International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.14-44, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

In vitro Glucose Uptake in Yeast Cells Facilitated by *Abelmoschus esculentus L.* (okra seed) for Management of Type 2 Diabetes

Morris, T. Aloysius[1], Andreou, Eleni[2], Kyriacos, Felekkis[3], C. Petrou[4], Egwim Chidi Evans[5]

1. Department of Life and Health Sciences, Nutrition and Dietetics, University of Nicosia, 46 Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus; aloysius.m@live.unic.ac.cy; terfamorris@yahoo.com
2. Programs coordinator Nutrition and Dietetics, Department of Life and Health Sciences, University of Nicosia, 46 Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus
3. Department of Life and Health Sciences, Human Biology, University of Nicosia, 46 Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus
4. Department of Life and Health Sciences, Pharmacy, University of Nicosia, 46 Makedonitissas Ave, CY-2417, P.O. Box 24005, CY-1700, Cyprus
5. Department of Biochemistry, Faculty of Sciences, Federal University of Technology, P.M.B. 65, Minna, Niger State, Nigeria;

doi: https://doi.org/10.37745/ijnmr.16/vol2n11444 Published November 17, 2024

Citation: Morris, T. A., Andreou, E., Kyriacos, F., Petrou C., Egwim C.E. (2024) In vitro Glucose Uptake in Yeast Cells Facilitated by Abelmoschus esculentus L. (okra seed) for Management of Type 2 Diabetes, *International Journal of Nutrition and Metabolism Research*, Vol.2, No.1, pp.14-44

Abstract: *In vitro glucose uptake in yeast cells facilitated by Abelmoschus esculentus L. (okra seed) for management of type 2 diabetes was studied. Plant material was collected, identified, processed, and stored for further use. 80% methanol was employed for extraction and sonicated to release anti-diabetic-bioactive component in solution and was filtered, concentrated, freeze-dried, and fractionated using standard techniques. Glucose uptake at an initial concentration of 5mM/L and 10mM/L by the crude extract was consistent to that of the known standard drug while at 25mM/L glucose concentration was equivalent with the crude extract. Also, at 0.625 mg/mL the linear equations, and R2 shows that the crude extract was high in dose predictability than the standard drug as presented by the equation; y = 35.754x - 57.822, and R^2 = 0.9502 (95%). The extract-fractions were employed to evaluate the ability of yeast cell line culture to take up glucose from the system through 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP), lipid peroxidation and anti-diabetes effect of extract-fraction assays. Extract-fractions were found to poses antioxidant activity high enough to inhibit stress-related diseases. The extract fractions were active both at low and high concentrations and were better compared with the standard drug and standard antioxidant was comparable. The high bioactive extract fractions require encapsulation with a*

International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.14-44, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

nanoparticle as a drug candidate for diabetics. Further studies will be necessary to monitor the in vivo performances of the extract fractions and subsequent trials.

Keywords: Glucose uptake; Yeast cell line; *Abelmoschus esculentus L.*; Extract fractions; Type 2 diabetes; drug candidate

INTRODUCTION

Abelmoschus esculentus L. (okra) may be a high-value crop because it characterizes a source of nutrients that are significant to human health, e.g., vitamins, potassium, calcium, carbohydrates, dietary fiber, and unsaturated fatty acids like linolenic and oleic acids, and similarly of bioactive chemicals (Moyin-Jesu, 2006; and Habtamu et al; 2014). Okra may be a versatile crop thanks to the various use of its leaves, buds, flowers, pods, stems, and seeds (Mihretu et al; 2014). Okra has long been a vegetable and a source of dietary drug (Maganda et al; 2009; Benchasr, 2012; Messing et al; 2012; and Roy et al; 2014). Indeed, apart from its nutritional role, it is appropriate for a few therapeutic and industrialized uses (Benchasr, 2012). The profile of the bioactive constituents in several parts of okra is well accepted: for okra pod polyphenolic mixtures, carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid, and amino acids (Roy et al; 2014; Jain et al; 2012; Gemede et al; 2016; Petropoulos et al; and 2018) for okra seed polyphenolic mixtures, mostly oligomeric catechins and flavonol byproducts, protein (i.e., high lysine levels), and oil segment (in specifically,)(Durazzo et al; 2017) its resultant oil is rich in palmitic, oleic, and linoleic acids) (Durazzo et al; Arapitsas, 2008; Adelakun et al; 2009; Adelakun et al; 2011; Jarret et al; 2011; Dong et al; 2014; Hu et al; 2014; Stryn et al; 2014; and Durazzo, 2017) for root carbohydrates and flavonol glycosides and primarily minerals, tannins, and flavonol glycosides for leaves (Wei et al; 2016; Idris et al; 2009; Calueta et al; 2014) called the occurrence in various proportions of the whole phenolics and total flavonoids and antioxidant properties in an exceedingly diverse part of plants, i.e., flower, fruit, leaf, and seed. Several okra constituents (flavonoids, polysaccharides, and vitamins) own significant biological activities (Liao et al; 2012). The valuation of relations of bioactive constituents during the quantifying of antioxidant properties (Liao et al; 2012) characterizes a first stage for understanding their biological actions and beneficial properties.

Figure 1.1: *Abelmoschus esculentus* (Okra)

Diabetes is a persistent disorder characterized by hyperglycemia, insufficient insulin secretion and disrupted metabolic pathways. It affects over 400 million individuals worldwide aged 18 years and above, particularly in low and middle – income countries, and is projected to be the 7th leading cause of death by 2030 (WHO, 2016). Hyperglycemia constitutes noninsulin-dependent hyperglycemia, which accounts for 90% of all cases, and is mainly caused by insulin resistance, partial insulin deficiency, and abnormal postprandial glucose elevation (WHO, 2016; Kwon et al; 2007; and Odebode et al; 2017).

The difficult nature of type 2 diabetes in developing countries is due to changes in nutrition and lifestyle from traditional meals, which are high in nutrients of food plant-based like grains, legumes and fruits, and vegetables to more Westernized sort of meals that are high in sugars, fat, and animal-source diets (Popkin, 2002; Kapoor and Anand, 2002; Sun et al; 2010; Eleazu and Okafor, 2012). These have also led to the high prevalence of prolonged and declining diseases. The content of food plants nutrients in the controlling of diabetes in a particular geographic area includes *Digitaria exilis* (acha), Treculia Africana (breadfruit), bean plant (beans), and (Undie and Akubue, 1986) and *Abelmoschus esculentus L*. (Okra seed) (Smit et al; 2013). However, because of the limitation of current therapy to manage all the physiology of abnormal characteristics of the disorder alternative strategies of employing a number of the phytonutrient content of plants mentioned are urgently needed (WHO, 2002).

Therapeutic plants generally from the earliest times were useful to discover bioactive compounds for the formulation of medication WHO, 2008). The challenges of cell resistance encountered, high budgeting, inaccessibility, and the increase in amount of toxin in human as a result of intake from the surrounding injurious substances continual or excess use of the conventional drugs, attention is now turning to natural bioactive compounds with improved therapeutic capabilities, modest, less injurious or un-harmful and quickly accessible to be used (Balogun et al; 2017; Akpovona et al; 2016, Mfotie et al; 2014). It is captivating to note that the research work from the World Health Organization, 2008 indicated that over 80% of the world, particularly those from developing countries, relies to a high extent on therapeutic plants for everyday vital health (Toiu et al; 2018). At the moment, about 20% of the currently existing medications contain high percentage of phytochemicals as a part of their bioactive constituents (WHO, 2008). The bulk of the human diseases emerging from the activities of microorganisms, infections, disordered conditions from metabolic difficulties, and illnesses related to oxidative stress use therapeutic plants (Umar et al; 2019; Onukogu et al; 2019; Madaki et al; 2016, and Lawal et al; 2015). The glucose toxicity theory recommends that constant exposure to modest increases in glucose over a

prolonged period seriously affect cells. Substantially, the effect of type 2 diabetes, hyperglycemia, is projected as a secondary reason behind continued cell decline.

Equally, there is attention growing in using natural products from plants as substitutes to current medications. Plant sources have become the most targets for getting new drugs to help manage diabetes.

Antioxidants are substances that are able to retard chemical reactions, no matter the concentrations; hence, antioxidants have various physical as well as chemical roles within the body. Moreover, antioxidants perform similar to the secondary substances accustomed to stopping thermal oxidation by responding with the reactive radicals and destroying them to quieten down activeness, less harmful, and long-sustained materials than those radicals. Antioxidants could also deactivate free radicals by accepting or donating electron(s) to get rid of the unpaired status of the novel (Krishnanmurthy, 2012).

The condition of stress where there are differences between production and accumulation within the cells and tissues of the body as well as detoxifying the product of the reaction because of absence in antioxidant or enlarged reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) formation, could create a possibility of destroying the cells (Aziz et al; 2016, and Law et al; 2017). The reactive oxygen species could be accustomed combined and including all extreme reactions of oxygen varieties, comprising molecular species able to exist alone which contains an unpaired electron in the atomic orbital. These reactive oxygen species are grouped into hydroxyl group (OH•), per hydroxyl group (HO_2•), hypochlorous acid (HOCl), Superoxide anion group ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), nitric oxide gas group (NO•), hypochlorite group (OCl•), peroxynitrite (ONOO), and several organic substances, and peroxides. On the other hand, reactive nitrogen species comes from the nitric oxide gas group as a result of reaction with oxygen ion to produce peroxynitrite ion however, reactive sulfur species is well-formed after the reaction with thiols and reactive oxygen species (Aziz et al; 2016, and Krishnamurthy; 2012).

