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IN-VITRO INHIBITION OF TYPE 2 DIABETES KEY ENZYMES; EFFECT OF EXTRACTS AND SOLVENT-SOLVENT FRACTIONS OF DANIELLIA OLIVERI (ROLFE) HUTCH. & DALZIEL

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Daniellia oliveri is a tree species belonging to the subfamily Caesalpinioideae (Leguminosae), whose young leaves are used locally to manage type 2 diabetes in Nigeria. This study aims at evaluating the inhibitory potentials of its crude ethanolic extract (Do-C) and solvent-solvent fractions (n-hexane (Do-H), diethyl ether (Do-D), and ethyl acetate (Do-E)) obtained from Do-C on α-amylase, α-glucosidase activities in-vitro and 1,1-diphenyl-2-picraylhydrazine (DPPH) radical scavenging activity using standard protocols. Our findings showed that the Do-C and its fractions had significant TFC and TPC composition. All fractions inhibited DPPH free radicals effectively, with Do-E having excellent inhibition when compared with BHT. In this study, Do-C and its solvent-solvent fractions (Do-D, Do-E, and Do-H) inhibited α-amylase and α-glucosidase in a dose-dependent pattern. However, compared to acarbose, the Do-E exhibited similar inhibitory activity against α-amylase (P≤ 0.05). Nevertheless, the Do-E (IC50 35.02 ± 1.22 µg/ml) and Do-H (IC50 31.28 ± 1.23 µg/ml) had the best inhibitory activity against α-glucosidase comparatively after acarbose (25.97 ± 0.96 µg/ml). The inhibitory potential of Do-E could be linked to its TFC and TPC. Therefore, ethyl acetate fraction obtained from the crude ethanolic extract of D. oliveri could effectively inhibit key enzymes linked to type 2 diabetes (α-amylase and α-glucosidase). Further studies recommended to isolate antidiabetic compounds present.

INTRODUCTION

Hyperglycemia, or a high blood glucose level exemplified by a set of metabolic diseases, is caused by a defect in insulin secretion, action, or even both[1&2]. Diabetes mellitus is a prevalent disease that the World Health Organization classifies as an epidemic[3]. Diabetes was categorised into four classes based on pathogenicity and not its treatment[4]. Type 1 and type 2 diabetes mellitus are widespread diabetic conditions. Other kinds of diabetes include gestational diabetes and diabetes associated with other disorders or drug use[2&5]. Type 1 diabetes mellitus (T1DM) is due to β-cell damage, which results in absolute insulin insufficiency and is mainly mediated by immunological processes[6&7]. β-cell destruction prevents insulin release and reduces the rate of glucose absorption into muscles and adipose tissues[8].

Hyperglycemia caused by impaired insulin production, insulin action, and excessive glucagon secretion causes type 2 diabetes mellitus (T2DM), a chronic metabolic condition with life-threatening consequences[9,10]. As documented, 90% of diabetes incidence worldwide is T2DM[11]. It is most prevalent in obese people and is linked to hypertension and dyslipidemia. The medication to manage the disease condition improves insulin sensitivity while also increasing insulin secretion[12]. In 2015, it was projected that 415 million individuals globally had diabetes[13], which grew to 451 million (age 18-99 years) in 2017, with about half of the cases

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undiagnosed[14]. International Diabetes Foundation (IDF) reported that there were 463 million persons with diabetes globally in 2019, with projections of 578 million by 2030 and 700 million by 2045[11].

Natural α-amylase and α-glucosidase inhibitors derived from food-grade plants are commonly used to treat rise in blood sugar after the meal in type 2 diabetic patients[15]. α-glucosidase inhibitors, viz; acarbose[16], trestatin[17], and amylostatin[18] isolated from microorganisms, were reported to regulate glucose uptake in the intestine. The use of these inhibitors, particularly acarbose, was associated with adverse consequences viz; stomach distention, flatulence, meteorism, and diarrhoea[19&20].

