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Article

In vitro Glucose Uptake in Yeast Cell Facilitated by *Abelmoschus esculentus L.* (Okra Seed) for Management of Type 2 Diabetes

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Abstract: In vitro glucose uptake in yeast cell 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 antidiabetic-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 R^2 demonstrations shows that the crude extract was higher 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 DPPH, 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 as drug candidates 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 nanoparticle as a drug candidate for type 2 diabetes patients. And an animal trial of the drug candidate may be necessary to monitor the in vivo performances of the extract fractions and subsequent human volunteered trial.

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

1. Introduction

Diabetes a prolonged disease condition described as hyperglycemia, insufficient insulin secretion and affects metabolic pathways (carbohydrate, protein, and lipid) affecting over 400 million people ages from eighteen years and above exist with diabetes globally, and it is prevalent in low and middle-class income countries and is projected to be the seventh primary source of death by 2030 [1]. On the other hand, type 2 diabetes, is noninsulin-dependent diabetes, widespread kind of diabetes

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totaling up to 90% of all cases, and it is a disorder primarily termed as insulin resistance, partial-insulin deficiency, and an uncommon upsurge in glucose rapidly after a meal, seen as postprandial hyperglycemia [1–3].

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 [4–7]. 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 [8] and *Abelmoschus esculentus L.* (okro seed) [9] 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 [10].

Therapeutic plants generally from the earliest times were useful to discover bioactive compounds for the formulation of medication [11]. 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 [12–14]. 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, rely to a high extent on therapeutic plants for everyday vital health [15]. At the moment, about 20% of the currently existing medications contain high percentage of phytochemicals as a part of their bioactive constituents [11]. 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 [16–19].

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 target for getting new drugs to help manage diabetes.

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 [49,50].

1.1. Rationale

The use of this nutrient from food plants within the scope of diabetes is own to the fact that this food plant extracts control glucose inflow into the cells and at the same time inhibit cell resistance.

1.2. Glycemic Control

Reports of health-related states and events globally have shown a strong association between glycemic regulation and microvascular difficulties, decreasing hemoglobin A1c (HbA1c, a blood colouring that transmits oxygen bound to glucose. Blood HbA1c levels are pointers of how well diabetes is regulated) levels less than seven percent have decreased microvascular difficulties of diabetes [20]. Every one percent decrease in HbA1c levels have led to a fourty percent decrease in diabetes-related microvascular difficulties [21,22].

2. Materials and Methods

2.1. 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),

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ferrous sulfate, acetic acid (TCA), baker's yeast, ascorbic acid (vitamin C), and metronidazole (standard diabetes drug).

2.2. 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°C for three days to scale down the moisture content.

2.3. 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.

2.4. Methanol Extraction of Plants 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°C under shaking condition. For every six hours, the solution was sonicated for twenty minutes to get the precise antidiabetic 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°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.

2.5. 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.

2.6. 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°C.

2.7. Freeze-Drying

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

2.8. 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

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separating funnel. The resultant fractions were dried at a reduced temperature of 40°C 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.* [23] 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 1).

Table 1. Showing the fractions from the partitioning of crude extract with n-Hexane, chloroform, ethyl acetate, and aqueous solution.

Sample	Hex	$CHCl_3$	EtOAc	Aqueous
Okra Seed (OS)	OH_1, OH_2	-	OE	OA

2.9. 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 [24] 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, 2 mL 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

2.10. 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.[24] 0.0005g of crude extracts and ascorbic acid as control were weighed and dissolved in 100 mL of methanol (1000µg/mL), from which different concentrations of 31.25, 62.5, 125, 250, and 500µg/mL were prepared. During this assay, 1 mL of each plant extracts, vitamin C, 1 mL of 0.2 M sodium orthophosphate buffer, and 1 mL of 1% Potassium hexacyanoferrate (III) were mixed together, and incubated at 50°C for twenty minutes. After that, 1 mL of 10% TCA was added to 1 mL of each concentration of the extracts and was mixed with 1 mL of water and 0.2 mL 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.