Oxidative stress damage to proteins

The covalent modification of protein made both through direct reactions with reactive oxygen species or indirect reactions with secondary products produced after production of oxidative stress can result in the change in organic compounds like amino acid, the disintegration of the peptide chain, a combination of cross-linked reaction products, as well as improved electrical charges. In addition, covalent modification of protein made both through direct reactions with reactive oxygen

species or indirect reactions with secondary products produced after production of oxidative stress are more prone to the partial or complete breakdown of protein to peptides as well as amino acids by enzymes responsible for proteolysis reactions, as well as a rise in oxidized proteins could be the cause for the damage of certain functions as well as chemical and biological roles. The imbalances between production and accumulation of oxygen reactive species impairment to proteins could perform a function within the connection of cataracts and aging (Aziz et al; 2016 and Kumar, 2011).

Oxidative stress damage to lipids

Lipids have a vital fundamental and useful part in cell membranes. However, after necrobiosis, membrane lipids are liable to peroxidation and this method can originate a mix-up of some lipid peroxidation analyzes. Specifically, polyunsaturated fatty acids are prone to attacks for reactive oxygen species outbreaks. The essential reactive fragment of a molecule as well as a source of chemical species that react with a monomer for reactive oxygen species chain reaction and lipoperoxidation of polyunsaturated is hydroxyl (Kurutas, 2016). Thanks to lipid peroxidation, numerous mixtures are formed, like alkanes, malondialdehyde, as well as isoprostanes. Furthermore, these mixtures are employed as signs in lipid peroxidation analysis and are established in ailments comprising neurogenerative ailments, cardiopathy, and diabetes (Aziz et al; 2016 and Bagchi and puri, 1998).

Oxidative stress damage to DNA

Molecular oxygen is powerful as an oxidant and mediator that releases oxygen-imbalances between production and accumulation of oxygen reactive species, such as ionizing particle emission, promoting impairment in DNA those results in removal, alterations, as well as added serious inborn errors. Moreover, through this DNA impairment, sugar and base molecule, as well as a source of chemical species that react with a monomer, are prone to oxidation, resulting in base breaking down into its separate parts, breaking arising when only one strand of the DNA duplex is disconnected besides cross-link to proteins. In addition, atom impairment to DNA is linked to the connection of cancer and enhanced aging process (Aziz et al; 2016; Lü et al; 2012, Zadak et al; 2009).

Oxidative stress damage to carbohydrates

Pertaining to carbohydrates, the manufacture of oxygen-imbalances between production and accumulation of oxygen reactive species throughout initial extemporaneous non-enzymatic response of free-reducing sugars with free amino groups, DNA, as well as lipids can add to glycoxidative impairment. Therefore, through the first steps of related to a system not produced by enzyme reaction of carbohydrate bound to a hydroxyl of a different molecule so as to produce

a glycoconjugate, asexual splitting of an organism into fragments as a system of production of oligosaccharides like glycolaldehyde whose chain is too short to undergo reactions with a molecule to develop association to a different molecule to produce a closed ring and is therefore vulnerable to extemporaneous oxidation of a compound in the air, forming the superoxide of molecules with an unpaired electron which could bring about the production of β-dicarbonyls, established substances that bring about alterations in the DNA of a cell (Benov and Beema, 2003). Carbohydrates atom oxidation mechanisms are similar to those of lipids.

Monosaccharides, like glucose, mannitol, and deoxyribose, are established to connect hydrogen oxide to developed molecular substances produced from reactants, which do not interfere with nutrition value (Polumbryk et al; 2013).

The complaint of stress where there are modifications amid creating and buildups within the cells and, tissues of the body, and cleansing the product of the reaction due to lack of antioxidant or distended reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) formation, may generate a risk of terminating the lives of the cells (UK Prospective, 1998; Stratton et al; 2000, and CDC, 2011).

Glucose uptake in cells

A sodium ion combined dynamic transporter method and a family of fundamentally associated sodium ion-self-regulating glucose transporter glycoproteins. Moreover, this final, termed glucose transporter (GLUT) family enables the enhancement of glucose through the cytomembrane declining to its substance inclining each one into or out of the cells. They are particular for D-glucose and do not seem to have any energy-demanding constituents, like Adenosine Triphosphate breakdown of substances by water or a hydrogen ion slope. The enhanced glucose transporters are different from the sodium ion-reliant transporters, which vigorously store this carbohydrate. These several performed influences of hormone on adrenaline and cortisol functions are typically subordinate to glucose absorbing into a living organism, a stage in which almost every tissue (with the important exclusion of the cell of the central parenchyma tissue of the liver as well as the cell originate in pancreatic islets that produce and release insulin as well as amylin), is controlled by the degree of glucose transporter manifestation at the cell exterior. The presence of several glucose transporter isoforms through diverse motion material goods and well-ordered cell exterior manifestation gives the idea for the factors of a model must be adjusted very exactly in order to fit with assured interpretations of glucose integrating into a living organism, biochemical reactions in the body's cells that convert food into energy, as well as permitting regulation of all distinctiveness of an indicator in demand to reserve the resembling a cell as well as whole-body

conversion of food substance to energy reliability. Entire mammalian cells comprise of one or added associates of the glucose transporter family (Gould et al; 1993, and Medina and Owen, 2002). These proteins exhibit great amount of the material goods of a substance reaction through a distinct reactant producing imbalanced combination of stereoisomers throughout a non-stereospecific formation, giving for the two ways opposite direction transport of substrate, with the movement from region of higher concentration to that of lower concentration declining its concentration slope. The glucose transporters control the enhancement of glucose among outside the cell as well as inside the cell sections, thus sustaining a relentless source of this vital element. The glucose transporter protein family fits to membrane transporters' main facilitator super family (MFS). Glucose transporters are approximately five hundred amino acids and own twelve proteins in membrane typically nonpolar as well as hydrophobic; interface through polar molecule produce pores transport and single N-linked oligosaccharides. The glucose transporter associates of the family may be categorized into three diverse group as a result of their categorization resemblances (Joost et al; 2002). Fourteen glucose transporter proteins in humans comprise transporters for substrates separately from glucose, comprising fructose, myoinositol, as well as urate. The initial role as well as actions of living matter substrates for at least half of the fourteen glucose transporter proteins exists moreover as unreliable or unfamiliar. Furthermore, the category I: molecules glucose transporter one to four are utmost widely categorized and are recognized to possess separate controlling as well as motion material goods that reproduce their precise functions in concerning to a cell and whole-body glucose actively maintaining fairly stable conditions necessary for survival (Thorens and Muekler, 2010).

In summary, two groups of glucose transporters exist firstly in the brain: referred to as glucose transporter proteins (GLUTs) which transport glucose via a facilitative diffusion (a form of passive transport), as well as sodium-dependent glucose transporters (SGLTs) which utilizes energy-coupled mechanism (active transport). Therefore, Glucose cannot move through a cell membrane by simple diffusion due to its large size and is straightaway disallowed by the hydrophobic tails (water hating). As an alternative, it passes through facilitated diffusion that includes molecules permitting it through the membrane by going through a network of proteins. The present study was conceived with the main objective of utilizing okra seeds to achieve glucose uptake in yeast cells.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were of analytical grade and products of Sigma Aldrich. Chemicals include methanol, phosphate buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium hexacyanoferrate (III), ferric chloride, thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), ferrous sulfate, acetic acid (TCA), baker's yeast, ascorbic acid (vitamin C), and metronidazole (standard diabetes drug).

Plant materials: Collection and identification

Abelmoschus esculentus L. (Okra seed) was acquired from a commercial source in Benue and Nasarawa States, Nigeria; a botanist identified the plant. The plant was washed, cut - opened to remove the fresh seeds and was air-dried at 37^{o}C for three days to scale down the moisture content.

Grinding process (pulverization)

The plant sample *Abelmoschus esculentus L.* (okra seed or OS) for the study was grinded to powder using an electronic grinder model Nima Japan. The grinded sample was packed into polystyrene (nylon) bag sailed and placed in a desiccator with colloid (desiccant) to forestall sample from absorbing moisture from the atmosphere. The dried pulverized (powdered) plant sample material was stored in a desiccator until use.

Methanol extraction of plant sample

Fine powdered material was extracted in order to obtain active substances with a suitable solvent (methanol). For the preparation of methanol extract, 100g each of

powdered *Abelmoschus esculentus L.*, was separately weighed into a 1000ml beaker and was thoroughly extracted by adding 80% methanol for eighteen hours at a sonicating temperature of 30^{o}C under shaking condition. For every six hours, the solution was sonicated for twenty minutes to get the precise anti-diabetic agents (bioactive component) of the plant sample which was followed by filtration to yield a final volume of 1litre (1000mL). The extract was filtered with Whitman paper No.1 and was concentrated to dryness under reduced pressure and controlled

temperature (40-50^{o}C) in a digital controlled water bath and was fractionalized sequentially (partitioning) by n-Hexane, Chloroform and Ethyl ethanoate (Ethyl acetate). n-Hexane,

Chloroform, and Ethyl ethanoate extract fractions were evaporated under reduced pressure.