Daniellia oliveri, commonly known as West African Copal tree, African copaiba balsam, Ilorin balsam, and Benin gum copal, is a member of the Caesalpinioideae subfamily (Family Leguminosae). *Daniellia oliveri* is the lone member of *Daniellia* subgenera *Paradaniellia.* The species "*oliveri*" is the *Daniellia's* most extensively dispersed species and are found throughout the lowland Savannah south of the Sahel[21]. Traditionally, the powdered dried leaf is administered orally to treat yellow fever and backache[22]. It has been documented that a decoction of *D. oliveri* roots and the root of *Sarcocephalus latifolius* is used as an antihyperglycemic treatment in southern Nigerian folk medicine[23]. Ringworm, scrotal elephantiasis, diarrhoea, syphilis, typhoid fever, and earache have been reportedly treated with the roots, stem barks, and leaves of *D. oliveri*[24]. In Nigeria, the young, tender leaves of *D. oliveri* are eaten[25]. The aqueous extract of the leaves was reported to be a safe and efficient treatment for T2DM[26].

Notwithstanding the increasing usage of medicinal plants in most parts of the world as hypolipidemic[27] and hypoglycaemic agents [28&29] in managing chronic diabetes and its related complication, some medicinal plants are still understudied, one of which is *D. oliveri*. Various parts of *D. oliveri* have been reported in therapeutic applications. However, there is limited empirical information on using fresh young leaves to manage type 2 diabetes. Hence, our study investigates the *in-vitro* DPPH radical scavenging activity, α-amylase, and α-glucosidase inhibitory potential of the ethanolic crude extract of *D. oliveri* and its derived solvent-solvent fractions.

MATERIALS AND METHOD

Reagents

Folin-Ciocaltue reagent, 1,1-diphenyl-2-picraylhydrazine (DPPH), Porcine Pancreatic α-amylase, α-glucosidase, 3,5-dintrosalicylic Acid (DNS), Acarbose, Butylated Hydroxytoluene (BHT), Ethanol, Diethyl ether, ethyl acetate, and n-hexane were products of SRL Pvt limited, India; *p*-Nitrophenyl-α-D-glucopyranoside was a product of Hi-media. All buffers were prepared using standard procedures. All reagents were supplied locally by Dualife Science PVT limited, Kaveri Hebitet, Surat, India.

Plant Materials Collection and Authentication

The young leaves of *Daniellia oliveri* were collected from the natural habitat in the University of Ilorin, Ilorin, Nigeria. The plant was verified at species level in the Department of Plant Biology Herbarium, University of Ilorin, Nigeria. A voucher specimen was deposited for reference (UILH/001/1291/2021).

Preparation of Extracts

The leaves were sorted to make sure that only the fresh ones were selected. The sorted leaves were thoroughly rinsed under running water to ensure debris and dust were thoroughly removed. The washed leaves were air-dried for 21 days and then powdered using an electric blender. 400 g of the powdered was extracted with 2000ml distilled ethanol using the cold maceration method. The resulting extract was filtered under vacuum using Whatman filter paper (No. 1). The extract was concentrated using a rotary evaporator and a water bath to eliminate all the solvents.

Partitioning of the Crude Extract

The solvent-solvent fractionation of the resulting crude extract from above was carried out using standard protocols[30,31] with modifications in partitioning solvents (n-hexane, Diethyl ether, and Ethyl acetate). The crude ethanolic extract was dissolved in water and stirred on a magnetic stirrer at 60^{o}C. The water-soluble fraction was separated and exhaustively extracted by a consecutive partition with n-hexane (200ml×3), diethyl ether (200ml×3), and ethyl acetate (200ml×3) using a separating funnel. The fractions obtained were evaporated to dryness and

labelled Do-C (*D. oliveri* Ethanolic Crude extract), Do-D (Diethyl ether fraction of Do-C), Do-E (Ethyl Acetate fraction of Do-C), Do-H (n-Hexane fraction of Do-C).

Qualitative Phytochemical Assessment

The phytochemical assessment of the crude and the fractionated extracts was accomplished using established procedures[32-35].

