Annals of Science and Technology - A, Vol 6 (1): 26-33, 2021

ISSN: 2 544 6320

sciendo

ARTICLE

Inhibition of Haemoglobin Glycosylation, Glucose Uptake and *In Vitro* Antidiabetic Activities of Solvent Fractions from *Daucus Carota* Seed

Habibu Tijjani* and Sadiq Adamu Imam

Department of Biochemistry, Bauchi State University, Gadau 751105, Nigeria

Received 20th February, 2021, Accepted 2nd May, 2021
DOI: 10.2478/ast-2021-0004

**Corresponding author*
Habibu Tijjani E-mail: haatscific@gmail.com
Tel: +234-8037327138

Abstract

Diabetes mellitus (DM) is a metabolic disorder which result from either absolute or relative insulin deficiency and may lead to dysfunction in some organ systems. Pancreatic α- glucosidase and α- amylase inhibition are an effective strategy to decrease levels of postprandial hyperglycemia through starch control breakdown. The aim of the study is to assess the *in vitro* antidiabetic activities of fractions from *Daucus carota* seed extract, a plant used traditionally for the treatment of diabetes mellitus. The aqueous extract (AQE) of *Daucus carota* seed was partitioned in ethyl acetate (EAE), n – hexane (HEX) and diethyl ether (DEE) to obtain the various fractions. The AQE and EAE expressed significant α – amylase inhibitory activity with IC_{50} values of 637.0±18.6 µg/mL and 603.0±25.8 µg/mL respectively. AQE, EAE, HEX and DEE expressed α – glucosidase inhibitory activity with IC_{50} value of 135.85±1.21, 147.59±0.57, 132.64±1.17, and 143.56±0.49 µg/mL respective compared with acarbose (ACA) with 5.42±0.20 µg/mL. Furthermore, DEE fraction expressed inhibitory effects on % glucose uptake in yeast cell comparable with metronidazole. All *Daucus carota* fractions expressed various inhibitory effects on haemoglobin glycosylation at a concentration of 200 – 1000 µg/mL. The results show that fractions from the aqueous seed extract of *Daucus carota* possess *in vitro* antidiabetic potentials with EAE and HEX fractions having most promising inhibitory activities against α-amylase and α-glucosidase respectively.

Keywords: *Daucus carota;* seed; *in vitro;* antidiabetic activities; glucose uptake; haemoglobin glycosylation

1.0 Introduction

Diabetes mellitus (DM) is a metabolic disorder with disturbances in carbohydrate, fat and protein metabolism characterized by a chronic hyperglycemic conditions. DM may result from either absolute or relative insulin deficiency and may lead to dysfunction in some organ systems (WHO, 2009). DM has shown a tremendous increase in occurrence through their transition from epidemiology studies in a recent study (Uloko *et al*., 2018). DM has also pose real challenges to the health sector through finances and budgeting (Uloko *et al*., 2018). Recent prevalence figures published by International Diabetes Federation (IDF) indicated about 463 million adults are living with DM worldwide, with 1 in 2 (about 232 million) living with diabetes undiagnosed and about 4.2 million deaths (International Diabetes Federation, 2019). Due to the developing economy of Africa and Asia, their economy contributes a significant fraction of the observed prevalence figures. More so, there are concerns arising from the complications of DM alongside the observed increased prevalence of the disease (Uloko *et al.,* 2008). Effective management and treatment of DM are therefore of immense importance and area of research.

Daucus carota L. (Carrot) is an important crop from the family *Apiaceae* with worldwide distribution. Carotenoids and anthocyanins are among the major antioxidant pigments found in carrots (Olatunde *et al*., 2020). Traditionally, *D. carota* seed are used to treat a spectrum of diseases, including DM which is associated with oxidative stress and hyperlipidaemia (Khaki, 2011; Pouraboli and Ranjbar, 2015, Tijjani *et al*., 2020a; 2020b). Furthermore, the seeds have analgesic (Vasudevan *et al*., 2010), anti-inflammatory (Vasudevan *et al*., 2010), hepatoprotective (Singh *et al*., 2012), *in vitro* and *in vivo* antioxidant activities (Tijjani *et al*., 2020a; 2020b; 2020c). Medicinal plant is moving from a simple to diverse used with a large number of researches reporting the benefits of whole plants and specific components in them for medicinal usage. Their components haven't reported side effects, which have been earlier, reported by synthetic drugs, making traditional plant based treatments preferred choice for treatment of diseases (Tijjani *et al.,* 2017). The present studies evaluated the *in vitro* antidiabetic activities of the various fractions of *Daucus carota* seed.

2.0 Experimental

Plant Material

Daucu scarota L. seeds were purchased from AlheriManoma, Musty Agroallied Nigeria, LTD and were identified by Mr Azila Joseph, a curator with the Federal College of Forestry, Jos, Plateau state and a voucher specimen with the number FHJ 288 was deposited.