2.11. 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.* [25] with slight modification. Briefly, 0.5 mL of 10% egg homogenate was added to 0.1 mL 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 1 mL of water was added. Afterward, 0.05 mL of FeSO4 was added to the mixtures and incubated for half-hour. Then, 1.5 mL 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 5 mL of butanol was added to each of the reaction mixtures and centrifuged at 1200 rpm

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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.

2.12. Antidiabetic Effect of *Abelmoschus esculentus L. of Glucose Uptake in Yeast Cells*

The assay was constant with the method of Cirillo [26] 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 1 mg w/v of plant crude extracts and extract fractions was mixed with dimethyl sulfoxide ($DMSO_4$). 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 540 nm. 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).

2.13. 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.

3. Results

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

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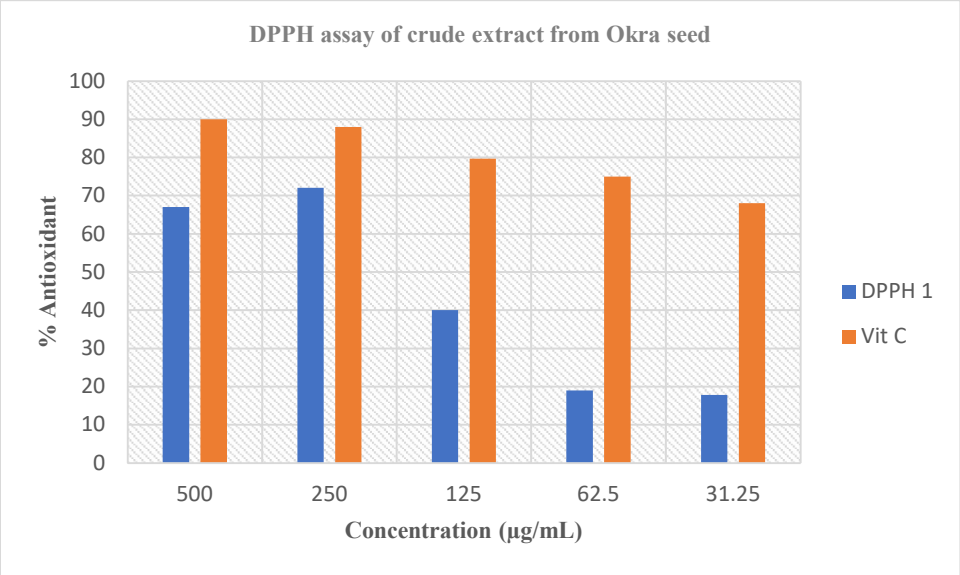

Figure 1. Percentage DPPH radical scavenging activities of crude extract of *Abelmoschus esculentus L.* (Okra Seed or OS). Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P < 0.05$.

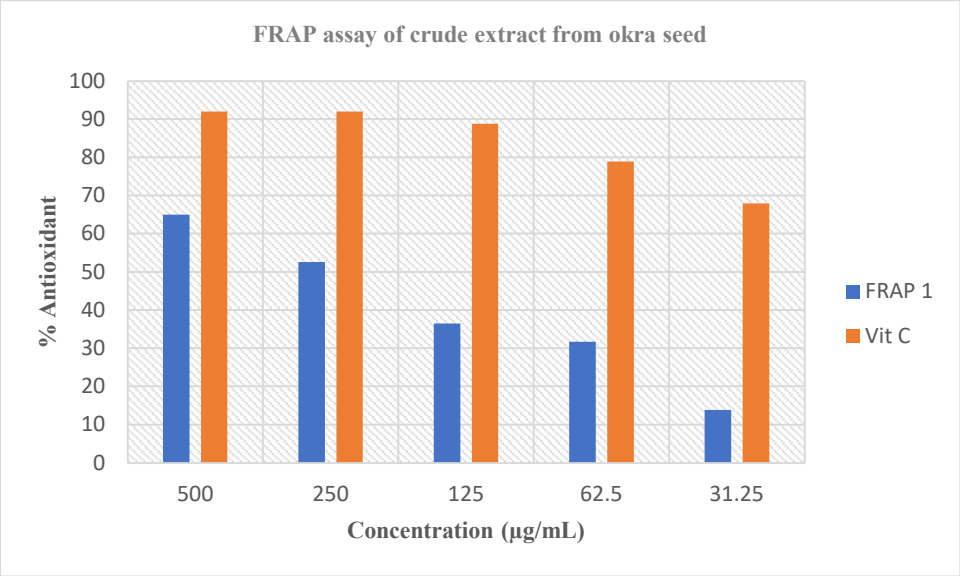

Figure 2. Percentage ferric reducing powers of crude extract of *Abelmoschus esculentus L.* (Okra Seed or OS). Values are presented as mean ± standard deviation of triplicates. Values with high ferric reducing power concentration are significantly different at $P < 0.05$.

