IN VITRO ANTIOXIDANT POTENTIAL AND INHIBITORY EFFECT OF HYDRO-ETHANOLIC EXTRACT FROM AFRICAN BLACK VELVET TAMARIND (DIALIUM INDIUM) PULP ON TYPE 2 DIABETES LINKED ENZYMES

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ABSTRACT
The alarming rate of diabetes mellitus (DM) globally is bothersome and has drawn the search light of researchers on naturally endowed phytonutrients being an alternative in managing the menace. Therefore, the current study was designed to investigate some antioxidant parameters embedded in the extract of Dialium indium (DI) fruit pulp and also, to elucidate its antidiabetic potentials through the inhibition of two key carbohydrate-metabolizing enzymes such as α-amylase and intestinal α-glucosidase. Hydro-ethanolic extract of DI fruit pulp was used for the antioxidants and enzyme inhibitory bioassays through various convectional antioxidant assay methods in vitro. In the results, total phenolic content of the extract had; 6.74 ±3.38 mg GAE.g⁻¹, total flavonoid contents; 0.02 ±0.01 mg QE.g⁻¹ and FRAP; 0.84 ±0.47 mg AAE.g⁻¹ dried sample. Also, there was a marked significant (p<0.05) difference observed in the inhibition of α-amylase and intestinal α-glucosidase by the different concentrations of the extract used in concentration-dependent manner with their different EC₅₀. The inhibition demonstrated against these two carbohydrate metabolizing enzymes possibly could be through the embedded antioxidant potentials of the fruit pulp and this if properly harnessed, it could be helpful in the management of type 2 diabetes.

Keywords: Type 2 Diabetes; α-amylase; Dialium indium; α-glucosidase; antioxidant

INTRODUCTION
Type 2 diabetes (T2D) is a common and chronic disorder caused by impaired cellular carbohydrate metabolism characterized by elevated serum glucose level (Beverley and Eschwege, 2003). Pancreatic beta-cell damage has been major cause of this disorder, leading to failure of β-cells to appropriately secrete insulin (Porte and Kahn, 2001). Extremely elevated glucose level, if not treated could induce the mitochondria and non- mitochondria generation of free radicals (Oyedemi et al., 2017), that subsequently could lead to damage by the free radicals in various tissues (Afolabi et al., 2018a; Nishikawa et al., 2000; Dave and Kalia, 2007). The prolonged exposure of pancreatic β-cells to these reactive oxidative species, utters insulin genetic expression and insulin production as a result of little antioxidant enzymes’ capacity (Robertson and Harmon, 2007). Other innate to disease factors namely; hyperinsulinemia, insulin resistance, damaged insulin production, declined insulin mediated reducing sugar absorption and metabolism have been evident as the cause of this difference between blood glucose intake and pancreatic insulin production (Gruenwald et al., 2010). However, there are indications that, environmental and lifestyle factors also are of major considerations in the cause of T2D (Zimmet et al., 2001). T2D is more prevalent than type 1 diabetes and rapidly growing globally with accounts for over ninety-five percent of the world diabetic population (Inga et al., 2008). Regulation of postprandial hyperglycemia level is important in delaying or preventing T2D (Madhusudhan and Kirankumar, 2015), however, therapeutic approach is a common strategy for decreasing postprandial hyperglycemia in T2D patients, through which there is inhibition of sugar metabolizing enzymes such as alpha-glucosidase and alpha-1,4-glucan-4-glucanohydrolases in the gastrointestinal tract (GIT) (Krentz and Bailey, 2005; Shim et al., 2003). These inhibitions cause reduction in the carbohydrate digestion process and subsequently lessening the postprandial plasma glucose rise (Rhabasa-Lhoret and Chiasson, 2004). The choice pharmaceuticals
in managing diabetes which has taken the highest trend have been reported to have side effects (Stein et al., 2013; Singh et al., 2007; Hung et al., 2012), likely to be low-sugar level, diarrhea, gassiness, and bowel swelling and these have reduced their application in managing T2D.

Alpha- amylases and alpha-glucosidases inhibition have been demonstrated by plants’ naturally enriched secondary metabolites with promising effects than many commercially available α-glucosidase inhibitors and starch blockers (α-amylase inhibitors) used in the management of T2D (Jalalpure et al., 2004; Hilary et al., 1998; Erasto et al., 2005). These plants are characteristically endowed with polyphenolic compounds, with the ability to interact with proteins, inhibit enzymatic activity, boost the immune system and causes inhibition of pathogenesis in the disease condition and as well has a terminal effect on pathogenic agents (Afolabi and Oloyede, 2014; McCue et al., 2005; Dawra et al., 1988).