Chemicals and Reagents

Sucrase, α – amylase, α – glucosidase and 3, 5-dinitrosalicylic acid (DNSA) were purchased from Sigma-Aldrich, Germany. Glucose kit was purchased from Randox Laboratory. All other reagents used were of analytical grade.

Preparation of Aqueous Carrot Seed Extract

Daucus carota L. seed was pulverized into powder and 190 g of the powder was dissolve in 1900 mL of distilled water for 24 hours. The aqueous extract (AQE) was filtered using Whatman filter paper (No 1). The filtrate was concentrated at 40^{o}C using a rotary evaporator.

Partitioning of Crude Aqueous Extract

The aqueous extract was partitioned in water (100 mL) and ethyl acetate (EAE, 200 mL) in a separation funnel. The various partitioned fractions (EAE and Aqueous EAE) was concentrated at 40^{o}C using a rotary evaporator to obtain the ethyl acetate (EAE) and aqueous extract. The procedure was repeated for n – hexane (HEX) and diethyl ether (DEE) by partitioning the aqueous fractions obtained from each step of extraction.

α-amylase Inhibition Assay

A 500 µL of AQE, EAE, HEX, DEE and acarbose (ACA) at 100 – 1000 µg/mL was added to 500 µL of phosphate buffer (0.02 M, pH 6.9) containing sodium chloride (6 mM) and α-amylase (0.5 mg/mL; 0.04 units Porcine Pancreatic α-amylase (PPA)) solution and incubated for 10 min at 37°C. After which, 500 µL of starch solution (0.5%) in sodium phosphate buffer (0.02 M, pH 6.9) was added to each tube and incubated for 10 min at 37^{o}C. The reaction was stopped by adding 1 mL of 3, 5-dinitrosalicylic acid (DNSA) colour reagent. The reaction mixture was incubated in a boiling water bath for 5 min, cold to room temperature and then diluted with 10 mL distilled water. The absorbance was measured at 540nm. Control represent 100% enzyme activity and was conducted by replacing fraction and standard with vehicle (Kuppusamy *et al*., 2011).

α-glucosidase Inhibition Assay

α-glucosidase inhibitory assay was determined by incubating 1 mL solution of starch substrate (2 % w/v) with 1 mL Tris buffer (0.2 M, pH 8.0) and various concentrations of AQE, EAE, HEX, DEE and acarbose (ACA) at 37°C for 5 min. The reaction was initiated by adding 1 mL of α-glucosidase enzyme (1 U/mL) to it followed by incubation at 37°C for 10 min. The reaction was stop by heating the reaction mixture for 2 min in boiling water bath. The amount of glucose liberated was measured using glucose oxidase peroxide method (Andrade-Cetto *et al*., 2008; Kuppusamy *et al*., 2011).

Determination of Glucose Uptake in Yeast Cells

Commercial baker's yeast was dissolve in distilled water and subjected to centrifuge repeated at 3000g for 5 min, until a clear supernatant fluids is obtained. From the suspension mixture a 10% (v/v) was prepared in distilled water. AQE, EAE, HEX, DEE and metronidazole (200-1000 µg/mL) were pipetted to 1 mL of glucose solution (5, 10, 25 mM) and incubated for 10 min at 37^{o}C. Reaction was initiated by adding 100 µL of the prepared 10% yeast suspension followed by vortexing and incubation at 37^{o}C for 60 min. The preparation were centrifuged at 2500g for 5 min and amount of glucose estimated in the supernatant using glucose oxidase method (Cirillo, 1962). Metronidazole was used as reference drug.

Inhibition of Haemoglobin Glycosylation

To 1 mL of haemoglobin solution, 25µL of gentamycin and 25µL of AQE, EAE, HEX, DEE and gentamycin was pipetted into a test tube. The reaction was initiated by the addition of 1 mL of 2% glucose in phosphate buffer (0.01M, pH 7.4) and incubate at room temperature in the dark. The concentrations of glycated haemoglobin at the incubation period of 0, 24 and 72 hours was estimated spectrophotometrically at 443nm (Adisa *et al.,* 2004).

Data Analysis

Data are presented as mean ± standard error of mean (SEM) following Duncan multiple range test using SPSS version 20, SPSS Inc., Chicago. IL, USA. The significant values were determined at $p<0.05$.