Filtration of extracted sample

After the sonication of the sample, there was a transparent separation of the supernatant from the residue cemented at the bottom of the conical flask. However, the filtration process prevented tiny residues from entering the filtrate if decanted. Whitman paper No1 was folded twice into a plastic funnel and the funnel over a conical flask's mouth. The solution separated was poured into the funnel with filter paper, gradually the filtrate was collected at the bottom of the conical flask and the residue was retained by the filter paper.

Concentration of the filtrate

The filtrate collected contained both methanol and water alongside the extract. In order to remove the methanol used for extraction a digital regulated water bath, was used. The digitally controlled water bath allowed the evaporation of methanol at 40^{o}C.

Freeze-drying

The concentrated extract contained water after the methanol was evaporated from the filtrate. The extracts were frozen to -20^{o}C and dried in a vacuum-compressed system (dryer). Freeze dryer; model number LGJ-18 fitted with compressor pump was used.

Fractionation of crude extract (partitioning)

Fractionation of the methanol crude extracts (Partitioning), 10g of the extracts was dissolved in 100 ml of distilled water and partitioned into n-hexane, chloroform, and ethyl acetate fractions in increasing order of the solvent polarity (n-hexane < chloroform < ethyl acetate < distilled water) using separating funnel. The resultant fractions were dried at a reduced temperature of 40oC with digital regulated water bath. The weight of the fractions was taken. The fractions reacted with yeast cells for viability, DPPH, FRAP, Lipid peroxidation, and yeast cell glucose uptake assay. The method of Kabir et al; 2005 was used to identify the bioactive fractions.

After fractionation of OS, a total of four (4) fractions were partitioned (table 1).

The coding of the fractions used two alphabets and a number. Where the prefix is the name of the food plant, the suffix is the name of the solvent used for extraction of that particular fraction while the number is fractions partitioned numbered according to their displacement from the separating funnel (table 2.1).

Table 2.1: Showing the fractions from the partitioning of crude extract with n-Hexane, chloroform, ethyl acetate, and aqueous solution

Sample	Hex	CHCl3	EtOAc	Aqueous
Okra Seed (OS)	OH1, OH2	-	OE	OA

Table 2.2: Percentage yield of fractions from 10g of the crude extracts

	Hex	CHCl3	EtOAc	Aqueous
Wt. of Solvent used	100%	100%	100%	100%
Wt. of fractions (g)	OH1 =2.0 OH2 =3.1	-	OE =2.0	OA =3.0
% Wt. of the fractions	OH1 =20 OH2 =31	-	OE =19	OA =30

In vitro antioxidant of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant activities of the plant extract were estimated using DPPH free radical scavenging assay as described by Oyaizu, (1986). Different concentrations of crude extracts and ascorbic acid (vitamin C as control) at concentrations of 31.25, 62.5, 125, 250, and 500 µg/ml as well as that of the fractions with similar concentrations were prepared from stock solutions (1000µg/ml) was prepared by weighing and dissolving 0.0005g of the crude extract and ascorbic acid in 100mL of methanol. Later, 2ml of 0.004% DPPH in methanol was added to 1 ml of varied concentrations of crude extract and extract fractions as well as ascorbic acid. The reaction mixtures were incubated at 25°C for half-hour. The absorbance of each test mixture was read against a blank at 517nm engaging a double beam Shimadzu UV-1800 series spectrophotometer. The experiment was carried out in triplicates. The percentage antioxidant activity was calculated using the formula below:
Percentage scavenging activity = Absorbance of blank minus Absorbance of the sample divided by Absorbance of blank multiplied by 100

Ferric reducing antioxidant power (FRAP) assay

Valuation of antioxidant activity of the crude extracts and extract fractions through ferric reducing antioxidant power assay was constant with the method of Oyaizu (1986). 0.0005g of crude extracts and ascorbic acid as control were weighed and dissolved in 100ml of methanol (1000µg/ml), from which different concentrations of 31.25, 62.5, 125, 250, and 500µg/ml were prepared. During this assay, 1ml of each plant extracts, vitamin C, 1ml of 0.2 M sodium orthophosphate buffer, and 1ml of 1% Potassium hexacyanoferrate (III) were mixed together, and incubated at 50°C for twenty minutes. After that, 1ml of 10% TCA was added to 1ml of each concentration of the extracts and was mixed with 1ml of water and 0.2ml of 0.1% Ferric chloride. The absorbance of the test samples was read at 700 nm with distilled water as blank. The percentage of antioxidant activity was calculated using the formula: Percentage activity = absorbance of sample minus absorbance of $blank$ divided by absorbance of $sample$ multiplied by 100.

Inhibition of lipid peroxidation (L.P) by crude extracts and extract fractions assay
The inhibitory effects of crude extracts and extract fractions on lipid peroxidation were determined using the method of Halliwell et al (1995) with slight modification. Briefly, 0.5ml of 10% egg homogenate was added to 0.1ml of crude extracts and fractions and ascorbic as control at various concentrations of 31.25, 62.5, 125, 250, and 500µg/ml as well as 1ml of water was added. Afterward, 0.05ml of FeSO4 was added to the mixtures and incubated for half-hour. Then,

1.5ml of carboxylic acid and thiobarbituric acid (TBA) in sodium dodecyl sulfate was added. The resulting reaction mixture was vortexed and incubated at 95°C for one hour. Reaction mixtures were allowed to cool, and 5ml of butanol was added to each of the test tubes and centrifuged at 1200 rpm for ten minutes, and the absorbance of the samples was read at 532nm.

The percentage inhibition of lipid peroxidation was calculated with the formula: Percentage Inhibition = absorbance of *blank* minus absorbance of *sample* divided by absorbance of *blank*multiplied by 100.

Antidiabetic effect of *Abelmoschus esculentus L*. of glucose uptake in yeast cells

The assay was constant with the method of Cirillo (1962) with slight modification. Commercial baker's yeast was dissolved in distilled water to prepare 1% suspension. The suspension was kept overnight at a temperature of 37◦C. The following day, yeast cells suspension was centrifuged at 4200 rpm with High-speed refrigerated 4 bucket centrifuge model, LR10 – 2.4A, 50/60 Hz, and 220–240 V for five minutes. The method was repeated by adding water to the pallet until a transparent supernatant was obtained. Exactly ten parts of the clear supernatant fluids was mixed with 90 parts of distilled water to get a 100ml v/v suspension of the yeast cells. Due to the extract's solubility, about 1mg w/v of plant crude extracts and extract fractions was mixed with dimethyl sulfoxide (DMSO4). A serial dilution of extract was done at the concentration of 0.625, 1.25, 2.5, and 5mg/ml for the crude extract as well as 31.25, 62.5, 125, 250 and 500µg/ml for extract fractions respectively. Samples were reacted with concentrations of 5, 10, and 25mM/L of 1mL of glucose solution and incubated for ten minutes at 37◦C. The reaction was initiated with the addition of 100ml of yeast suspension to the samples of glucose and extracts. The samples were vortexed and incubated for one more hour at 37◦C. After the incubation, 3,5-dinitrosalicylic acid (DNSA) was added to the tubes and was placed in boiling water for five minutes (but the tubes were not allowed to boil – this was to permit for the rapid reaction of the extracts, glucose as well as the yeast cells) the glucose uptake was read by engaging a spectrophotometer (UV - 1800 SHIMADZU) at 540nm. Absorbance for the control was carried out on a similar wavelength. The percentage increase in glucose uptake was calculated with the formula: Percentage increase in glucose uptake = absorbance of control minus absorbance of the sample divided by absorbance of control multiplied by 100, where control was the solution containing all reactants except the test sample. Metronidazole was used as the standard drug (control).

Statistical analysis

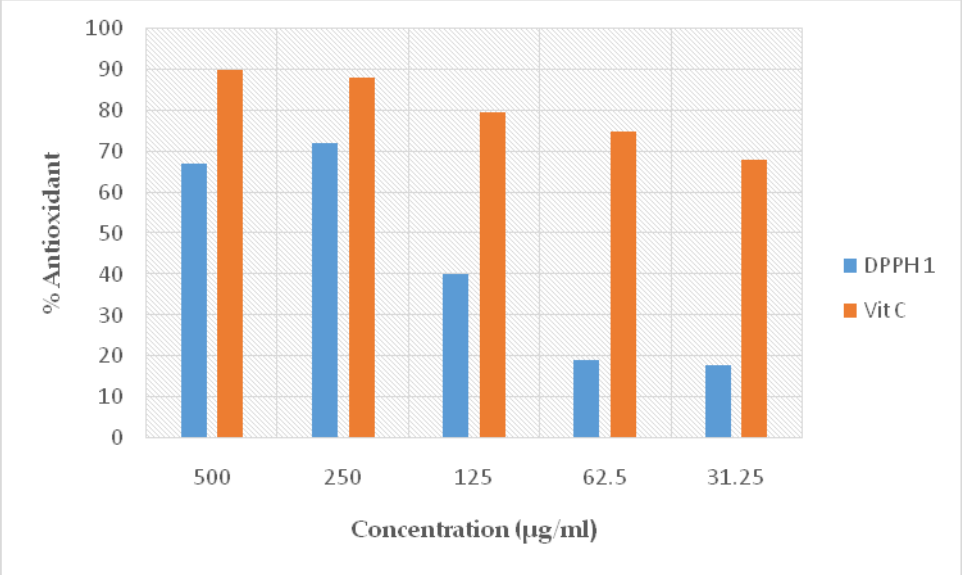
Data were collected using a one-way, and two-way analysis of variance (ANOVA) as well as independent T-test, and paired T-test analysis. Groups were considered significant if $P<0{\cdot}05$ and,

a F-value was significant for ANOVA; the differences between all pairs were carried out using Ducan Post Hoc Test; SPSS version 26, and Microsoft excel windows 10 were used for statistical analysis, and data figure generation.