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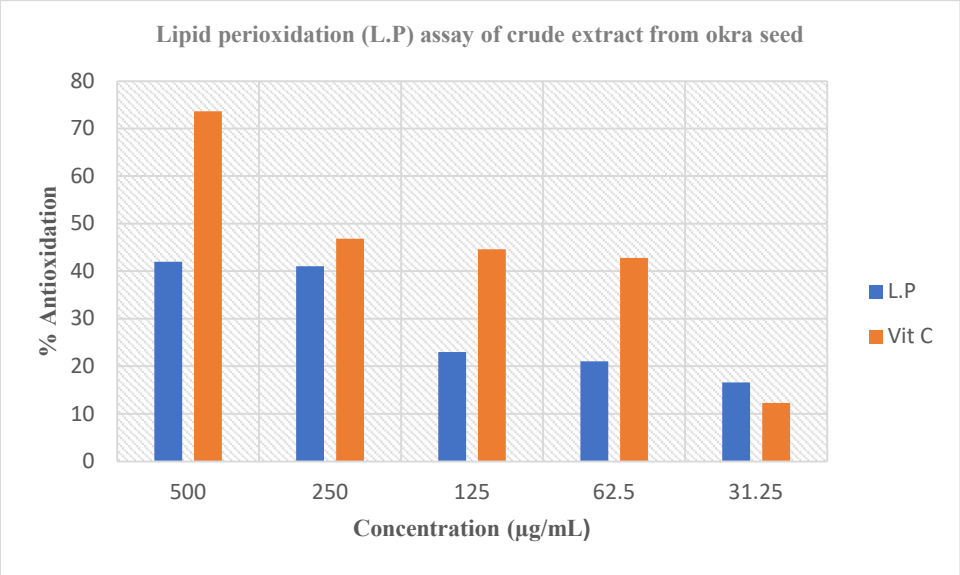

Figure 3. Percentage inhibitory activities of crude extracts of *Abelmoschus esculentus L.* (Okra Seed or OS) on lipid peroxidation (L.P). Values are presented as mean ± standard deviation of triplicates. Values with higher lipid peroxidation activity concentration are significantly different at $P < 0.05$.

3.2. Antioxidant Effect of DPPH 2, FRAP 2 and Lipid Peroxidation 2 on Extract Fractions

Table 2. 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

Table 3. 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

Table 4. 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
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

3.3. Antidiabetic Effect of Crude Extract in Yeast Cell

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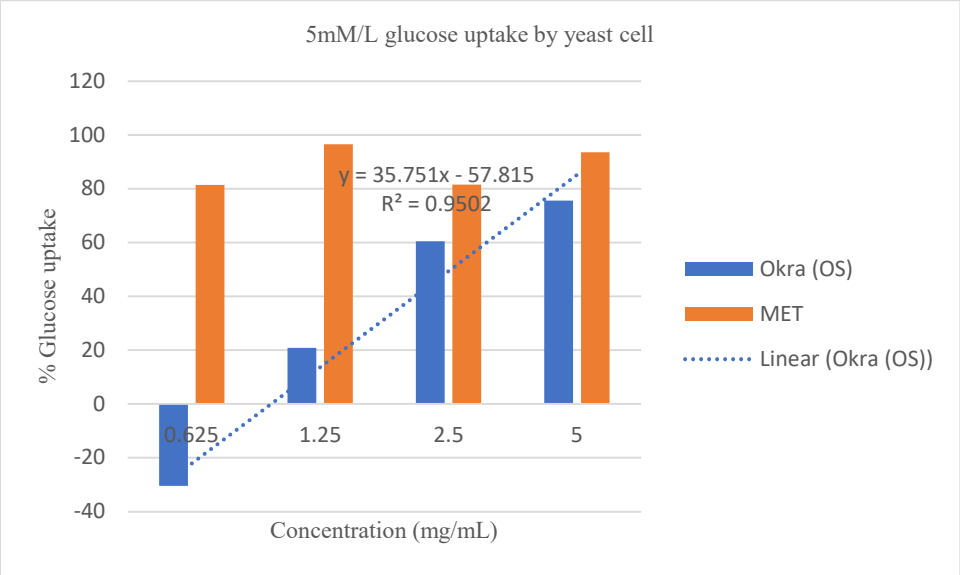