3.0 Results and Discussion

The fractions obtained express significant *in vitro* antidiabetic properties. AQE and EAE expressed α – amylase activities with IC_{50} values of 637.0±18.6µg/mL and 603.0±25.8µg/mL (Table 1). However, HEX, and DEE expressed α – amylase inhibitory activities greater than 1000µg/mL compared with acarbose with IC_{50} values of 7.2±0.8µg/mL. The IC_{50} values for α – glucosidase of the various fraction ranges between 132 – 148µg/mL with HEX having the least IC_{50} value 132.64±1.17µg/mL (Table 2). The fractions expressed different inhibitory effects on glucose uptake by yeast. At the concentration rang of 200 – 1000µg/mL, AQE, EAE, HEX and DEE expressed 18.96±6.68%, 38.93±16.20%, 36.16±7.41%, and 21.59±2.43% inhibitory effects at glucose concentration of 20mg/mL (Table 3) compared with 18.89±3.39% for reference drug metronidazole. Incubation of the fractions for 0, 24 and 72 hours inhibited the formation of glycated haemoglobin at 2, 10 and 20mg/mL concentration of haemoglobin (Tables 4 - 6).

Table 1: *In vitro* α-amylase inhibitory activities of *Daucus carota* seed extracts

α-amylase activities									
ACA		**AQE**		**EAE**		**HEX**		**DEE**	
Conc. (µg/mL)	%	Conc. (µg/mL)	%	Conc. (µg/mL)	%	Conc. (µg/mL)	%	Conc. (µg/mL)	%
2	18.53	200	36.25	200	31.45	200	12.18	200	12.26
4	29.06	400	36.93	400	32.20	400	21.75	400	13.84
6	38.46	600	41.74	600	37.20	600	25.53	600	20.96
8	60.82	800	42.96	800	42.52	800	29.73	800	22.57
10	68.66	1000	44.41	1000	45.18	1000	30.68	1000	24.00
*IC_{50} = 7.2±0.8[a]		*IC_{50} = 637.0±18.6[b]		*IC_{50} = 603.0±25.8[b]		*IC_{50} = >1000[c]		*IC_{50} = >1000[c]	

ACA = Acarbose, AQE = Aqueous extract, EAE = Ethyl acetate extract, HEX = n-hexane extract, DEE = Diethyl ether extract, IC_{50} = Half maximal inhibitory concentration
*Values are mean ± Standard Error of Mean (SEM, triplicated determination), Values with different superscripts are significantly different ($p<0.05$)

Table 2: *In vitro* α-glucosidase inhibitory activities of *Daucus carota* seed extracts

α-glucosidase activities									
ACA		**AQE**		**EAE**		**HEX**		**DEE**	
Conc. (µg/mL)	%	Conc. (µg/mL)	%	Conc. (µg/mL)	%	Conc. (µg/mL)	%	Conc. (µg/mL)	%
2	36.73	200	94.87	200	90.66	200	95.31	200	91.36
4	46.53	400	95.00	400	90.79	400	95.35	400	92.32
6	48.27	600	95.09	600	91.01	600	95.44	600	92.94
8	55.88	800	95.61	800	91.01	800	95.66	800	93.16
10	60.64	1000	96.36	1000	91.58	1000	95.83	1000	93.42
*IC_{50} = 5.42±0.20[a]		*IC_{50} = 135.85±1.21[b]		*IC_{50} = 147.59±0.57[c]		*IC_{50} = 132.64±1.17[d]		*IC_{50} = 143.56±0.49[e]	

ACA = Acarbose, AQE = Aqueous extract, EAE = Ethyl acetate extract, HEX = n-hexane extract, DEE = Diethyl ether extract, IC_{50} = Half maximal inhibitory concentration
*Values are mean ± Standard Error of Mean (SEM, triplicated determination), Values with different superscripts are significantly different ($p<0.05$)

Table 3: Glucose uptake by yeast

Glucose uptake (%)						
Glucose concentration	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**
Fractions (µg/mL)		AQE			EAE	
200	45.82±4.12[a]	46.76±7.09[a]	52.97±2.23[a]	44.33±2.50[a]	47.10±6.75[a]	12.55±3.64[a]
400	28.88±0.54[a]	27.67±8.77[a]	31.58±3.37[ab]	42.78±0.67[a]	41.23±11.54[a]	30.63±13.36[b]
600	55.20±13.23[a]	30.90±2.83[a]	24.70±8.77[b]	45.14±1.15[a]	45.75±3.64[a]	38.73±17.72[b]
800	46.63±16.40[a]	34.21±1.96[a]	10.12±7.42[b]	47.77±17.54[a]	44.33±2.50[a]	30.90±2.70[b]
1000	41.03±4.72[a]	31.04±17.68[a]	18.96±6.68[b]	46.56±13.09[a]	48.79±4.12[a]	38.93±16.20[b]
Fractions (µg/mL)		HEX			DEE	
200	56.00±5.39[a]	41.83±5.58[a]	28.20±5.28[a]	47.36±8.42[a]	46.28±8.99[a]	19.04±8.35[a]
400	14.70±9.85[b]	28.47±5.34[a]	24.29±4.98[a]	28.20±5.28[b]	17.54±3.60[c]	36.43±7.47[a]
600	28.34±8.10[b]	57.75±9.84[b]	17.13±9.01[a]	17.27±3.54[c]	25.50±6.73[c]	61.53±8.62[b]
800	17.13±9.01[b]	33.60±3.39[a]	41.16±5.94[b]	18.21±4.75[c]	46.82±8.10[a]	33.87±3.44[a]
1000	33.46±8.86[b]	14.84±4.84[c]	36.16±7.41[a]	40.75±5.35[a]	19.44±6.94[c]	21.59±2.43[a]
Standard drug		Metronidazole				
1000 µg/mL	14.84±4.80[a]	28.21±5.13[b]	18.89±3.39[a]			