RESULTS

Antioxidant activity of the crude extract and extract fractions of *Abelmoschus esculentus L.*

DPPH is an appropriate free radical compound generally used for testing the free-radical scavenging activities of different types of samples. The DPPH radical scavenging test mainly depends on the ability of a compound to donate hydrogen atoms; therefore, stabilizing the free radicals which in turn prevent oxidation of biomolecules (Kusuma et al; 2014). Although the DPPH radical does not conform with any biological compounds (and thus has a relatively little significance in living organisms), nevertheless, DPPH assay is generally considered as an indicator of the ability of plant extracts to extinguish free radicals, as well as their hydrogen atom or electron donation ability, in the absence of any enzymatic action (Mileva et al; 2014). The most advantageous reason for the use of DPPH in assessing in vitro antioxidant activities of drugs and plant extracts is owing to its higher stability than hydroxyl and superoxide radicals (Li et al; 2009). Hence, the antioxidant activities exhibited by the extracts through DPPH scavenging capacities.



International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.14-44, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

Figure 3.2: Percentage DPPH radical scavenging activities of crude extract of *Abelmoschus esculentus L.* (okra seed or OS). Values are presented as mean ± standard deviation of triplicates.
Values with high activity concentration are significantly different at $P < 0.05$.

Figure 3.2 may be said to be predominantly due to their hydrogen atom or electron donation ability. The hydrogen-donating ability, equally, may be traceable to the presence of phenolic compounds in the extracts as these secondary metabolites have been established to possess antioxidant activities (Gruz et al; 2011). Therefore, it is rational to deduce that the higher activity of the crude extract of *Abelmoschus esculentus L.* extract may be as a result of higher concentrations of phenolics in the crude extract. The electron-donating ability of antioxidants in the crude extracts is generally reproduced by the ability of such antioxidants to reduce iron (Fe) in the oxidation state of Fe3+ to Fe2+. Therefore, the higher the activity of the antioxidants indicates the higher electron-donating ability (reducing ability) (Amari et al; 2014). Therefore, the significant activities of the extracts suggest that they were able to reduce Fe3+ to Fe2+, revealing their electron-donating ability, which in turn suggests the possibility of using the extracts in preventing oxidation of biomolecules in cells.

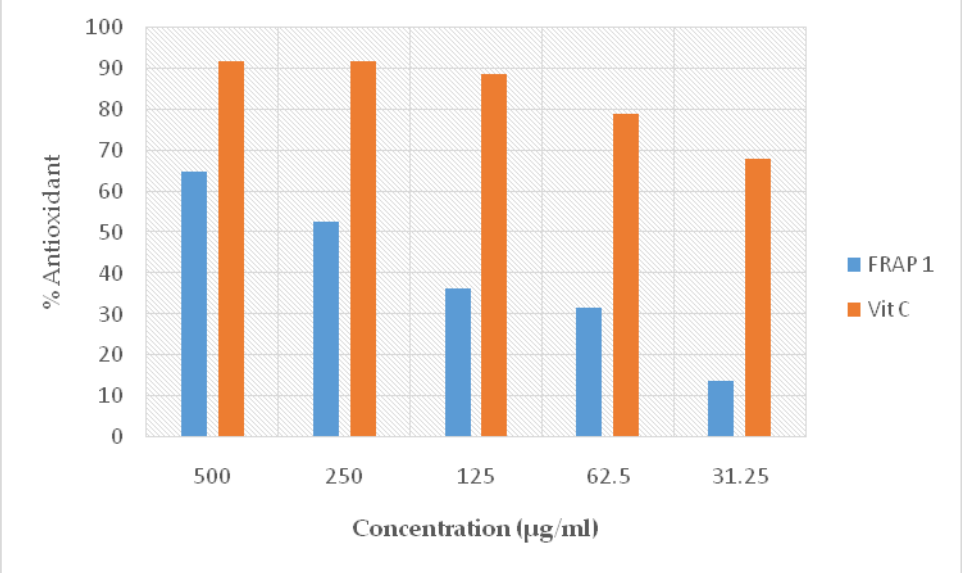


Figure 3.3: Percentage ferric reducing powers of crude extract of *Abelmoschus esculentus L.* (okra seed or OS). Values are presented as mean ± standard deviation of triplicates. Values with high ferric reducing power concentration are significantly different at $P < 0.05$.

The results obtained for the *Abelmoschus esculentus L*. extract and the percentage inhibition obtained in figure 3.3 were higher. The same reasons mentioned in the DPPH radical scavenging assay may also be responsible for the differences in this assay. Nevertheless, *Abelmoschus esculentus L*. extract presented significant ferric reducing power figure 3.

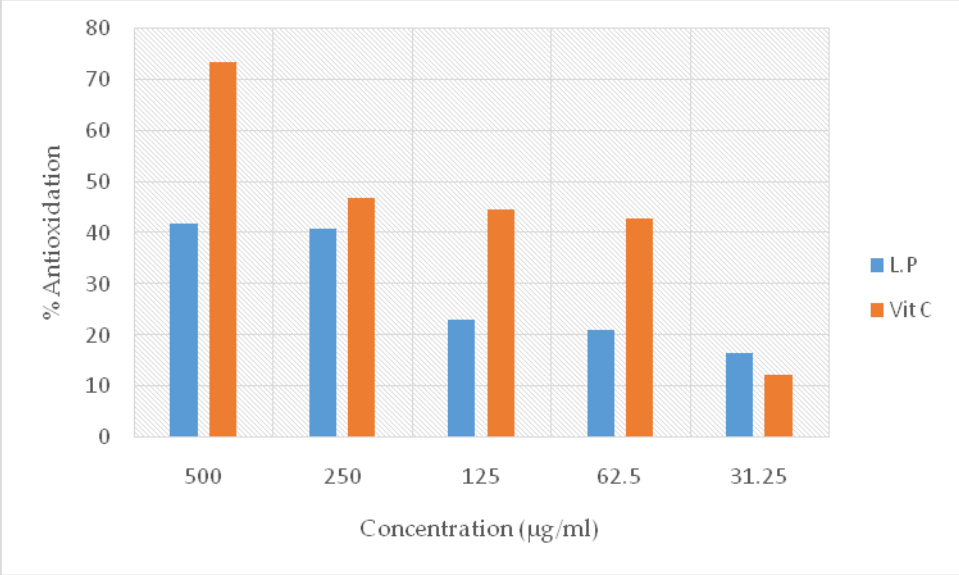


Figure 3.4: Percentage inhibitory activities of crude extracts of *Abelmoschus esculentus L*. (okra seed or OS) on lipid peroxidation (L.P). Values are presented as mean ± standard deviation of triplicates. Values with higher lipid peroxidation activity concentration are significantly different at $P < 0.05$. Lipid peroxidation has been described as an oxidative breakdown of lipids, a process in which free radicals' abstract electrons from cell membrane lipids (this mostly affects polyunsaturated fatty acids due to the presence of double bonds). The process of lipid peroxidation has been proposed to occur through a free radical chain reaction, which has been linked with the damage of cell bio-membranes (Halliwell, 1989). The damage caused has been established to influence the disease conditions of many individuals, such diseases as cardiovascular diseases, cancer, and diabetes (Usuk et al; 1981). Therefore, the ability of the extracts to significantly inhibit lipid peroxidation of the egg homogenate implies that they were able to extinguish the actions of the free radicals by preventing the concept of the electrons from cell membrane lipids by the free radicals and therefore may be used in shielding humans from chronic diseases and other oxidative stress-related diseases

International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.14-44, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

In figure 3.4, the result showed increase inhibition in L.P with a corresponding increase in concentration. Even though the standard antioxidant was higher in all concentrations (500 to 62.5µg/ml) except in the lower concentration of 31.25 µg/ml.

The results obtained in this study suggest that the crude extracts of *Abelmoschus esculentus L.*, possess antioxidant activities and may therefore be used in the treatments and management of oxidative stress-related diseases.

Antioxidant effect of DPPH 2, FRAP 2 and Lipid peroxidation 2 on extract fractions

Table 3.1: Showing the percentage scavenging antioxidant activity of extract fractions DPPH 2.

µg/ml	% OH1	% OH2	% OA	% OE	% Vit C
500	48.21	38.42	40.33	59.19	82.58
250	43.44	24.11	44.15	46.54	53.22
125	42.96	19.09	36.28	37.71	46.3
62.5	40.81	16.47	23.87	41.05	47.02
31.25	25.78	6.92	18.62	42.96	45.58

The percentage scavenging antioxidant activity (also referred to as DPPH) of the extract fractions table 3.1 showed decreased scavenging antioxidant ability with decreased extract fraction concentration. The extract fraction OE (59.19%) revealed higher antioxidant percentage activity compared to other fractions partitioned. All of the higher activity was exhibited at the concentration of 500µg/ml.

Table 3.4: Showing the percentage ferric reducing antioxidant power (FRAP) of extract fractions.