In-vitro* antioxidant activity of extracts of *D. oliveri

Estimation of total flavonoid content (TFC)

1ml of Quercetin (reference standard) and extract solution (various concentrations) was taken in a test tube. 3ml methanol was added, along with 200 µl of 10% AlCl3 and 200 µl of a 1M potassium acetate solution. After adding distilled (5.6 ml) water to the reaction, the test tubes were incubated at room temperature to complete the reaction. The solution's absorbance was determined at 420 nm using a UV-visible spectrophotometer (Shimadzu UV-1800) in comparison to a blank.

The TFC in the plant extract and fractions (mg/g quercetin equivalent) was estimated using the formula:

$$C = \frac{(c \times V)}{M}$$

C= Total flavonoid contents, mg/g Quercetin equivalent (QE)
c= Quercetin concentration calculated from the calibration curve, mg/ml
V= Volume of extract, ml
M= The weight of the plant extract used, gm.

Estimation of total phenolic content (TPC)

The modified method of Singleton *et al.*,[36] was used. 0.5 ml of methanolic solution of the extracts/Gallic Acid, standard reference (10-200 µg/ml) was mixed with 2.5ml 10% FCR and 2.5 ml 7.5% $NaHCO_3$. The mixture was incubated for 45 min. (at 45°C). The absorbance was read at 765 nm with UV-visible Spectrophotometer (Shimadzu UV-1800). The Gallic Acid Calibration curve was constructed by plotting the OD vs. concentrations.

The total phenolic contents in the plant extract in Gallic Acid Equivalent (GAE) was evaluated employing the formula;

$$C = \frac{(c \times V)}{M}$$

C= Total Phenolic Content, mg/g Gallic Acid Equivalent (GAE)
c= Gallic acid concentration determined from the calibration curve, mg/ml
V= Volume of extract (ml)
M= Extract weight used (gm).

Evaluation of DPPH radical scavenging activity

The method of Shen *et al.*[37] was employed with modifications. Varying concentrations of fractions/Standard (3ml in methanol) were dispensed with 1 ml DPPH (0.1 mM) in test tubes. After rapidly agitating the mixture, it was allowed to stand at room temperature for thirty minutes in the dark. A UV-visible spectrophotometer (Shimadzu UV-1800) was used to determine the absorbance at 517 nm. As a standard, butylated hydroxyl toluene was used. The control was prepared similarly to the standard/fractions but with methanol instead of standard/fractions. The reaction mixture's reduced absorbance indicated increased free radical scavenging activity.

The ability to scavenge the DPPH radicals was expressed as %Inhibition (%I) using the formula;

$$\%\,I = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Additionally, the antioxidant activity of the extract was also reported as an IC_{50} value. The IC_{50} value was defined as the concentration needed to reduce DPPH radical production by 50%, as determined by plotting inhibition percentage vs. extract concentration.

***In-vitro* Alpha-amylase Inhibitory Assay**

Varying concentrations (100 µl of 2, 4, 8, 10 and 15 µg/ml) of Acarbose, Do-C, Do-D, Do-E, and Do-H were incubated with 200 µl of 20 mM of phosphate buffer (pH 6.9 with 6.7 mM NaCl) comprising alpha-amylase at 37ºC for 20 min. Then, 100 µl (1%) starch solution prepared in 20 mM phosphate buffer was added to the reaction mixture and incubated at 37ºC for 10 min. The reaction was stopped by adding 200 µl of 3,5-dinitro salicylic acid (DNSA). The reaction mixture was placed in a water bath to boil for 5 minutes, after when it was set aside

to cool to room temperature. The reaction mixture was diluted by adding water (2.2 ml), and absorbance was read at 540 nm against the blank in spectrophotometer UV-VIS (Shimadzu UV-1800). The control, representing 100% enzyme activity, was prepared similarly, without the extract/fractions[38]. The study was in triplicate, and the amylase inhibitory activity was expressed as percentage inhibition (% Inhibition).

$$\%Inhibition = (Ac-As)/Ac \times 100$$

Ac is the absorbance of the control, and As is the sample absorbance.