AQE = Aqueous extract, EAE = Ethyl acetate extract, HEX = n-hexane extract, DEE = Diethyl ether extract
Values are mean ± Standard Error of Mean (SEM, triplicated determination), Values with different superscripts are significantly different ($p<0.05$)

Table 4: Inhibition of haemoglobin glycosylation at zero hour of incubation

Inhibition of haemoglobin glycosylation (%, 0 hour)						
Glucose concentration	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**
Fractions (µg/mL)	AQE			EAE		
200	28.27±1.07[a]	36.94±4.18[a]	36.94±12.31[a]	34.48±1.28[a]	12.42±6.21[a]	24.73±18.31[a]
400	22.38±10.60[a]	23.45±2.68[b]	24.20±3.64[a]	34.48±7.28[a]	37.90±3.43[b]	30.30±11.67[a]
600	33.08±2.46[a]	31.37±2.68[a]	10.92±1.21[b]	32.76±3.85[a]	29.44±3.75[a]	11.03±4.18[b]
800	23.66±11.24[a]	25.70±2.78[b]	20.66±7.60[a]	38.54±4.71[a]	18.52±2.03[a]	28.91±2.36[a]
1000	26.23±18.31[a]	17.02±12.31[b]	26.23±4.39[a]	24.20±11.56[a]	22.81±3.10[a]	15.20±7.71[b]
Fractions (µg/mL)	HEX			DEE		
200	47.86±4.54[a]	49.79±3.32[a]	48.93±1.18[a]	28.69±14.99[a]	9.64±1.93[a]	20.24±4.82[a]
400	47.43±5.03[a]	25.80±15.10[a]	27.30±17.67[a]	31.37±15.52[a]	31.66±20.80[b]	7.28±0.43[b]
600	33.62±1.71[b]	43.15±1.18[a]	37.90±16.70[a]	20.56±6.42[a]	16.57±2.86[c]	13.67±0.89[c]
800	51.93±4.75[a]	44.11±3.00[a]	37.26±3.43[a]	10.81±6.96[b]	22.06±11.78[b]	18.60±1.53[c]
1000	37.15±2.46[b]	32.12±7.92[b]	26.12±9.42[a]	10.17±3.53[b]	26.87±2.25[b]	21.09±2.89[a]
Standard drug	Gentamycin					
30 µg/mL	6.42±1.83[a]	6.20±1.95[a]	6.63±1.16[a]			

AQE = Aqueous extract, EAE = Ethyl acetate extract, HEX = n-hexane extract, DEE = Diethyl ether extract
Values are mean ± Standard Error of Mean (SEM, triplicated determination), Values with different superscripts are significantly different ($p<0.05$)

Table 5: Inhibition of haemoglobin glycosylation at twenty-four hours of incubation

	Inhibition of haemoglobin glycosylation (%, 24 hours)					
Glucose concentration Fractions (µg/mL)	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**
		AQE			EAE	
200	12.39±7.87[a]	12.82±1.19[a]	16.06±1.11[a]	22.74±3.56[a]	12.07±3.66[a]	17.89±1.22[a]
400	17.35±5.50[a]	26.29±4.96[b]	10.67±2.05[a]	7.00±4.20[b]	15.84±4.6[a]	9.81±7.22[a]
600	23.28±8.41[a]	10.78±8.84[a]	25.65±2.37[a]	14.22±1.72[c]	18.64±3.99[a]	21.34±4.09[b]
800	1.08±0.43[b]	3.13±2.48[c]	19.07±10.45[a]	13.15±7.97[c]	32.05±4.32[b]	16.81±9.05[a]
1000	2.05±1.62[b]	15.84±13.25[ab]	10.88±1.54[a]	15.84±10.88[a]	15.09±7.97[a]	5.82±5.17[c]
Fractions (µg/mL)	HEX			DEE		
200	15.59±3.38[a]	11.42±0.43[a]	20.91±12.50[a]	28.88±15.73[a]	10.34±1.94[a]	20.37±4.20[a]
400	19.40±0.22[a]	26.72±13.15[a]	32.44±5.19[a]	31.57±16.27[a]	28.34±3.81[b]	6.68±0.43[b]
600	17.24±6.90[a]	13.58±8.84[a]	6.36±1.19[b]	15.41±1.83[b]	8.62±5.82[c]	8.73±5.28[b]
800	10.02±3.34[a]	5.39±1.29[b]	12.72±2.80[c]	10.24±7.00[b]	21.55±11.85[a]	14.33±6.57[a]
1000	18.21±11.31[a]	14.66±11.42[a]	10.78±1.94[c]	9.59±3.56[c]	27.69±2.26[b]	21.23±2.26[a]
Standard drug	Gentamycin					
30 µg/mL	23.92±2.41[a]	14.95±6.97[a]	13.14±6.55[a]			