Figure 4. Glucose uptake by yeast cells at 5mM/L initial concentration of glucose in the presence of OS; crude extract of *Abelmoschus esculentus L.* (okra seed or OS); Metronidazole (MET) is a synthetic diabetes drug. The error bars represent ± SE of triplicate data. The bars are significantly different at *P* = 0.05.

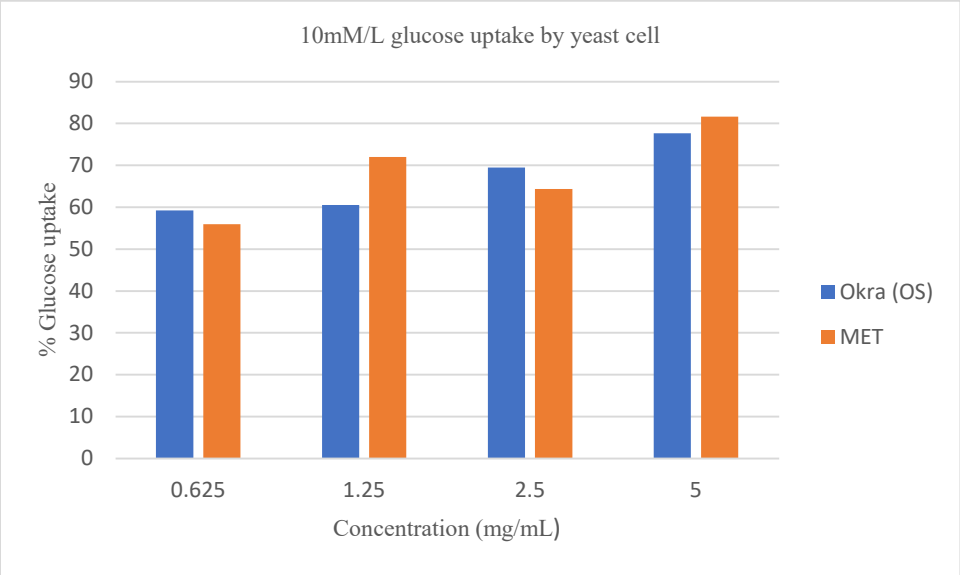

Figure 5. Glucose uptake by yeast cells at 10mM/L initial concentration of glucose in the presence of OS; crude extract of *Abelmoschus esculentus L.* (okra seed or OS); Metronidazole (MET) is a synthetic diabetes drug. The error bars represent ± SE of triplicate data. The bars are significantly different at *P* = 0.05.

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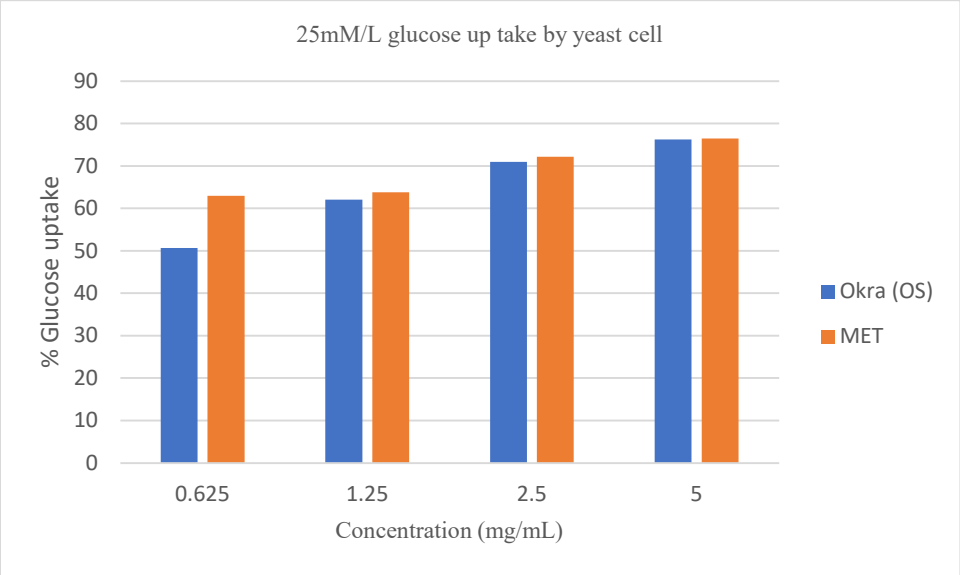