In-vitro Alpha-Glucosidase Inhibitory Assay

The inhibitory activity of alpha-glucosidase was evaluated using a revised version of a previously reported approach[39,40]. To 100 µl of (2, 4, 8, 10, 15 µg/ml) plant extract/fractions/Acarbose, 200 µl α-glucosidase was added and the mixture incubated at 37ºC for 20 min. 100 µl 3 mM *p*-nitrophenyl -Glucopyranoside (p-NPG) was added to the reaction mixture and incubated at 37 ºC for 10 min. The reaction was ended by adding 2 ml 0.1 M Na_2CO_3. The α-glucosidase activity was read at 405 nm in a UV-VIS spectrophotometer (Shimadzu UV-1800) by measuring the quantity of α-nitrophenol released from *p*-NPG.

The test was in triplicate, and the alpha-glucosidase inhibitory activity was expressed as percentage inhibition (% I).

$$I\ \% = (Ac-As)/Ac \times 100$$

Ac is the absorbance of the control, and As is the absorbance of the sample

Statistical Analysis

The data obtained were subjected to statistical analysis using IBM SPSS Statistics Software (Version 20) and GraphPad Prism 8. One-way analysis of variance (ANOVA) followed by Duncan multiple range tests (DMRT) was used for multiple comparisons at a 5% probability level ($P \leq 0.05$).

RESULTS AND DESCUSSION

Results

Phytochemical Analysis

Table 1 summarises the qualitative phytochemical composition of the *D. oliveri* leaf extracts. Alkaloids, flavonoids, and Phenols are present in Do-C and its fractions. Only the ethyl acetate fraction (Do-E) contains cardiac glycoside.

Table 1: Qualitative Phytochemical Composition of *Daniellia oliveri* extract and fractions

Phytochemicals	Do-C	Do-D	Do-E	Do-H
Alkaloids	+	+	+	+
Carbohydrate	-	-	-	-
Flavonoids	+	+	+	+
Cardiac Glycosides	-	-	+	-
Phenols	+	+	+	+
Saponins	+	-	+	+
Tannins	+	-	+	+

Legend: + = Present; - = Absent

Do-C: *Daniellia oliveri* Ethanolic Crude extract; **Do-D**: Diethyl ether fraction of Do-C; **Do-E**: Ethyl Acetate fraction of Do-C; **Do-H**: n-Hexane fraction of Do-C.

Antioxidant activity of extracts of *Daniellia oliveri*

Total flavonoid and total phenolic contents

Figure 1 illustrates the TFC and TPC of the crude ethanolic extract (Do-C) and its fractions (Do-D, Do-E, and Do-H). However, Do-H had the highest total flavonoid content (TFC) (134.22 ± 1.18mg/g QE), although it was not significantly different from Do-D (132.72 ± 6.65mg/g QE) at $P \leq 0.05$. However, the determined value for Do-E (112.94 ± 0.77 mg/g QE) is higher than the parent extract, Do-C (79.22 ± 2.44 mg/g QE), which has the lowest TFC. Meanwhile, Do-E's total phenolic content (TPC) (211.55 ± 5.48 mg/g GAE) was considerably higher and differed significantly from the other fractions examined ($P \leq 0.05$). The lowest concentration was found in Do-H (29.22 ± 0.93 mg/g GAE).