AQE = Aqueous extract, EAE = Ethyl acetate extract, HEX = n-hexane extract, DEE = Diethyl ether extract
Values are mean ± Standard Error of Mean (SEM, triplicated determination), Values with different superscripts are significantly different ($p<0.05$)

Table 6: Inhibition of haemoglobin glycosylation at seventy-two hours of incubation

	Inhibition of haemoglobin glycosylation (%, 72 hours)					
Glucose concentration Fractions (µg/mL)	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**	**2 mg/mL**	**10 mg/mL**	**20 mg/mL**
	AQE			EAE		
200	15.06±2.13[a]	18.32±4.04[a]	19.57±8.78[a]	42.91±7.94[a]	13.66±2.73[a]	18.24±4.10[a]
400	12.74±5.71[a]	11.48±3.46[a]	12.30±4.39[a]	22.96±5.90[b]	9.52±2.49[b]	17.75±7.09[a]
600	17.19±2.92[a]	7.59±2.33[b]	15.37±6.59[a]	9.41±7.65[c]	15.43±6.65[a]	16.04±3.16[a]
800	18.57±7.65[a]	33.75±6.40[c]	34.19±8.77[b]	7.38±4.50[c]	16.29±4.03[a]	14.81±6.90[a]
1000	39.21±2.57[b]	39.52±7.82[c]	28.04±7.51[a]	9.16±1.25[c]	13.68±7.90[a]	26.27±7.64[a]
Fractions (µg/mL)	HEX			DEE		
200	36.57±3.83[a]	6.46±1.69[a]	22.88±6.61[a]	35.26±12.08[a]	31.22±14.57[a]	27.42±2.69[a]
400	25.22±8.44[a]	43.48±7.67[b]	36.20±8.64[b]	18.32±6.27[b]	26.16±12.99[a]	13.80±2.13[b]
600	30.99±7.30[a]	17.86±1.93[a]	17.74±3.85[a]	18.32±3.92[b]	15.43±11.67[b]	3.26±0.63[c]
800	22.08±2.51[a]	5.27±1.75[a]	43.16±5.25[b]	22.33±3.05[b]	5.90±3.26[c]	11.52±1.49[b]
1000	23.88±6.98[a]	16.44±2.25[a]	28.11±8.71[b]	11.42±1.51[b]	45.80±6.02[a]	12.34±1.55[b]
Standard drug	Gentamycin					
30 µg/mL	12.04±1.75[a]	24.21±3.81[b]	21.70±2.64[b]			

AQE = Aqueous extract, EAE = Ethyl acetate extract, HEX = n-hexane extract, DEE = Diethyl ether extract
Values are mean ± Standard Error of Mean (SEM, triplicated determination), Values with different superscripts are significantly different ($p<0.05$)

4.0. Discussion

Diabetes mellitus (DM) are among the common disorders of the endocrine system resulting in metabolic disorders and which further leads to multiple organ damage syndrome. The inhibition of key enzymes such as α-amylase and α-glucosidase which are involved in the digestion of carbohydrates can significantly lower observed postprandial increases of blood glucose after a mixed diet. The inhibition of these key enzymes are important strategy in the management of the level of postprandial blood glucose in type 2 diabetic patients and among borderline patients (Ali *et al.,* 2006). α-amylase inhibitors including voglibose, miglitol and acarbose (a reference drug use in the present study) are among clinical drugs use as treatment, but their prices are high and clinical side effects are reported (Khoo, 2017). More so, intestinal α-glucosidases are key

enzyme for carbohydrate digestion that are located at the epithelium of the small intestine. α- glucosidase are key enzymes recognized as a therapeutic target for the modulation of postprandial hyperglycemia, the initial metabolic abnormality observed in Type 2 DM (Scott *et al.,* 2000).