Figure 6. Glucose uptake by yeast cells at 25mM/L initial concentration of glucose in the presence of OS; crude extract of *Abelmoschus esculentus L.* (okra seed or OS); Metronidazole (MET) is a synthetic diabetes drug. The error bars represent ± SE of triplicate data. The bars are significantly different at *P* = 0.05.

3.4. Antidiabetic Effect of Extract Fractions in Yeast Cell

Table 5. 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 6. 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 7. 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

DPPH is the appropriate free radical compound typically utilized for analyzing free-radical scavenging actions of numerous forms of samples. The DPPH radical scavenging analysis generally

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relies on the ability of a compound to give hydrogen atoms; therefore, steadying the free radicals which in turn discontinue oxidation chemical mixtures well-known in lively organisms [27]. However, the DPPH radical prevents imitation of any biological combinations (and therefore has a comparatively slight significance in lively organisms), though, DPPH test is typically replicated as an indicator of the ability of plant extracts to quench free radicals, as well as their hydrogen atom or electron giving capacity, in the nonappearance of any enzymatic exploit [28]. The greatest beneficial purpose for the utilization of DPPH in evaluating in vitro antioxidant actions of medications and extracts from plant due to its greater strength than hydroxyl and superoxide radicals [29]. Therefore, the antioxidant actions showed by the extracts via DPPH scavenging capabilities Figure 1 could be said to be largely owing to their hydrogen atom or electron contribution capacity. The hydrogen-contributing ability, likewise, may be link to the existence of phenolic mixtures in the extracts as these lesser products have been proven to retain antioxidant activities [30]. Hence, it is rational to deduce that the higher activity of the crude extract of *Abelmoschus esculentus L.* extract could be as a consequence of greater concentrations of phenolics in the crude extract. The electron-contributing capacity of antioxidants in the crude extracts is usually replicated by the capacity of that particular antioxidants to decrease iron (Fe) in the oxidation form of iron 3+ (Fe^{3+}) to iron 2+ (Fe^{2+}). Thus, the greater the action of the antioxidants shows the greater electron-contributing capacity (decreasing capacity) [31]. Thus, the important actions of the extracts propose that they remained capable to decrease iron 3+ ($Fe3^{+}$) to iron 2+ (Fe2+), demonstrating their electron-contributing capacity, which evitable proposes the prospect of consuming the extracts in preventing oxidation of chemical mixtures proven in lively cells. The results gotten for the *Abelmoschus esculentus L.* extract and the percentage (%) inhibition gotten in Figure 3 remained greater. The similar aims stated in the DPPH radical scavenging assay could likewise be liable for the changes in this assay. Though, *Abelmoschus esculentus L.* extract offered important ferric decreasing power Figure 2. Lipid peroxidation has remained defined as an oxidative analysis of lipids, a procedure in which free radical's theoretical electrons from cell membrane lipids (this typically touches polyunsaturated fatty acids owing to the existence of dual bonds). The method of lipid peroxidation has been suggested to take place via a free radical chain response, which has been associated with the destruction of cell by a system of opening membrane that separate the cell from external settings [32]. The destruction initiated has been recognized to impact the ailment disorders of several persons, such ailments as cardiovascular ailments, cancer, and diabetes [33]. Thus, the capacity of the extracts to significantly prevent lipid peroxidation of the egg homogenate proposes that they remained capable to quench the activities of the free radicals by preventing the idea of the electrons from cell membrane lipids by the free radicals and thus could do the work of protecting individuals from protracted ailments and other oxidative stress-related ailments.