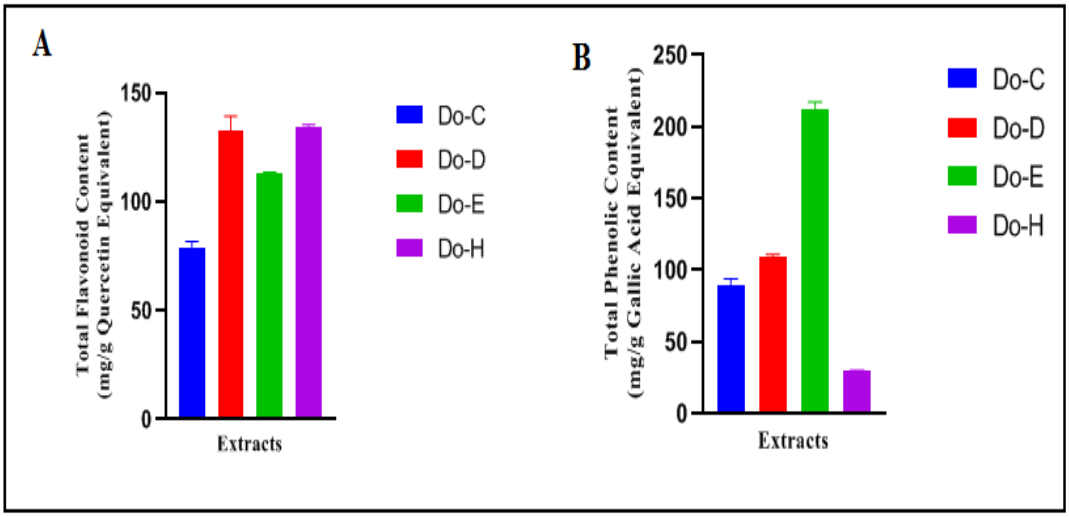

Fig. 1: Estimation of Antioxidant phytochemicals of extract and fractions of *D. oliveri*. (A) Total flavonoid content (mg/g Quercetin Equivalent); (B) Total Phenolic Content (mg/g Gallic Acid Equivalent); **Do-C**: *Daniellia oliveri* Ethanolic Crude extract; **Do-D**: Diethyl ether fraction of Do-C; **Do-E**: Ethyl Acetate fraction of Do-C; **Do-H**: n-Hexane fraction of Do-C. Values are mean of three replicates ± Standard Error of Mean (SEM).

DPPH radical scavenging activity

In comparison to Butylated Hydroxytoluene (BHT), Do-C and its fractions (Do-D, Do-E, and Do-H) showed excellent DPPH scavenging activity (Table 2). The studied drugs had a dose-dependent pattern of activity. The lowest activity was found at 1µg/ml, whereas the maximum inhibition was reported at 100 g/ml. When compared to BHT (98.46 ± 0.44%), Do-D (94.60 ± 1.82%) and Do-E (93.06 ± 0.59%) had a significantly the same effect in inhibiting DPPH radicals at 100µg/ml. Do-H had the lowest inhibition (40.27±5.03%) at 100g/ml. Except in Do-H, the mean effective concentration required to inhibit DPPH radical production by 50% was not significantly different ($P \leq 0.05$). However, in all fractions examined, Do-E had the lowest IC_{50} (11.57 ± 0.42 µg/ml).

Table 2: DPPH Radical scavenging Activity of Fractions of *Daniellia oliveri*

Concentration (µg/ml)	DPPH INHIBITION (%)				
	BHT	**Do-C**	**Do-D**	**Do-E**	**Do-H**
1	$14.11\pm4.72^{a*}$	3.04 ± 0.86^{bc}	10.27 ± 1.09^{ab}	4.50 ± 1.06^{bc}	0.00 ± 0.00^{c}
3	35.65 ± 1.31^{a}	6.92 ± 0.65^{b}	10.90 ± 3.27^{b}	7.13 ± 1.63^{b}	0.00 ± 0.00^{c}
5	60.06 ± 1.66^{a}	13.63 ± 0.75^{b}	16.66 ± 4.72^{b}	17.93 ± 5.42^{b}	2.51 ± 0.83^{c}
10	74.70 ± 4.13^{a}	44.64 ± 3.60^{b}	54.79 ± 1.27^{b}	66.34 ± 0.64^{a}	23.05 ± 5.34^{c}
20	91.78 ± 0.93^{a}	49.13 ± 4.17^{b}	53.76 ± 4.59^{b}	75.98 ± 2.24^{c}	16.38 ± 4.91^{d}
30	94.99 ± 1.46^{a}	36.03 ± 2.22^{b}	59.02 ± 2.24^{c}	75.34 ± 1.90^{d}	22.03 ± 1.70^{e}
50	92.04 ± 1.34^{a}	46.82 ± 9.42^{b}	70.71 ± 3.09^{c}	82.79 ± 0.13^{ac}	32.56 ± 3.48^{b}
100	98.46 ± 0.44^{a}	76.11 ± 2.32^{b}	94.60 ± 1.82^{a}	93.06 ± 0.59^{a}	40.27 ± 5.03^{c}
IC50 (µg/ml)	6.33 ± 0.72^{a}	18.20 ± 1.51^{a}	15.92 ± 0.94^{a}	11.57 ± 0.42^{a}	115.53 ± 15.65^{b}