Dialium indium (DI) commonly known as black velvet tamarind (Figure 2) is among the list of plants that exhibit this natural biological response against infectious and non-infectious diseases. The current work was put together to study in vitro the antioxidant potentials, alpha-amylase and alpha-glucosidase inhibitory ability of combined solvent extract (Water: Ethanol, 70:30) of DI pulp commonly eaten in Southeastern part of Nigeria (Nwaukwu and Ikechi, 2012).

**Scientific hypothesis**

If different mg.mL⁻¹ of the same extracts is in the future subjected to the same assays as found in the current work, it could be scientifically assumed that same trend would be achieved, only if the same methods and procedures are engaged.

**MATERIAL AND METHODOLOGY**

**Chemicals and Reagents**

Chemicals and reagents used such as thiobarbituric acid (TBA), gallic acid, Folin-coicoalteau’s reagent, intestinal α-glucosidase (EC 3.2.1.20), pancreatic α-amylase (EC 3.2.1.1), p-nitrophenyl-α-D-glucopyranose (PNPG), Sodium carbonate, Aluminum chloride (AlCl₃), FeCl₂ (Iron II chloride), Sodium hydroxide (NaOH) and Potassium ferricyanide were used with 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trichloroacetic acid (TCA) sourced from Sigma-Aldrich, Inc. (St Louis, MO). All these and other chemicals used were of analytical grades and prepared in all-glass apparatus using sterilized distilled water.

**Sample collection and preparation**

*Dialium indium (DI)* fruits were obtained from a popular place in Ikare Akoko, Ondo State, Nigeria. The voucher sample was dropped at the herbarium of the Department of Plant Science in Ekiti State University, Nigeria. where it was authenticated. Thereafter, the dried sample was blended and kept at moderate temperature.
Preparation of 70% hydro-ethanolic of DI pulp
The blended DI fruit pulps were air-dried to a constant weight at 37 °C ± 2 °C, about 120 g of the sample was weighed and soaked in 70% ethanol for 72 h. Thereafter, the mixture was processed and the extract concentrated at 50 °C to dryness.

Antioxidant Content assays

Total phenolic content assay (TPC)
Hydro-ethanolic extract of DI pulp was used to determine TPC using the method of Singleton et al. (1999). An appropriate volume of the extract was added to 10% folin-ciocalteau’s solution and Sodium carbonate (anhydrous). The mixture was later incubated for 45 °C for 40 min. The absorbance was read at 700 nm.

Total flavonoid content assay (TFC)
Total flavonoid content of hydro-ethanolic of DI pulp was determined according to the method of Bao et al. (2005). An appropriate volume of the extract was added to 5% sodium nitrate at zero time. 5 min later, AlCl₃ was added and after 6 min, NaOH was added followed by the addition of distilled water. The absorbance of the mixture was taken at 510 nm.

Antioxidant Activity Assays
Ferric reducing antioxidant power (FRAP)
The ferric reducing power of hydro-ethanolic of DI pulp was carried out using Pulido et al. (2000). Appropriate volume of the extract was mixed with 200 mM of sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, thereafter 250 µL of 10% trichloroacetic acid was added and centrifuged at 2000 rpm for 10 minutes, 250 µL of one percent FeCl₃ was added to appropriate volume of the supernatant and the absorbance taken in the spectrophotometer at 700 nm.

1, 1 diphenyl, 2-picrylhyrazine (DPPH) radical scavenging ability assay
Inhibitory effect of hydro-ethanolic extract of DI pulp was carried out on DPPH by Gyanifi et al. (1999). The same proportion of the extract and methanolic solution of the DPPH was left in the dark in tubes for 30 min and the absorbance taken in the spectrophotometer at 516 nm.

Hydroxyl radical scavenging assay
Hydro-ethanolic extract of DI pulp inhibitory potential against degradation of deoxyribose was determined by method described by Halliwell and Gutteridge (1981). Appropriate dilution of the extract was added to a reaction mixture containing 20 mM deoxyribose, 0.1 M phosphate buffer (pH 7.4), 20 mM hydrogen peroxide and 500 µM FeSO₄ and this was made up to 800 µL with distilled water. The reaction was incubated at 37 °C for a while, and the reaction stopped by the addition of 2.8% trichloroacetic acid, then TBA solution as the colour forming agent. The reaction was boiled for 20 min and the absorbance taken in the spectrophotometer at 532 nm.