The *in vitro* α-amylase and α-glucosidase inhibitory studies demonstrated that *Daucus carota* extract has both α- glucosidase and α-amylase inhibitory activities. The percentage inhibition at 200, 400, 600, 800 and 1000µg/mL concentrations of plant extract showed a concentration dependent percentage inhibition (Table 1 and Table 2). α - amylase inhibitors are also called starch blockers since they prevent or slow down the absorption of starch mainly by blocking the hydrolysis of 1, 4-glycosidic linkages of starch during digestion and other oligosaccharides into maltriose, maltose and other simple sugars (Wadkar *et al.,* 2008). In the study, the ethyl acetate (EAE) and n-hexane (HEX) extract expressed higher α- amylase inhibitory and α-glucosidase inhibitory activities. In the study acarbose was used as the positive control, it inhibited the α-amylase activity and α- glucosidase inhibitory activities better when compared with the extracts. The results obtained from the study indicated that the ethyl acetate and n-hexane fractions are also potent α-amylase and α- glucosidase inhibitions.

Glycosylated haemoglobin (glycohaemoglobins) are formed when a ketoamine reaction occurs between the N-terminal amino acid of the β chain of haemoglobin and glucose. Glycohaemoglobins level is important indicators for long-term blood glucose control (McCowen and Smith, 2005). The results suggest that the plant extract inhibits the binding of glucose to haemoglobin with evidence from the glycated haemoglobin assay (Table 4 – 6). Advance glycation end product (AGEs) is a known source of free radicals in diabetes, and their accumulation aggravates the state of an increased oxidative stress in DM (Adisa *et al.,* 2004). Thus, hindering the formation of AGEs implies decreased levels of free radicals in diabetes, and could reduce further diabetic complication. In management of diabetic mellitus, reducing properties of monosaccharides are important. More so, direct glycosylation reactions of monosaccharide lead to enolize, reduce molecular oxygen and yielding hydrogen peroxide, α-ketoaldehydes and free radical intermediates (Wolff and Dean, 1987). The occurrence of this process i*n vivo*, contributes significantly to the elevated plasma peroxides levels in diabetics complication and may contribute to protein modification reactions perform with glucose *in vitro*.

The mechanism of glucose transport across the cell membrane of yeast has received attention, and are utilized as *in vitro* screening methods for various compounds/medicinal plants to access their hypoglycaemic potentials (Maier *et al.,* 2002). The results of the present study revealed that *Daucus carota* extract decrease glucose uptake in yeast cells at various glucose concentrations of 5, 10 and 20 mg/mL. This was measured by the amount of glucose remaining in the medium after a specific time which serve as an indicator of glucose uptake by the yeast cells (Ahmed and Urooj, 2010). Recent studies by Teusink *et al.* (1998) on the means of transport of non-metabolizable sugars and glycosides across yeast cell membrane indicates that the sugars are transported across membranes through stereospecific membrane carriers. *Saccharomyces cerevisiae* glucose transport are extremely complex and generally, it is suggested that glucose are transported across yeast cells by a facilitated diffusion process (Teusink *et al.,* 1998). Facilitated carriers process are specific types of process that transport solutes (glucose) down concentration gradient. The effective transport is only attained when there is effective removal of intracellular glucose (Teusink *et al.,* 1998). Thus, glucose transport will occur only if the intracellular glucose is effectively reduced by the means of their utilization for metabolic processes. The present study, suggests that *Daucus carota* seed extract is capable of inhibiting glucose uptake, which in turn suggests that it can regulate glucose uptake through the control of blood glucose levels as also suggested by Ahmed *et al.* (2009).

Medicinal plants and their products usually contain phytochemicals which includes phenolic, flavonoids, terpenoids, coumarins and some other constituents, which help in controlling blood glucose levels. Thereby, the serve as alternative medicines to treat diabetes due to the presences of relevance chemical composition. AQE and DEE of *Daucus carota* contains flavonoids, alkaloids, saponins, steroids, tannins and phenols (Tijjani *et al*., 2019). The maintenance of plasma glucose levels for an extended period of time under a variety of dietary conditions is an important and closely regulated processes (Nair *et al.,* 2013). Presence of fibers in diet, along with some phytochemicals are important in combating diabetes disorders, through several proposed mechanisms which could include enhancing insulin releasing activity, manipulation of glucose transporters, inhibition of carbohydrate metabolizing enzymes and β-cell regeneration (Jenkins *et al*. 1978; Lopez *et al*. 1996; Sairam and Urooj, 2013).

Conclusion

The results show that fractions from aqueous seed extract of *Daucus carota* possess *in vitro* andiabetic potentials with ethyl acetate (EAE) and n-hexane (HEX) fractions having most promising inhibitory activities against α-amylase and α-glucosidase respectively. The fractions decreased % glucose uptake by yeast, however, not in the same manner with metronidazole and prevented haemoglobin glycosylation which were however, lower in most cases when compared with gentamicin. Further studies may ascertain the observed preventive haemoglobin glycosylation activities *in vivo*.