*Values are mean of three replicates ± Standard Error of Mean (SEM)

Values with the same superscript across the rows are not significantly different (P≤0.05)

Do-C: *Daniellia oliveri* Ethanolic Crude extract; **Do-D**: Diethyl ether fraction of Do-C; **Do-E**: Ethyl Acetate fraction of Do-C; **Do-H**: n-Hexane fraction of Do-C. Values are mean of three replicates ± Standard Error of Mean (SEM)

***In-vitro* α-amylase and α-glucosidase Inhibitory Assays**

The observed inhibitory potential of the various fractions of *D. oliveri* against alpha-amylase and alpha-glucosidase is dose-dependent, as shown in figure 2(A & B). The standard drug, acarbose, exhibited remarkable inhibition as expected. In the alpha-amylase inhibition assay, the activity exhibited by Do-E was significantly at par with that of the standard drug but different from that of Do-D. The crude extract has the lowest inhibition percentage (Fig. 2A). The activity of the fractions in the alpha-glucosidase inhibitory assay followed the same pattern as in the alpha-amylase inhibitory assay, except that Do-C exhibited more significant inhibition than Do-D (Fig. 2B).

The quantitative measures that indicate how much of the Do-C and its derived fractions needed to inhibit α-amylase and α-glucosidase by 50% (IC_{50}) were recorded (Table 3). Evaluating the IC_{50} values of alpha-amylase, the ethyl acetate fraction of Do-C (Do-E) exhibited the lowest IC_{50} (84.32 ± 2.33µg/ml), and IC_{50} values of Do-C, Do-D, and Do-H were 147.56 ± 5.30 µg/ml, 89.63 ± 2.33 µg/ml, and 115.66 ± 4.60 µg/ml, respectively. The IC_{50} of the standard drug, acarbose (77.84 ± 1.09 µg/ml), was not distinct from Do-E ($P \leq 0.05$). Meanwhile, Do-H exhibited the lowest IC_{50} (31.23 ± 1.23 µg/ml) among the test fractions in α-glucosidase inhibition assay, while it was not significantly different from the IC_{50} of Do-E (35.02 ± 1.22 µg/ml) at $p \leq 0.05$. The IC_{50} for acarbose was 25.97±0.96 µg/ml. The IC_{50} of acarbose, Do-E, and Do-H, on the other hand, did not differ significantly ($p \leq 0.05$).

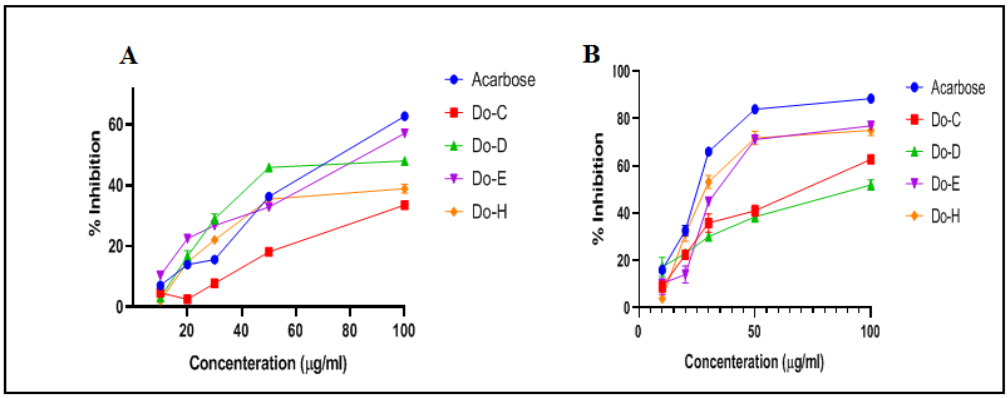

Fig. 2: *In-vitro* antidiabetic evaluations of extract and fractions of *D. oliveri*. α-amylase inhibitory assay (A); α-Glucosidase Inhibitory assay (B).