Nitric oxide radical scavenging ability
Inhibitory effect of DI pulp hydro-ethanolic extract on NO radical was carried out according to the method of Mercocci et al. (1994). An appropriate volume of the extract was incubated with 25 mM Sodium nitroprusside solution and subsequently incubated for 2 h at room temperature. 500 µL of the incubated mixture was added to 300 µL of Griess solution. The absorbance of the mixture was read in the spectrophotometer at 570 nm and the results expressed as percentage radical scavenging ability.

Enzymes inhibitory Assays
Pancreatic alpha-amylase (EC 3.2.1.1) inhibitory in vitro assay
Inhibitory potential of hydro-ethanolic extract of DI pulp against amylase was carried out according to the method of Shai et al., 2010. Different volumes of the Hydro-alcoholic extract were added to an appropriate volume of the enzyme (2 U.mL⁻¹) in 0.1 M Sodium phosphate buffer (pH 6.8) and incubated at 37 °C for 20 min. 1% soluble starch in 0.1 M Sodium phosphate buffer (pH 6.8) was added to the reaction mixture and incubated at 37 °C for 1 h. 1 µL of 3.5 DNSA reagent was added to the reaction mixture boiled for 10 min. The absorbance of the test was taken in the spectrophotometer at 540 nm.

Intestinal alpha-glucosidase (EC 3.2.1.20) inhibitory in vitro assay
Inhibitory potential of hydro-ethanolic extract of DI pulp against glucosidase was carried out according to the

Figure 2 Different diagrams of African black velvet tamarind (Dialium indium).
method of Ademiluyi and Oboh (2013). Diluted volumes of the extract were added to alpha-glucosidase (1U.mL⁻¹) solution in 0.1 M Sodium phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, pNPG solution (0.005 M) in 0.1 M Sodium phosphate buffer (pH 6.8) was added and the mixture was kept at room temperature for 20 min. The absorbance of the p-nitrophenol released was measured in the spectrophotometer at 405 nm.

**Determination of IC₅₀/EC₅₀**

Determination of IC₅₀/EC₅₀ values was carried out from the plot of percentage inhibition caused by the various concentrations of the extract against different concentrations of the extract concentrations. The IC₅₀ / EC₅₀ was then calculated using a linear regression curve.

**Statistical analysis**

All experiments were carried out in duplicate. Data were expressed as mean ± standard deviation (SD). Differences were evaluated by one-way analysis of variance (ANOVA) followed by Duncan’s multiple test (Zar, 1984). Significance was accepted at p-value < 0.05.

**RESULTS**

**Antioxidant Contents**

Table 1. Shows the possible antioxidant contents in the hydro-ethanolic extract from DI pulp. The total phenolic content revealed significant (p <0.05) in value (mg GAE.g⁻¹ of the dried sample) higher than both the total flavonoid (mg QE.g⁻¹ of the dried sample).

**Antioxidant Activities**

Table 1 Shows ferric reducing potential antioxidant activity (mg Ascorbic acid equivalent/g of the dried sample) of the hydro-ethanolic extract from DI pulp.

| Table 1 | The results for total phenolic contents, total flavonoid and ferric reducing property (FRAP) of DI pulp hydro-ethanolic extract. |
| --- | --- | --- |
| Total Phenolic (mg GAE.g⁻¹) | Total Flavonoid content (mg QE.g⁻¹) | Ferric reducing power (mg AAE.g⁻¹) |
| 6.74 ±3.38 | 0.02 ±0.01 | 0.84 ±0.47 |

The results were expressed as mean values ±SD (n=2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The IC₅₀ values in µg.ml⁻¹ (Concentration of the extracts that will cause 50 percent inhibition) of hydro-ethanolic extract of DI pulp were calculated from a linear regression curve of the percentage (%) inhibitions against various concentrations of the extracts.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitory concentration (IC₅₀)</td>
<td>DPPH Radical Scavenging 179.08 ±1.66</td>
</tr>
<tr>
<td></td>
<td>Hydroxyl radical scavenging ability 362.05 ±0.01</td>
</tr>
</tbody>
</table>

Results represent mean values of duplicated sample ±SD, n = 2. Various concentrations were used for the assay to determine the IC₅₀.

| Table 3. | The EC₅₀ values in µg.ml⁻¹ (Concentration of the extracts that will cause 50 percent inhibition of the enzyme) of hydro-ethanolic extract of DI pulp. |
| --- | --- | --- |
| Enzyme | α-Amylase | α-glucosidase |
| EC₅₀ | 0.52 ±0.06 | 0.45 ±0.02 |

The results were expressed as mean values of duplicated sample ±SD, n = 2.
Carbohydrate-metabolizing enzymes have been considered to be important in monitoring diabetes mellitus most especially in people living with T2D (Krentz and Bailey 2005). More also, the inhibitory and ameliorating potentials exhibited in the management of diabetes by plants with medicinal values have been credited to the presence naturally endowed polyphenols, which are considered to be potential antioxidants due to the reduction-oxidation properties of their hydroxyl groups (Afolabi and Oloyede, 2014; Materska and Perucka 2005). These plant secondary metabolites fight against generation/proliferation of metabolically induced free radicals which are potential causative agents involve in diabetes. They are readily available in plants in isomeric forms of flavonoids and phenolic acids (Oberley 1988; Bravo, 1998). Most recent studies have implicated flavonoids to have shown inhibition against ROS generation (Jo et al., 2009).