Declaration of Conflict of Interests

The authors declare no conflict of interests.

Authors' Contributions

Conception: [HT]
Design: [HT]
Execution: [SAI]
Interpretation: [HT]
Writing the paper: [HT, SAI]

References

Adisa, R. A, Oke, J, Olomu, S. A., Olorunsogo, O., 2004, Inhibition of human haemoglobin glycosylation by flavonoid containing leaf extract of *Cnestis ferruginea*. Journal of the Cameroon Academy of Sciences, **4**:

351-359. https://africaneditors.org/journal/JCAS/abstract/37623-79307

Ahmed, F., Sairam, S., Urooj, A., 2009, Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an in vitro study. J Pharm Res., **2**:563-8.

Ahmed, F., Urooj, A., 2010, *In vitro* studies on the hypoglycemic potential of *Ficus racemosa* stem bark. J Sci Food Agric., **90**:397-401. DOI: 10.1002/jsfa.3828

Ali, H., Houghton, P.J., Soumyanath, A., 2006, α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. J Ethnopharmacol, **107**: 449–455. DOI: 10.1016/j.jep.2006.04.004

Andrade-Cetto, A., Becerra-Jimennez, J., Cardenes, R., 2008. Alpha glucosidase inhibiting activity of some Mexican plants used in treatment of type 2 diabetes mellitus. Journal of ethnopharmacology, **116**: 27-32. DOI: 10.1016/j.jep.2007.10.031

Cirillo, V.P., 1962, Mechanism of glucose transport across the yeast cell membrane. J Bacteriol, **84**: 485-91. doi:10.1128/JB.84.3.

International Diabetes Federation, (IDF) Diabetes atlas. 8th ed. Brussels: International Diabetes Federation; 2019.

Jenkins, D.J.A., Wolever, T.M.S., Leeds, A.R., Gassul, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., Alberti, K.G.M.M., 1978, Dietary fibers, fiber analogues and glucose tolerance: importance of viscosity. Brit Med J, **1**:1392–1394. doi: 10.1136/bmj.1.6124.1392

Khaki, A., 2011, Effect of Ethanolic Extract of Carrot seed on Blood Glucose level and Liver Tissue in Streptozotocin-Induced Diabetic Rat. Medical Journal of Tabriz University of Medical Science, **33**:3.

Khoo, C. M., 2017, Diabetes Mellitus Treatment, Editor(s): Stella R. Quah, International Encyclopedia of Public Health (Second Edition), Academic Press, 288-293.

Kuppusamy, A., Muthusamy, U., Swashanmugum, T., Subhadradevi, V., Kalyanasubramaniam, R., Sambathkumar, R., 2011, In vitro (α-Glucosidase and α-Amylase) inhibition and *in vivo* antidiabetic property of Phytic acid (ip_6) in Streptozotocin-Nicotinamide-induced type 2 Diabetes mellitus (NIDDM) in rats. Journal of complementary and integrative medicine, **8**: 1553-3840. dOI: 10.2202/1553-3840.1483

Lopez, G., Ros, G., Rincon, F., Periago, M.J., Martinez, M.C., Ortuno, J., 1996, Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke. J Agric Food Chem, **44**:2773–2778. https://doi.org/10.1021/jf9507699

Maier, A., Völker, B., Boles, E., Fuhrmann, G.F., 2002, Characterisation of glucose transport in Saccharomyces cerevisiae with plasma membrane vesicles (countertransport) and intact cells (initial uptake) with single Hxt1, Hxt2, Hxt3, Hxt4, Hxt6, Hxt7 or Gal2 transporters. FEMS Yeast Res., **2**(4): 539-50. DOI: 10.1111/j.1567-1364.2002.tb00121.x

McCowen, K.C., Smith, R.J., 2005, Diabetes Mellitus Classification and Chemical Pathology, Editor(s): Benjamin Caballero, Encyclopedia of Human Nutrition (Second Edition), Elsevier, 543-551.

Nair, S. S., Kavrekar, V., Mishra, A., 2013, Evaluation of in vitro antidiabetic activity of selected plant extracts. Int J Pharm Pharm Sci Invent., **2**:12-9.

Olatunde, A., Tijjani, H., Ishola, A.A., Egbuna, C., Hassan, S., Akram, M., 2020, Carotenoids as Functional Bioactive Compounds. In: Egbuna C., DableTupas G. (eds) Functional Foods and Nutraceuticals. Springer, Cham. Pp 415-444. https://doi.org/10.1007/978-3-030-42319-3_20

Pouraboli, I., Ranjbar, B., 2015, The effect of *Daucus carota* seeds extract on lipid profile, LFT and kidney function indicators in streptozocin-induced diabetic rats. *Int J Plant Sci Ecol*. **3**:84-7.