Do-C: *Daniellia oliveri* Ethanolic Crude extract; **Do-D**: Diethyl ether fraction of Do-C; **Do-E**: Ethyl Acetate fraction of Do-C; **Do-H**: n-Hexane fraction of Do-C. Values are mean of three replicates ± Standard Error of Mean (SEM).

Table 3: *In vitro* Antidiabetic evaluations (IC_{50}) of Fractions of Ethanolic extract of *Daniellia oliveri*

Extracts	α-amylase Inhibitory Activity IC_{50} (µg/ml)	α-glucosidase Inhibitory Activity IC_{50} (µg/ml)
Standard*	$77.84 \pm 1.09^{a**}$	25.97 ± 0.96^{a}
Do-C	147.56 ± 5.30^{b}	64.98 ± 8.10^{b}
Do-D	89.63 ± 2.33^{c}	89.28 ± 11.26^{c}
Do-E	84.32 ± 2.33^{ac}	35.02 ± 1.22^{a}
Do-H	115.66 ± 4.60^{d}	31.23 ± 1.23^{a}

*Standard: **Acarbose** for α-amylase and α-glucosidase inhibitory assays; **Do-C**: *Daniellia oliveri* Ethanolic Crude extract; **Do-D**: Diethyl ether fraction of Do-C; **Do-E**: Ethyl Acetate fraction of Do-C; **Do-H**: n-Hexane fraction of Do-C.

**Values are mean of three replicates ± Standard Error of Mean (SEM)

Values with the same superscript down the column are not significantly different (P≤0.05)

Discussion

Medicinal plants are rich in essential secondary metabolites that could be used to produce new therapeutic agents[41]. The secondary metabolites are critical contributors to the healing ability of medicinal plants and their derivatives[42]. Therefore, the solvent-solvent partitioning of the ethanolic extract of *D. oliveri* leaf is a viable step in identifying potential antidiabetic chemicals.

The *in-vitro* antioxidant, alpha-amylase, and alpha-glucosidase activities of *D. oliveri* ethanolic extract and its solvent-solvent partitions, were remarkable. Several researchers have claimed that the phytochemicals found in these extracts have significant medicinal potential, particularly in treating diabetic mellitus[43-48]. It is worth noting that alkaloid was found in Do-C and its derived fractions (Do-D, Do-E, Do-H). Natural alkaloids have been reported to have potent inhibitory efficacy against carbohydrate-hydrolysing enzymes[43]. Alkaloids isolated from natural sources, especially medicinal plants, have been found to impede the actions of alpha-glucosidase enzymes and alpha-amylase enzymes[44&45]. Alkaloids in Do-C and its fractions may be responsible for their therapeutic effect in inhibiting alpha-amylase and alpha-glucosidase enzymes.

Flavonoids observed in the crude ethanolic extract of *D. oliveri* leaf and its fractions, on the other hand, are antioxidants and free radical scavengers[46,47]. Flavonoids have been linked to medicinal plants' ability to regulate diabetic-induced oxidative stress[48]. The *in-silico* insulin-mimetic activity of flavonoids is reportedly linked with a reduced risk of type 2 diabetes[49&50].

In diabetes, phenolic substances extracted from medicinal plants have been shown to limit the absorption of alpha-amylase[45]. According to Lin et al.[51], polyphenols, phenolic acids, and tannins are essential inhibitors of the alpha-amylase and alpha-glucosidase enzymes. However, it has been observed that the presence of phenolics in plants aids in inhibiting digestive enzymes by attaching to them and altering their bioactivity[52&53] and serving as natural therapeutic intermediates in managing diabetes and its complications[54]. The tannins in Do-C and its fractions (Do-E and Do-H) may have the capacity to impede the activities of alpha-amylase and alpha-glucosidase enzymes efficiently.