µg/ml	% OH1	% OH2	% OA	% OE	% Vit C
500	85.29	65.35	78.62	93.62	95.25
250	80.47	61.94	63.99	92.06	95.25
125	62.48	23.22	57.73	83.56	93.42
62.5	48.4	32.22	46.6	74.58	86.37
31.25	36.1	17.12	34.64	60.46	77.28

The percentage ferric reducing antioxidant power (FRAP) of the extract fractions table 3.4 showed a increased FRAP percentage capacity with anincrease in extract fraction concentration. Extract fraction OE (93.62%) produced very high FRAP percentage capacity compared to other fractions. Though, the other fractions were also very high at concentrations of 500µg/ml and 250µg/ml respectively.

Table 3.5: Showing the percentage inhibitory activity of antioxidant of extract fractions Lipid peroxidation (L.P).

µg/ml	% OH1	% OH2	% OA	% OE	% Vit C
500	70.54	57.91	59.52	84.59	91.36
250	65.26	49.13	55.52	72.97	84.58

International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.14-44, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

125	61.19	46.23	48.6	62.03	72.97
62.5	59.51	15.71	46.17	58.01	65.28
31.25	49.19	9.26	26.92	42.69	49.25

The percentage inhibitory activity of antioxidants (also referred to as lipid peroxidation) for all extract fraction table 3.5 showed increased inhibitory activity with increase in extract fraction concentration. Extract fractions OE (84.59%) and OH (70.54%) gave higher inhibitory ability than other extract fractions. The standard antioxidant was higher than the extract fractions in all concentrations of the antioxidant assays (i.e. DPPH, FRAP, and lipid peroxidation).

Anti diabetic effect of crude extract in yeast cells

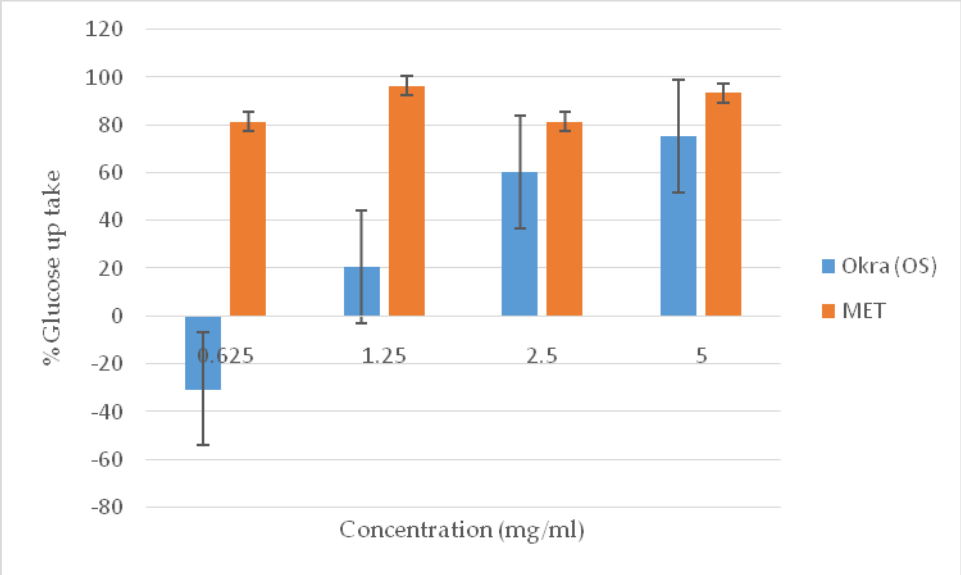


Figure 3.5: Glucose uptake by yeast cells at 5mM/L initial concentration of glucose in the presence of OS; crude extract of *Abelmoschus esculentus L*. (okra seeds or OS); Metronidazole (MET) is a synthetic diabetes drug. The error bars represent ± SE of triplicate data. The bars are significantly different at $P = 0.05$.

International Journal of Nutrition and Metabolism Research
Vol.2, No.1, pp.14-44, 2024
Print ISSN: 2399-7052
Online ISSN: 2399-7060
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

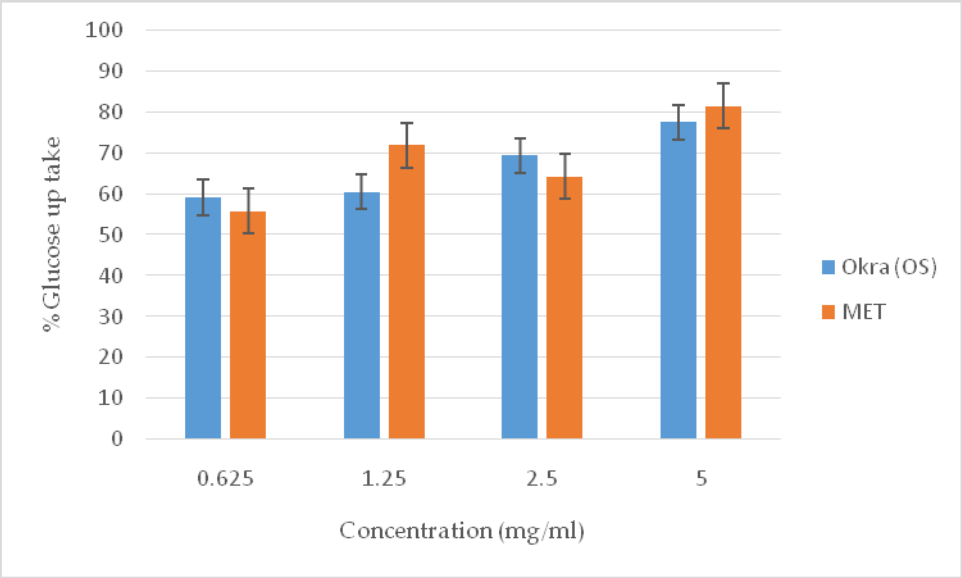


Figure 3.6: Glucose uptake by yeast cells at 10mM/L initial concentration of glucose in the presence of OS; crude extract of *Abelmoschus esculentus* ***L***. (okra seeds or OS); Metronidazole (MET) is a synthetic diabetes drug. The error bars represent ± SE of triplicate data. The bars are significantly different at $P = 0.05$.

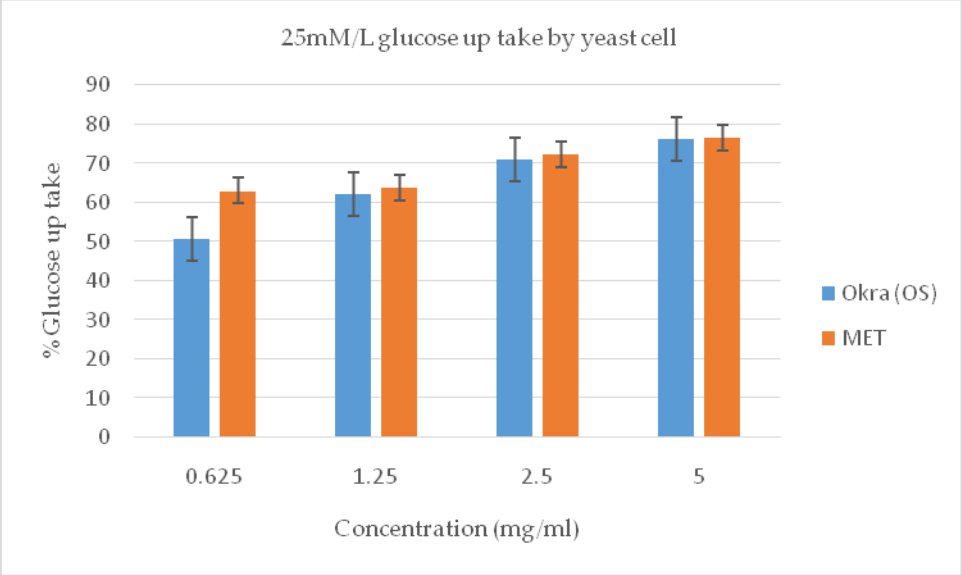


Figure 3.7: Glucose uptake by yeast cells at 25mM/L initial concentration of glucose in the presence of OS; crude extract of *Abelmoschus esculentus L*. (okra seeds or OS); Metronidazole (MET) is a synthetic diabetes drug. The error bars represent ± SE of triplicate data. The bars are significantly different at $P = 0.05$

Effect of crude extract of *Abelmoschus esculentus L*. on glucose uptake ability by yeast cells. The crude extract of *Abelmoschus esculentus L*. stimulated the uptake of glucose through the partially but not entirely permeable membrane of yeast cells figures 3.5, 3.6, and 3.7 respectively. The glucose uptake at an initial concentration of 5mM/L and 10mM/L by the crude extract of *Abelmoschus esculentus L*. was consistent to that of the known standard drug (figures 5 and 6). However, the effect of Metronidazole on glucose uptake by the yeast cell at 25mM/L glucose concentration was at par with the crude extract of *Abelmoschus esculentus L* (figure 3.7).