Sairam, S., Urooj, A.. 2013, Artocarpusaltilis-mode of anti hyperglycemic activity: elucidation by suitable in-vitro and exvivo techniques. Int J Pharm Sci Res., **4**:3013.
DOI: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(8).3013-19

Scott, L.J., Spencer, C.M., 2000, Miglitol: a review of its therapeutic potential in type 2 diabetes mellitus. Drugs, **59**: 521-549. DOI: 10.2165/00003495-200059030-00012

Singh, K., Singh, N., Chandy, A., Manigauha, A., 2012, *In vivo* antioxidant and hepatoprotective activity of methanolic extracts of *Daucus carota* seeds in experimental animals. Asian Pac J Trop Biomed., **2**:385-8. DOI: 10.1016/S2221-1691(12)60061-6

Teusink, B., Diderich, J. A., Westerhoff, H. V., van Dam, K., Walsh, M. C., 1998, Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. Journal of bacteriology, **180**(3): 556–562.
DOI: 10.1128/JB.180.3.556-562.1998

Tijjani, H., Adegunloye, A.P., Uba, H., Joel, E.B., Olatunde, A., 2020b, Antioxidant activities of aqueous and ethyl acetate fractions of *Daucus carota* L. seed against triton X-100 induced oxidative stress in mice. *Scientific African*. **8**, e00429.
https://doi.org/10.1016/j.sciaf.2020.e00429

Tijjani, H., Mohammed, A., Adegunloye, A.P., 2019, Phytochemicals and *in vitro* antioxidant studies of *Daucus carota* L. seed extracts, 5th International Electronic Conference on Medicinal Chemistry, 1-30. https://sciforum.net/paper/view/6278. DOI:10.3390/ECMC2019-06278

Tijjani, H., Mohammed, A., Ahmed, F., Yahaya, H., Zakka, N., 2020c, *In vitro* antioxidant activity-guided fractionation of *Daucus carota* L. seed extract. Proceedings of the Nigerian Academy of Science, **13**(2): 74 – 85. https://nasjournal.org.ng/index.php/pnas/article/view/279

Tijjani, H., Mohammed, A., Idris, Z.L., Adegunloye, P.A., Luka, C.D. and Alhassan, A.J., 2017, Current status of antidiabetic plants in the *Lamiaceae*family, OmniScriptin GmbH & Co. KG Publisher, Germany, ISBN 198-3-330-86902-8, Pp 1-64.

Tijjani, H., Mohammed, A., Muktar, S., Musa, S., Abubakar, Y., Adegunloye, A. P., Ishola, A. A., Joel, E. B., Luka, C. D., Alhassan, A. J., 2020a, Antioxidant and antihyperlipidemic effects of aqueous seed

extract of *Daucus carota* L. in triton ×100-induced hyperlipidemic mice. *J App Biol Biotech*., **8**(1):76-83. DOI: 10.7324/JABB.2020.80113

Uloko, A. E., Musa, B. M., Ramalan, M. A., Gezawa, I. D., Puepet, F. H., Uloko, A. T., Borodo, M. M., Sada, K. B., 2018, Prevalence and Risk Factors for Diabetes Mellitus in Nigeria: A Systematic Review and Meta-Analysis. Diabetes therapy: research, treatment and education of diabetes and related disorders, **9**(3):1307–1316. doi: 10.1007/s13300-018-0441-1

Uloko, A.E., Ofoegbu, E.N., Chinenye, S., Fasanmade, O.A., Fasanmade, A.A., Ogbera, A.O., Ogbu, O.O., Oli, J.M., Girei, B.A., Adamu, A., 2008, Profile of Nigerians with diabetes mellitus - Diabcare Nigeria study group (2008): Results of a multicenter study. Indian J Endocrinol Metab., **16**(4): 558-64. doi: 10.4103/2230-8210.98011

Mani, V., Parle, M., Ramasamy, K., Abdul Majeed, A.B., 2010, Anti-dementia potential of *Daucus carota* seed extracts in rats. Pharmacologyonline, **1**:552-65.

Wadkar, K.A., Magdum, C.S., Patil, S.S., Naikwade, N.S., 2008, Anti-diabetic potential and indian medicinal plants. Journal of Herbal Medicine and Toxicology, **2**(1): 45-50.

WHO, 2009, World Health Organization; African Region, Nigeria: Emerging epidemics. http://www.whoint/countries/nga/areas/hiv/en/index.html Accessed date 04/12/2019.

Wolff, S.P., Dean, R.T., 1987, Glucose auto-oxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. Biochem, **245**: 243-250. doi: 10.1042/bj2450243