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

The percentage scavenging antioxidant activity (also referred to as DPPH) of the extract fractions Table 2 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; The percentage ferric reducing antioxidant power (FRAP) of the extract fractions Table 3 shows a decreased FRAP percentage capacity with a decreased extract fraction concentration. Extract fraction OE (93.62%) produced higher FRAP percentage capacity compared to other fractions. Though, the other fractions were also very high at concentrations of 500µg/mL and 250µg/mL respectively.

Also, the percentage inhibitory activity of antioxidants (also referred to as lipid peroxidation) for all extract fraction Table 4 also showed decreased inhibitory activity with decreased extract fraction concentration. Extract fractions OE (84.59%) and OH (70.54%) gave higher inhibitory ability

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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).

Effect of crude extract of *Abelmoschus esculentus L.* on glucose uptake ability by yeast cell. The crude extract of *Abelmoschus esculentus L.* stimulated the uptake of glucose through the partially but not entirely permeable membrane of yeast cells figures 4, 5, and 6 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 4 and 5). 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 6).

Moreover, at 0.625 mg/mL the linear equations, and R^2 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 4). 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 R^2 value of 12.1% against the crude extract R^2 value of 92.3% respectively. On the other hand, figures 5 and 6 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 4, 5, and 6).

Remarkably, the fraction OE was higher than the standard drug at a lower extract fraction concentrations of 125µg/mL (79.55 %), 62.5µg/mL (81.58%), 31.25µg/mL (81.65 %) which showed a different trend of increase in percentage glucose uptake with a decrease in extract fraction concentration.

4. 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 [34]. 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 [35]. Similarly, indicators of the likely antidiabetic possibility of a medication is evaluated via numerous in vitro analyses, provided that evidences for its in vivo antidiabetic possibility. As well the antioxidant evaluates [36], numerous additional pointer analyses comprise (i) prospect of glucose uptake through the plasma membrane similar to that of yeast cells [37], others like fat cells [38], or cells of the muscle; [39] (ii) capacity of glucose taken on the surface [38]; (iii) stoppage of α-amylase and α-glucosidase enzymes are mainly engaged for in vivo investigations. For the extract fractions in this study to have high antioxidant activity (figures 4 to 5 and tables 5 to 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 [40], reported that a diabetic agent can exercise a beneficial effect by increasing insulin secretion, improving, and imitating insulin action. This statement agrees with

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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 antidiabetic 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.* (Okra Seed or OS) [41,42]. 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, dodecatetrae namide, and phenethylamine [43–47]. 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, bring about low blood sugar outcome [39]. 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 multicellular 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, (this current invastigation emphases on in vitro) 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.

5. Conclusions

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. This finding correlates with the report of Rehman *et al.* [47].

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 for type 2 diabetes patients.

And an animal trial of the drug will be necessary to monitor the in vivo performances of the drug and subsequent human volunteered trial.