Moreover, at 0.625 mg/ml the linear equations, and R2 shows that the extract was higher in dose predictability than the standard drug as shown by the equation; $y = 35.751x - 57.815$, and $R^2 = 0.9502$ (95%) for Abelmoschus esculentus L., while $y = 2.1324x + 82.881$, and $R^2 = 0.1213$ (12.1%) for Metronidazole when 5 mg/mL of crude extract of Abelmoschus esculentus L was used (figure 3.5). This suggests that increasing the concentration of crude extract of *Abelmoschus esculentus L*., increased the potential of yeast cells to take up more glucose from the environment; however, the standard drug; although it showed high glucose uptake capacity, but had a very low drug-dose predictability compared to the extracts as confirmed by the very low R2 value of 12.1% against the crude extract R2 value of 92.3% respectively. On the other hand, figures 3.6 and 3.7 showed a linear increase in the uptake of glucose by yeast cells with a gradual increase in the concentration of the crude extract. However, an inverse correlation to the molar concentration of glucose was observed, when glucose uptake by yeast cells was compared among 5mM/L, 10mM/L, and 25mM/L for the similar amount of crude extract of *Abelmoschus esculentus L*., (figures 3.5, 3.6, and 3.7).

Anti diabetic effect of extract fractions in yeast cells

Table 3.6: Showing glucose uptake ability at a concentration of 5mM/L.

µg/ml	% OH1	% OH2	% OA	% OE	% MET
500	58.13	49.82	48.5	70.66	88.18
250	55.93	42.29	44.49	66.54	81.93
125	33.34	34.1	42.15	55.53	76.5
62.5	28.05	27.99	37.31	50.27	54.29
31.25	18.43	23.64	34.93	40.48	49

Table 3.7: Showing glucose uptake ability at a concentration of 10mM/L.

µg/ml	% OH1	% OH2	% OA	% OE	% MET
500	75.97	76.5	64.57	73.4	92.35
250	68.59	71.32	63.39	59.56	89.37
125	64.09	62.42	60.11	51.59	85.39
62.5	57.33	58.54	54.59	44.63	71.21
31.25	55.56	56.9	49.64	43.88	57.47

Table 3.8: Showing glucose uptake ability at a concentration of 25mM/L.

µg/ml	% OH1	% OH2	% OA	% OE	% MET
500	60.07	67.54	63.49	70.15	97.43
250	54.92	64.27	58.52	74.55	93.67
125	49.65	63.92	56.12	79.55	74.09
62.5	42.97	51.51	52.83	81.58	69.91
31.25	41.63	44.68	48.42	81.65	64.86

Remarkably, in table 3.6, 3.7 and 3.8 the fraction OE was higher than all other extract fractions. But in table 8 extract fraction OE was higher than the standard drug at a lower extract fraction concentration of 125µg/ml (79.55 %), 62.5µg/mL (81.58%), 31.25µg/ml (81.65 %) respectively. This showed a different trend of increase in percentage glucose uptake with a decrease in extract fraction concentration.

DISCUSSION

Diabetes mellitus (DM) has a near link with several dietary anomalies; unique amongst the greatest anomalies is oxidative stress. Biological as well as chemical trainings have presented an improved group of reactive oxygen species (ROS) inside the cells and tissues of people suffering from hyperglycemia (Atlas, 2013). As a result, ROS is confronted due to the existence of strong antioxidants inside the body of an individual with diabetes is important since an antioxidant has the capacity of delaying or totally discontinuing the oxidation of additional constituents. Throughout this procedure, DPPH free radical scavenging assay is among the popular antioxidant analyses, first presented through Marsden Blois of Stanford University in 1958. Numerous investigators have applied this technique to study the antioxidant prospect of standard drugs and usual nutraceutical. Brand Williams and his associates have presented an improved form of the Blois technique in 1995, which is involved as an example by several sets of investigators lately (Lebeau et al; 2000). Similarly, indicators of the likely anti-diabetic possibility of a medication

are evaluated through numerous in vitro analyses, provided that evidences for its in vivo anti-diabetic possibility. As well the antioxidant evaluates, (Marinova and Batchvarov, 2011) numerous additional pointer analyses comprise (i) prospect of glucose uptake through the plasma membrane similar to that of yeast cells, (Bhutkar and Bhise, 2013) others like fat cells, (Gulati et al; 2015) or cells of the muscle; (Rajeswari and Sriidevi, 2014) (ii) capacity of glucose taken on the surface; (Gulati et al; 2015) (iii) stoppage of α-amylase and βglucosidase enzymes are mainly engaged for in vivo investigations. For the extract fractions in this study to have high antioxidant activity (figures 3.4 to 3.5 and tables 3.5 to 3.6) suggest that these fractions in helping the diabetic patient may be acting in one or more ways by helping to eliminate free radicals, in the process healing any inflammation on the pancreas such that insulin can be better released. Another way to explain the mechanism may be that by inhibiting free radical from locking up the cell membrane; in the process stopping the flexibility of the cell membrane. Furthermore, (Srividhya et al; 2017) reported that a diabetic agent can exercise a beneficial effect by increasing insulin secretion, improving, and imitating insulin action. This statement agrees with the findings of this research as the extract fractions facilitates the healing of the inflammation on the pancreas for better insulin release and in the process unlock the cell membrane for glucose uptake.

The anti-diabetic and antioxidant properties of methanol extract and the extract fractions may be attributed to the presence of bioactive compounds that may be present in *Abelmoschus esculentus L.* (okro seed or OS) (Gray and Flatt, 1997a). Previous research work has identified bioactive compounds in the extracts which include quercetin 3- O-glucosyl (1→6) glucoside (QDG) and quercetin 3-Oglucoside (QG) oxacyclododecane 2-one, imidazole, amentoflavone, bioflavonoids, eugenol, caryophyllene, -copaene, azulene, dodecatetraenamide, and phenethylamine (Shui and Leong, 2004; Atawodi et al; 2009; Rajagopal et al; 2013; Kadhim et al; 2016, Rehman; 2018). Thus, the existence of some of these mixtures in the extract could aid in the application of glucose in the current investigation. Generally, the application of glucose by muscles of the skeletons is due to the increase of efficient glucose conveying molecules in the membrane of the cell.

The glucose conveying molecules are controlled by leptocytes and myocytes in answer to the great release of insulin in the blood, bringing about low blood sugar (Rajeswari and Sriidevi, 2014). On the other hand, the investigation regarding the outcome of medications on the decrease of blood sugar immediately after a meal have been one of the significant parts in the control of hyperglycemia, which is a properly designed healing method to date. Moreover, glucose application by yeast cells could be diverse from that of other multi-cellular cells. Conveyance of glucose through yeast membrane could include enabled flow somewhat than the facilitation of a phosphate transfer of a biological catalyst or a protein system or any other unfamiliar method. The

glucose application by the yeast cells could be exaggerated by numerous changes, like glucose concentration classified the cells or the later breakdown of glucose. If greater part of the interior sugar is transformed freely into additional substances, the interior glucose concentration decreases and elevated uptake of glucose into the cell is carried on. Similarly, there are potentials that the glucose uptake by yeast cells in the existence of the extract is owed to both enabled diffusion and higher glucose breakdown. Surely, it will be quite exciting to discover the action of usual extract fractions in vivo, (these current investigation emphases which could aid in the improved glucose uptake by cells of the muscle and fat tissues of the body. The extract could fix glucose efficiently and convey it through the membrane of the cell for further breakdown. This finding correlates with the report of (Rehman et al; 2018).

The plant base protein sources in relation to insulin allow the conservation of glycemic outline which might remain a source of attention in the perspective of type 2 diabetes. Though, their mechanism of action is uncertain (Costa et al; 2020).

Also, okra seeds extract fraction presented anti-inflammatory activity by blocking possible membrane pathway and therefore aid healing of the pancreas, lower the expression of associated inflammatory factors and increase insulin release; this is consistent with the work of (Mengxin et al; 2022, and Gulali et al; 2023).

CONCLUSION

The extract fractions were active as drug candidates both at high and low concentrations and were better compared with the standard drug and standard antioxidant was comparable. From the results, it can be concluded that the higher the concentration of the extract in the solution, the higher the uptake of glucose by yeast cells. In addition, standard drugs are burdened with side effects as compared to the nutrients of the extract fractions which are natural and without side effects.

There is need for further research in encapsulating the high bioactive extract fractions separately and coding them according to their different functions as a drug candidate for diabetics. Further studies will be necessary to monitor the in vivo performances of the extract fractions and subsequent human trials.

Contributors

“Conceptualization, MA. and EA.; methodology, MA and EA. EE; software, MA and EA.; validation, KF., CP. and EE.; formal analysis, MA.; investigation, MA, EA, KF, CP and EE.; resources, MA and EA.; data curation, MA and EA.; writing - original draft preparation, MA.;

writing - review and editing, MA, EA; visualization, MA, EA, KF, CP, and EE; supervision, EA, KF, CP, and EE; project administration, EA; funding acquisition, MA, EA, KF, CP, and EE. All authors have read and agreed to the published version of the manuscript."

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of University of Nicosia; approval date is 15 January 2020.

Funding

This research received no external funding

REFERENCES

Amari, N. O. *et al.* (2014) 'Phytochemical screening and antioxidant capacity of the aerial parts of Thymelaea hirsuta L.', *Asian Pacific Journal of Tropical Disease*, 4(2), pp. 104–109. doi: 10.1016/S2222-1808(14)60324-8.

Atlas, I. D. (2013) 'Brussels, Belgium: international diabetes federation', *International Diabetes Federation* (IDF) 2014.

