wang J, Li X, Huang L, Gao W. Multicomponent analysis and activities for evaluation of Dioscorea oppositifolia and Dioscorea hamiltonii. *Food Agric. Immunol.* 2019 ;30(1):1148-61.
62. Adedayo BC, Ademiluyi AO, Oboh G, Akindahunsi AA. Interaction of aqueous extracts of two varieties of Yam tubers (Dioscorea spp.) on some key enzymes linked to type 2 Diabetes in vitro. *Int J Food Sci Technol.* 2012; 47: 703–709.
63. Gulcin İ, Alwasel SH. Metal ions, metal chelators and metal chelating assay as antioxidant method. *Processes*. 2022; 10(1):132.
64. Nikolova M. Screening of radical scavenging activity and polyphenol content of Bulgarian plant species. *Pharmacognosy Res.* 2011 Oct;3(4):256-9. doi: 10.4103/0974-8490.89746. PMID: 22224049; PMCID: PMC3249785.