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References

1. World Health Organization. Global report on diabetes. Geneva. 2016; Author.

2. Kwon YI, Apostolidis E, Kim YC, Shetty K. Health benefits of traditional corn, beans, and pumpkin: In vitro studies for hyperglycemia and hypertension management. J Med Food. 2007;10(2):266–75.
3. Odebode FD, Ekeleme OT, Ijarotimi OS, Malomo SA, Idowu AO, Badejo AA, et al. Nutritional composition , antidiabetic and antilipidemic potentials of flour blends made from unripe plantain , soybean cake , and rice bran. 2017;(July):1–9
4. Popkin BM. The shift in stages of the nutrition transition in the developing world differs from past experiences! Malays J Nutr. 2002;8(1):109–24.
5. Kapoor SK. Anand K. Nutritional transition: a public health challenge in developing countries. J Epidemiol Community Health. 2002; 56:804–805.
6. Sun, Q. et al. White rice, brown rice, and risk of type 2 diabetes in US men and women. Archiv.Intern. Med. 2010; 170:961–969.
7. Eleazu C, Okafor P. Antioxidant effect of unripe plantain (Musa paradisiacae) on oxidative stress in alloxan-induced diabetic rabbits. Int J Med Biomed Res. 2012;1(3):232–41.
8. Undie AS, Akubue PI. Pharmacological evaluation of Dioscorea dumetorum tuber used in traditional antidiabetic therapy. J Ethnopharmacol. 1986;15(2):133–44.
9. Smit, R., Neeraj, K. and Preeti, K. Traditional Medicinal Plants Used for the Treatment of Diabetes. Int. J. of Pharma 2013; 3(3):171-175.
10. World Health Organization Who launches the first global strategy on traditional medicine. Press Release WHO 38. World Health Organization, Geneva. 2002; Switzerland.
11. WHO Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO Expert Consultation. 2008; Geneva. Switzerland
12. Balogun, J. B. et al. In Vivo Anti-Trypanosomal Potential of Methanol Root Extract of Terminalia macroptera (Guill. And Perr.) In Trypanosoma Brucei Brucei Infected Wistar Rat. IOSR J Pharm Biol Sci. 2017; 12(5):12-17.
13. Akpovona AE, Onoagbe IO, Eze GI, Omonkhua AA. Acute and subchronic toxicity studies of ethanol extract of terminalia macroptera stem bark in wistar albino rats. J Med Biomed Res. 2016;15(1):62–73.
14. Mfotie Njoya E, Weber C, Hernandez-Cuevas NA, Hon CC, Janin Y, Kamini MFG, et al. Bioassay-Guided Fractionation of Extracts from Codiaeum variegatum against Entamoeba histolytica Discovers Compounds That Modify Expression of Ceramide Biosynthesis Related Genes. PLoS Negl Trop Dis. 2014;8(1):29.
15. Toiu A, Mocan A, Vlase L, Pârvu AE, Vodnar DC, Gheldiu AM, Moldovan C, Oniga I. Phytochemical Composition, Antioxidant, Antimicrobial and in Vivo Anti-inflammatory Activity of Traditionally Used Romanian Ajuga laxmannii (Murray) Benth. ("Nobleman's Beard – Barba Împaratului). Front. Pharmacol. 2018; 9:7.
16. Umar, S.I. et al. Antioxidant and antimicrobial activities of naturally occurring Flavonoids from M. heterophylla and the safety evaluation in Wistar rats. Iran. J. Toxicol. 2019; 13(4):39-44.
17. Onukogu, S. C. et al. In vitro antioxidants, antimicrobials and biochemical response of methanol leaf extract of Eucalyptus camaldulensis following sub-acute administration to rats. SJBR. 2019; 4(11):405-11.
18. Madaki FM, Kabiru AY, Mann A, Abdulkadir A, Agadi JN, Akinyode AO Phytochemical Analysis and In-vitro Antitrypanosomal Activity of Selected Medicinal Plants in Niger State, Nigeria IJBCRR, 11(3): 1-7, 2016; Article no. IJBCRR.24955.
19. Lawal B, Shittu O, Kabiru A, Jigam A, Umar M, Berinyuy E, et al. Potential antimalarials from African natural products: a review. J Intercult Ethnopharmacol. 2015;4(4):318.
20. UK Prospective Diabetes Study Group. 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. 1998; 352:837-853.
21. Stratton IM, Adler AI, Neil HA. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000; 321:405-412.
22. Centers for Disease Control and Prevention. National diabetes fact sheet, 2011. www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf. Accessed January 15, 2015
23. Kabir OA, Olukayode O, Chidi EO, Christopher CI, Kehinde AF. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. BMC Complement Altern. 2005; 5:6.
24. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. J. Nutr. 1986; 44:307-315.