Atawodi, S. E.; Atawodi, J. C.; Idakwo, G. A. (2009) 'Polyphenol composition and antioxidant potential of Hibiscus esculentus L. fruit cultivated in Nigeria,' *Journal of Medicinal Food*; 12(6), pp. 1316–1320.

Arapitsas, P. (2008) 'Identification and quantification of polyphenolic compounds from okra seeds and skins', *Food Chemistry*, 110(4), pp. 1041–1045. doi: 10.1016/j.foodchem.2008.03.014.

Adelakun, O. E. *et al.* (2009) 'Chemical composition and the antioxidative properties of Nigerian Okra Seed (Abelmoschus esculentus Moench) Flour', *Food and Chemical Toxicology*. Elsevier Ltd, 47(6), pp. 1123–1126. doi: 10.1016/j.fct.2009.01.036.

Adelakun, O. E.; Oyelade and O. J. (2011) *Chemical and Antioxidant Properties of Okra (Abelmoschus esculentus Moench) Seed. In Nuts and Seeds in Health and Disease Prevention*; Preedy, V. R., Watson, R. R., Patel, V. B., Eds.; Academic Press: Cambridge, MA, USA, pp. 841–846.

Akpovona, A. E. *et al.* (2016) 'Acute and subchronic toxicity studies of ethanol extract of terminalia macroptera stem bark in wistar albino rats', *Journal of Medicine and Biomedical Research*, 15(1), pp. 62–73. doi: 10.1055/s-0036-1596308.

Aziz, M. A. *et al.* (2016) 'The association of oxidant-antioxidant status in patients with chronic renal failure', *Renal Failure*, 38(1), pp. 20–26. doi: 10.3109/0886022X.2015.1103654.

Balogun, J. B. *et al*. (2017) 'In Vivo Anti-Trypanosomal Potential of Methanol Root Extract of Terminalia macroptera (Guill. And Perr.) In Trypanosoma Brucei Brucei Infected Wistar Rat', IOSR *Journal Pharmarcy Biological Sciences*; 12(5) pp. 12-17.

Benov, L.; Beema, A. F. (2003) 'Superoxide dependence of the short chain sugars induced mutagenesis,' *Free Radical Biology and Medicine*; 34 pp. 429-433.

Bagchi, K.; Puri, S. (1998) 'Free radicals and antioxidants in health and disease: A review,' *Eastern Mediterranean Health Journal*; 4(2) pp. 350-360.

Benchasr, S. (2012) Okra (Abelmoschus esculentus (L.) Moench) as a valuable vegetable of the World. *Ratar. Poverty*, 49, pp. 105–112.

Bhutkar, M. and Bhise, S. (2013) 'In vitro hypoglycemic effects of Albizzia lebbeck and Mucuna pruriens', *Asian Pacific Journal of Tropical Biomedicine*, 3(11), pp. 866–870. doi: 10.1016/S2221-1691(13)60170-7.

Caluete, M. E. E. *et al*. (2014) Nutritional, antinutritional, phytochemical status of okra leaves (Abelmoschus esculentus) subjected to different processes. *African Journal of Biotechnology*, 14, pp. 683–687.

Costa, Izael S.; Medeiros, Amanda F.; Piuvezam, Grasiela; Medeiros, Gidyenne C. B. S.; Maciel, Bruna L. L.; Morais, Heloneida Ana A. (2020) 'Insulin-Like Proteins in Plant Sources: A Systematic Review'. *Journal Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*; 13, pp. 3421 – 3431.

Centers for Disease Control and Prevention (2011) National diabetes fact sheet, 2011. www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed January 15, 2015

Cirillo, V. P. (1962) 'Mechanism of glucose transport across the yeast cell membrane', *Journal of Bacteriology*; 84(3), pp. 485– 491.

Durazzo, A. *et al.* (2019) 'Abelmoschus esculentus (L.): Bioactive components' beneficial properties-focused on antidiabetic role-for sustainable health applications', *Molecules*, 24(1). doi: 10.3390/molecules 24010038

Durazzo, A. (2017) 'Study approach of antioxidant properties in foods: Update and considerations', *Foods*, 6(3), pp. 1–7. doi: 10.3390/foods6030017.

Dong, Z.*et al*. (2014) The fatty oil from okra seed: Supercritical carbon dioxide extraction, composition and antioxidant activity; *Current Topics in Nutrition Research*, 12, pp. 75–84.

Eleazu, C. and Okafor, P. (2012) 'Antioxidant effect of unripe plantain (Musa paradisiacae) on oxidative stress in alloxan-induced diabetic rabbits', *International Journal of Medicine and Biomedical Research*, 1(3), pp. 232–241. doi: 10.14194/ijmbr.1311.

Gray, A. M. and Flatt, P. R. (1997a) 'Nature's own pharmacy: the diabetes perspective', *Proceedings of the Nutrition Society;* 56, pp. 507–517.

Gruz, J. *et al.* (2011) 'Phenolic acid content and radical scavenging activity of extracts from medlar (Mespilus germanica L.) fruit at different stages of ripening', *Food Chemistry*. Elsevier Ltd, 124(1), pp. 271–277. doi: 10.1016/j.foodchem.2010.06.030.

Gulati, V. *et al.* (2015) 'Exploring the anti-diabetic potential of Australian Aboriginal and Indian Ayurvedic plant extracts using cell-based assays', *BMC Complementary and Alternative Medicine*, 15(1), pp. 1–11. doi: 10.1186/s12906-015-0524-8.

Gemede, H. F. *et al.* (2016) 'Proximate, mineral, and antinutrient compositions of indigenous Okra (Abelmoschus esculentus) pod accessions: implications for mineral bioavailability', *Food Science and Nutrition*, 4(2), pp. 223–233. doi: 10.1002/fsn3.282.

Gould, G. W. Holman, G. D. (1993) 'The glucose transporter family: Structure, function and tissue-specific expression,' *Biochemistry Journal*; 295 pp. 329- 341.

Gulali Aktaş , Burcin Meryem Atak Tel , Ramiz Tel , Buse Balcı (2023) Treatment of type 2 diabetes patients with heart conditions. Expert Review of Endocrinology & Metabolism; 18(3), pp. 255-265.

Habtamu, F. *et al*. (2014) 'Nutritional quality and health benefits of okra (Abelmoschus esculentus): A review', *Journal of Food Science, Quality control and Management*; 33, pp. 87– 96.

Hu, L. *et al.* (2014) 'Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride', *BioMed Research International*, 2014. doi:
10.1155/2014/341291.

Halliwell, B. (1995) *How to characterize an antioxidant*: an update. In Biochemical Society Symposia 61, pp. 73-101). Portland Press Limited.

Halliwell, B. (1989) 'Protection against tissue damage in vivo by desferrioxamine: What is its mechanism of action?', *Free Radical Biology and Medicine*, 7(6), pp. 645–651. doi:
10.1016/0891-5849(89)90145-7.

Jothy, S. L. *et al.* (2011) 'Bioassay-directed isolation of active compounds with antiyeast activity from a cassia fistula seed extract', *Molecules*, 16(9), pp. 7583–7592. doi:
10.3390/molecules16097583.

Jain, N. *et al.A Review on: Abelmoschus esculentus.* 2012; *1 1 1 1*.

Joost, H. G.*et al*. (2002) 'Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators,' *American Journal of Physiology - Endocrinology and Metabolism*; 282 pp. E974E976 Jarret, R. L., Wang, M. L. and Levy, I. J. (2011) 'Seed oil and fatty acid content in okra (Abelmoschus esculentus) and related species', *Journal of Agricultural and Food Chemistry*, 59(8), pp. 4019–4024. doi: 10.1021/jf104590u.

Kwon, Y. I. *et al.* (2007) 'Health benefits of traditional corn, beans, and pumpkin: In vitro studies for hyperglycemia and hypertension management', *Journal of Medicinal Food*, 10(2), pp. 266–275. doi: 10.1089/jmf.2006.234.

Kapoor, S. K. and Anand, K. (2002) 'Nutritional transition: a public health challenge in developing countries', *Journal of Epidemiology and Community Health*, 56, pp. 804–805.

Krishnamurthy, P.; Wadhwani, A. (2012) 'Antioxidant enzymes and human health. In: El-Misery MA, editor. Antioxidant Enzyme,' *Croatia: In Tech*; 2012. pp. 3-18.

Kumar, S. (2011) 'Free radicals and antioxidants: Human and food system,' *Advanced in Applied Science Research*; 2(1) pp. 129-135.

Kadhim, M. J.; Mohammed, G. J.; Hameed, I. H. (2016) 'In vitro antibacterial, antifungal and phytochemical analysis of methanolic extract of fruit Cassia fstula', *Oriental Journal of Chemistry*; 32(3), pp. 1329–1346.

Kusuma, I. W.; Arung, E. T. and Kim, Y. U. (2014) 'Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia,' *Food Science and Human Wellness*; 3(3-4) pp. 191-196.

Kabir, O. A., Olukayode, O., Chidi, E. O., Christopher, C., I, Kehinde, A. F. (2005) 'Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity',BMC Complementary and Alternative Medicine;5:6.

Lü, J. M. *et al*. (2012) 'Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems,' *Journal of Cellular and Molecular Medicine*; 14(4) pp. 840860.

Liao, H.*et al*. (2012) 'Analysis and comparison of the active components and antioxidant activities of extracts from Abelmoschus esculentus L.', *Pharmacognosy Magazine*; 8, pp. 156– 161.