**Figure 3** DPPH· radical scavenging ability of hydro-ethanolic extract of DI pulp.

**Figure 4** Hydroxyl radical scavenging ability (%) hydro-ethanolic extract of DI pulp.
In the current finding, from Table 1, phytonutrients components in the hydro-ethanolic extract of DI pulp were revealed, total phenolic content showed predominant value over total flavonoid and ferric reducing power (FRAP) contents, the reason behind this could not be clearly explained, but it could be attributed to the antioxidant properties embedded in the extract as shown in table 1, however, the finding was in agreement with the result of a Inglett and Chen (2011), that showed high value of phenolic than flavonoid content in the pulp extract of Synsepalum dulificum Ziping et al. (2009), their results revealed higher polyphenolic content in the extracts of different Ziziphus jujube cultivars. However, in Figure 3 – 5, the ability of the hydro-ethanolic extract of DI pulp to inhibit the generation of free radicals were clearly shown. In Figure 3 the antioxidant potential of the extract was demonstrated with the IC₅₀ represented in Table 2. Several studies have shown the generation of free radicals to be underlying mechanism of action of some commonly available diabetogenic agents (Afolabi et al., 2018).

Antioxidant enriched extracts have been reported to play a pivotal role in the management of Diabetes by scavenging the various ROS generated in the disease. Similarly, as shown from the Figure 4, degradation of deoxyribose inhibitory ability of the hydro-alcoholic extract of DI pulp, in the presence of Fe²⁺/H₂O₂ exhibited percentage inhibitory rise in concentration dependent manner, the mechanism for the inhibition has not been reported in the current work but, it could be attributed to the antioxidant capacity of the extract as shown in Table 1. In the same vein, Figure 5, reveals the inhibitory potential of DI pulp against nitric oxide radical, the involvement of NO• radical has been implicated in various diseases through the combination with superoxide radical (O₂•⁻) generated through Fenton reaction that results in the generation of peroxynitrite (ONOO⁻), a potential cytotoxic molecule, through which oxidation can damage cellular proteins (Obafemi et al., 2016). In the result, all the concentrations of the extract examined caused noticeable changes in the inhibition against the latent production of peroxynitrite. The underlying reason(s) for the several evident inhibitory
potentials of DI pulp against oxidant species is/are not known but it could be credited to the presence of quantifiable antioxidant contents present in the hydroalcoholic extract of DI pulp mainly the phenolic acid compound as revealed in Table1.

To fully establish the antidiabetic effects of hydroethanolic extract of DI pulp, inhibitory potentials of the extract against enzymes involved carbohydrate metabolism were appraised. It has been reported that plants that are rich in secondary metabolites (i.e polyphenols) are potential inhibitors of α-amylases and α-glucosidases (Afolabi et al., 2018b; Kwon et al., 2007). Therefore, pancreatic α-amylase and intestinal alpha-glucosidase inhibitions are shown in the Figure 6 and Figure 7 respectively. In the Figures, it was clearly shown that hydro-alcoholic extract of DI exhibited inhibitory potentials in all the concentrations examined against these metabolizing enzymes. Apparently, it could be deduced that the inhibitory potentials shown against this carbohydrate-hydrolyzing enzymes could be attributed to the fact that, the extract exhibited considerable phenolic content, as revealed in the Table 1.

CONCLUSION
In the current study, inhibitory potential on the carbohydrate-hydrolyzing enzymes involved in T2D has apparently been so demonstrated by the hydro-alcoholic extract of DI pulp, this possibly could have been as a result of the embedded antioxidant contents through the free radicals scavenging ability. Howbeit, the possible mechanism (s) of most antidiabetic drugs is/are to target delay in carbohydrate break down process and to cause prolonged overall carbohydrate metabolism time frame, thereby reducing the activities of carbohydrate-metabolizing enzymes and rate of glucose absorption. Thus, the pulp could be suggested helpful as an alternative in the management of T2D when incorporated in diet.

REFERENCES


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