25. Halliwell B. How to characterize an antioxidant: an update In: Biochemical Society Symposia. 1995 61:73-101). Portland Press Limited.
26. Cirillo VP. Yeast Cell Membrane. 1962;485–91.
27. Kusuma IW, Arung ET, Kim, YU. Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Sci. Hum. Wellness. 2014; 3(3-4):191-196.
28. Mileva M, Kusovaski V, Krastev D, Dobreva A, Galabov A. Chemical composition, in vitro antiradical and antimicrobial activities of Bulgarian Rosa alba L. essential oil against some oral pathogens. Int J Curr Microbiol App Sci. 2014;3(7):11–20.
29. Li W, Hosseinian FS, Tsopmo A, Friel JK, Beta T. Evaluation of antioxidant capacity and aroma quality of breast milk. Nutrition [Internet]. 2009;25(1):105–14.
30. Gruz J, Ayaz FA, Torun H, Strnad M. Phenolic acid content and radical scavenging activity of extracts from medlar (Mespilus germanica L.) fruit at different stages of ripening. Food Chem [Internet]. 2011;124(1):271–7.
31. Amari NO, Bouzouina M, Berkani A, Lotmani B. Phytochemical screening and antioxidant capacity of the aerial parts of Thymelaea hirsuta L. Asian Pacific J Trop Dis. 2014;4(2):104–9.
32. Halliwell B. Protection against tissue damage in vivo by desferrioxamine: What is its mechanism of action? Free Radic Biol Med. 1989;7(6):645–51.
33. Usuki R, Endoh Y, Kaneda T. A simple and sensitive evaluation method of antioxidant activity by the measurement of ultraweak chemiluminescence. NSKG (LTWA). 1981; 28(11):583- 587.
34. Atlas ID. Brussels, Belgium: international diabetes federation. IDF. 2013; 2014.
35. Lebeau J, Furman C, Bernier JL, Duriez P, Teissier E, Cotelle N. Antioxidant properties of di-tert-butylhydroxylated flavonoids. Free Radic Biol Med. 2000;29(9):900–12.
36. Marinova G, Batchvarov V. Methods DPPH. Bulg J Agric Sci. 2011;17(1):11–24.
37. Bhutkar M, Bhise S. In vitro hypoglycemic effects of Albizzia lebbeck and Mucuna pruriens. Asian Pac J Trop Biomed. 2013;3(11):866–70.
38. Gulati V, Gulati P, Harding IH, Palombo EA. Exploring the anti-diabetic potential of Australian Aboriginal and Indian Ayurvedic plant extracts using cell-based assays. BMC Complement Altern Med. 2015;15(1):1–11.
39. Rajeswari R, Sriidevi M. Study of in vitro glucose uptake activity of isolated compounds from hydro alcoholic leaf extract of cardiospermum halicacabum linn. Int J Pharm Pharm Sci. 2014;6(11):181–5.
40. Srividhya M, Hridya H, Shanthi V, Ramanathan K. Bioactive Amento flavone isolated from Cassia fistula L. leaves exhibits therapeutic efficacy. 3 Biotech. 2017;7(1):1–5.
41. Gray, A. M. and Flatt, P. R. (1997a) 'Nature's own pharmacy: the diabetes perspective', *Proceedings of the Nutrition Society;* 56, pp. 507–517.
42. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Shin LN, et al. Bioassay-directed isolation of active compounds with antiyeast activity from a cassia fistula seed extract. Molecules. 2011;16(9):7583–92.
43. Arapitsas P. Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chem. 2008;110(4):1041–5.
44. Shui G, Leong LP. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. J Chromatogr A. 2004;1022(1–2):67–75.
45. Atawodi SE, Atawodi JC, Idakwo GA. Polyphenol composition and antioxidant potential of Hibiscus esculentus L. fruit cultivated in Nigeria. J. Med. Food. 2009;12(6):1316–1320.
46. Rajagopal, P. et al. Phytochemical and pharmacological review on Cassia fstula linn. The golden shower. Int. J. Pharm. 2013; 3(3).
47. Kadhim MJ, Mohammed GJ, Hameed IH. In vitro antibacterial, antifungal and phytochemical analysis of methanolic extract of fruit Cassia fstula. Orient J. Chem. 2016; 32(3):1329–1346.
48. Rehman G, Hamayun M, Iqbal A, Ul Islam S, Arshad S, Zaman K, et al. In vitro antidiabetic effects and antioxidant potential of cassia nemophila pods. Biomed Res Int. 2018;2018.
49. Aziz MA, Majeed GH, Diab KS, Al-Tamimi RJ. The association of oxidant-antioxidant status in patients with chronic renal failure. Ren Fail. 2016;38(1):20–6.
50. Law BMH, Waye MMY, So WKW, Chair SY. Hypotheses on the potential of rice bran intake to prevent gastrointestinal cancer through the modulation of oxidative stress. Int J Mol Sci. 2017;18(7).

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