Lawal, B. *et al.* (2015) 'Potential antimalarials from African natural products: a review', *Journal of Intercultural Ethnopharmacology*, 4(4), p. 318. doi: 10.5455/jice.20150928102856.

Li, W. *et al.* (2009) 'Evaluation of antioxidant capacity and aroma quality of breast milk', *Nutrition*. Elsevier Inc., 25(1), pp. 105–114. doi: 10.1016/j.nut.2008.07.017.

Lebeau, J. *et al.* (2000) 'Antioxidant properties of di-tert-butylhydroxylated flavonoids', *Free Radical Biology and Medicine*, 29(9), pp. 900–912. doi: 10.1016/S0891-5849(00)00390-7.

Law, B. M. H.*et al*. (2017) 'Hypotheses on the potential of rice bran intake to prevent gastrointestinal cancer through the modulation of oxidative stress,' *International Journal of Molecular Sciences*; 18 pp. 1-20.

Medina, R. A. Owen, G. I. (2002) 'Glucose transporters: Expression, regulation and cancer,' *Biological Research*; 35 pp. 9-26.

Marinova, G.; Batchvarov, V. (2011) 'Evaluation of the methods for determination of the free radical scavenging activity by DPPH', *Bulgarian Journal of Agricultural Science*; 17(1), pp. 11– 24.

Mengxin Li, Junhui Wang, Yunfang Ye, Shanqiang Xiong, Yong Liu (2022) 'Structural characterization and anti-inflammatory activity of a pectin polysaccharide AP2-c from the lignified okra. Journal of Food Biochemistry; 46(9): e14380.

Mileva, M.*et al*. (2014) 'Chemical composition, in vitro antiradical and antimicrobial activities of Bulgarian Rosa alba L. essential oil against some oral pathogens,' *International Journal of Current Microbiology and Applied Science*; 3(7) pp. 11-20.

Mihretu, Y.; Wayessa, G and Adugna, D. (2014) 'Multivariate Analysis among Okra
(Abelmoschus esculentus (L.) Moench) Collection in South Western Ethiopia' *Journal of Plant Science*; 9, pp. 43–50.

Maganha, E. G. *et al.* (2010) 'Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus', *Food Chemistry*, 118(1), pp. 1–10. doi:

10.1016/j.foodchem.2009.04.005.

Messing, J. *et al.* (2014) 'Antiadhesive properties of Abelmoschus esculentus (okra) immature fruit extract against Helicobacter pylori adhesion', *PLoS ONE*, 9(1). doi: 10.1371/journal.pone.0084836.

Mfotie Njoya E, Weber C, Hernandez-Cuevas NA, Hon CC, Janin Y, Kamini MFG (2014). Bioassay-Guided Fractionation of Extracts from Codiaeum variegatum against Entamoeba histolytica Discovers Compounds That Modify Expression of Ceramide Biosynthesis Related Genes,' *PLoS Neglected Tropical Diseases*; 8(1):29.

Moyin-Jesu, E. I. (2007) 'Use of plant residues for improving soil fertility, pod nutrients, root growth and pod weight of okra (Abelmoschus esculentum L)', *Bioresource Technology*, 98(11), pp. 2057–2064. doi: 10.1016/j.biortech.2006.03.007.

Madaki FM, Kabiru AY, Mann A, Abdulkadir A, Agadi JN, Akinyode AO (2016).

Phytochemical Analysis and In-vitro Antitrypanosomal Activity of Selected Medicinal Plants in Niger State, Nigeria,' *International Journal of Biochemistry Research & Review* CRR, 11(3): 17; Article no. IJBCRR.24955.

Onukogu, S. C.*et al*. (2019) 'In vitro antioxidants, antimicrobials and biochemical response of methanol leaf extract of Eucalyptus camaldulensis following sub-acute administration to rats,' *Saudi Journal of Biomedical Research*; 4(11) pp. 405-11.

Odebode, F. D. *et al.* (2017) 'Nutritional composition, antidiabetic and antilipidemic potentials of flour blends made from unripe plantain, soybean cake, and rice bran', *Journal of Food Biochemistry*, pp. 1–9. doi: 10.1111/jfbc.12447.

Oyaizu, M. (1986) 'Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine,' *Japanese Journal of Nutrition and Dietetics*; 44 pp. 307-315.

Petropoulos, S. *et al.* (2018) 'Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage', *Food Chemistry*, 242(July 2017), pp. 466–474. doi: 10.1016/j.foodchem.2017.09.082.

Popkin, B. M. (2002) 'The shift in stages of the nutrition transition in the developing world differs from past experiences!', *Malaysian Journal of Nutrition*, 8(1), pp. 109–124. doi: 10.1079/PHN2001295

Polumbryk, M.; Ivanov, S.; Polumbryk, O. (2013) 'Antioxidants in food systems. Mechanism of action.' *Ukrainian Journal of Food Science*; 1(1) pp. 15-40.

Rehman, G. *et al.* (2018) 'In vitro antidiabetic effects and antioxidant potential of cassia nemophila pods', *BioMed Research International*, 2018. doi: 10.1155/2018/1824790.

Rajagopal, P *et al*. (2013) 'Phytochemical and pharmacological review on Cassia fstula linn. "The golden shower' *International Journal of Pharmaceutical, Chemical & Biological Sciences*; 3(3).

Rajeswari, R. and Sriidevi, M. (2014) 'Study of in vitro glucose uptake activity of isolated compounds from hydro alcoholic leaf extract of cardiospermum halicacabum linn', *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(11), pp. 181–185

Roy, A., Shrivastava, S. L. and Mandal, S. M. (2014) 'Functional properties of Okra
Abelmoschus esculentus L. (Moench): traditional claims and scientific evidences', *Plant Science Today*, 1(3), pp. 121–130. doi: 10.14719/pst.2014.1.3.63.

Shui, G. and Leong, L. P. (2004) 'Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry', *Journal of Chromatography A*, 1022(1–2), pp. 67–75. doi: 10.1016/j.chroma.2003.09.055.

Srividhya, M. *et al.* (2017) 'Bioactive Amento flavone isolated from Cassia Fistula L. leaves exhibits therapeutic efficacy', *3 Biotech*. Springer Berlin Heidelberg, 7(1), pp. 1–5. doi: 10.1007/s13205-017-0599-7.

Stratton, I. M.; Adler, A. I. and Neil, H. A. (2000) 'Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study', British Medical Journal; 321, pp. 405-412.

Sun, Q.*et al*. (2010) 'White rice, brown rice, and risk of type 2 diabetes in US men and women', *Archives of Internal Medicine*, 170, pp. 961–969.

Smit, R., Neeraj, K. and Preeti, K. (2013) Traditional Medicinal Plants Used for the Treatment of Diabetes, *International Journal of Pharmaceutical and Psychopharmacological Research*, 3 (3), pp. 171-175.

Steyn, N. P. *et al.* (2014) 'Nutritional contribution of street foods to the diet of people in developing countries: A systematic review', *Public Health Nutrition*, 17(6), pp. 1363–1374. doi: 10.1017/S1368980013001158.

Thorens, B. Mueckler, M. (2010) 'Glucose transporters in the 21st century,' American *Journal of Physiology- Endocrinology and Metabolism*; 298 pp. E141-E145.

Toiu A, Mocan A, Vlase L, Pârvu AE, Vodnar DC, Gheldiu AM, Moldovan C, Oniga I (2018). Phytochemical Composition, Antioxidant, Antimicrobial and in Vivo Anti-inflammatory Activity of Traditionally Used Romanian Ajuga laxmannii (Murray) Benth. (Nobleman's Beard – Barba Împaratului), *'Frontier Pharmacology*; 9:7

Umar, S.I.*et al* (2019) 'Antioxidant and antimicrobial activities of naturally occurring flavonoids from M. heterophylla and the safety evaluation in Wistar rats,' *Iranian Journal of Toxicology*; 13(4) pp. 39-44.

UK Prospective Diabetes Study Group (1998) 'Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33) UK Prospective Diabetes Study (UKPDS) Group', *Lancet*; 352, pp. 837-853.

Usuki, R.; Endoh, Y.; Kaneda, T. (1981) 'A simple and sensitive evaluation method of antioxidant activity by the measurement of ultra-weak chemiluminescence,' *Nippon Shokuhin Kogyo Gakkaishi*; 28(11) pp. 583- 587.

Undie, A. S. and Akubue, P. I. (1986) 'Pharmacological evaluation of Dioscorea dumetorum tuber used in traditional antidiabetic therapy', *Journal of Ethnopharmacology*, 15(2), pp. 133–144. doi: 10.1016/0378-8741(86)90150-9.

World Health Organization (2016) *Global report on diabetes*, Geneva: Author.

World Health Organization (2002) *Who launches the first global strategy on traditional medicine*, Press Release WHO 38, World Health Organization, Geneva, Switzerland.

WHO (2008) *Diet, nutrition and the prevention of chronic diseases.* Report of a joint WHO/FAO Expert Consultation; Geneva, Switzerland.

Wei, C *et al* (2016) 'Fatty acid composition and evaluation on anti-oxidation activities of okra seed oil under ultrasonic wave extraction', *Journal of Chinese Cereals and Oils Association*; 31, pp. 89 – 93.

Zadák, Z. *et al.* (2009) 'Antioxidants and vitamins in clinical conditions', *Physiological Research*, 58(SUPPL.1). doi: 10.33549/physiolres.931861.