Cardiovascular disease is prone in people with reduced plasma antioxidants[55]. Thus, exogenous antioxidants are required for the balance of antioxidants and prooxidants in such people. Blois[56] developed the DPPH inhibitory assay, which is frequently used to assess antioxidant activity and test the therapeutic efficacy of drugs as radical scavengers or hydrogen donors. The antioxidant efficacy of Do-C and its fractions *in-vitro* agrees with the report of Muanda, et al.[57]. Based on the exhibited antioxidant capacity (IC_{50} of 15.49 ± 0.39 µg/ml), Atolani and Olatunji[58] concluded that the oleoresin from *D. oliveri* could be a favourable source of natural antioxidants.

Inhibiting free radicals' generation is a therapeutic strategy for preventing oxidative stress and concomitant diabetic vascular problems[59]. Free radical production that exceeds the scavenging capacity of endogenous antioxidants is also known to cause microvascular and macrovascular dysfunction and polyneuropathy[60]. As a result, *in-vitro* inhibition of DPPH scavenging radicals by our fractions implies that when administered *in-vivo* as an antidiabetic, they will effectively control diabetic complications.

In general, the α-amylase enzyme digests carbohydrates by hydrolysing polysaccharide's 1,4-glycosidic bonds to disaccharides, which are then converted to monosaccharides by the α-glucosidase enzyme. This process leads to postprandial hyperglycaemia[61&62]. Do-C and its fractions are potent inhibitors of these enzymes, with Do-E displaying particularly strong inhibitions compared to acarbose. Their enzymes inhibition ability indicates that they can delay carbohydrate digestion, hence lowering postprandial glucose levels.

Conclusion

In the current investigation, Do-C and its solvent-solvent fractions (Do-D, Do-E, and Do-H) efficiently and effectively inhibited the primary carbohydrate-hydrolysing enzymes (alpha-amylase and alpha-glucosidase) linked to type 2 diabetes. However, further study is required to characterise the bioactive components responsible for the reported activity in this investigation. Furthermore, the toxicological consequences of utilising these fractions and the resulting isolated chemicals should be thoroughly investigated.

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نشرة العلوم الصيدلية
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ثبيط إنزيمات رئيسية لمرض السكري من النوع الثاني في المختبر ؛ تأثير المستخلصات وأجزاءها لنبات دانيليا اوليفيري (رولف) هوتش. و دالزيل

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نبات دانيليا اوليفيري هو نوع من الأشجار ينتمي إلى الفصيلة السيزالبينودي (البقولية) ، والتي تُستخدم أوراقها الصغيرة محليًا لعلاج مرض السكري من النوع الثاني في نيجيريا. تهدف هذه الدراسة إلى تقييم امكانية تثبيط المستخلص الإيثانولي للنبات (دو– س) وأجزاء المستخلص المختلفة باستخدام المذيبات التالية الهكسان (دو– ه) و الدايايثيل ايثر (دو– د) و الايثيل اسيتات (دو– ي) لأنشطة انزيمات الالفا اميلز و الالفا جلوكوزيداز في المختبر تاثيرهم على دايفينيل –٢– بيكريلهيدرازين كمضادات للاكسدة باستخدام البروتوكولات القياسية. أظهرت النتائج التي توصلنا إليها أن دو–س واجزائه تحتوي على فينولات و فلافونويدات بنسبة كل الاجزاء لها تاثير كمضات اكسدة بشكل فعال ، دو– ي له تثبيط ممتاز عند مقارنته بـ بيوتيلاتيد هيدوكسي تولوين. اظهرت هذه الدراسة ان المستخلص الإيثانولي للنبات (دو– س) وأجزاء المستخلص المختلفة باستخدام المذيبات (دو– ه و دو– د و دو– ي) نشاطا نثبطا على انزيمات الالفا اميلز و الالفا جلوكوزيداز في نمط يعتمد على الجرعة. ومع ذلك ، بالمقارنة مع أكاربوز ، أظهر دو–ي نشاطًا مثبطًا مماثلًا ضد الالفا اميلز ($P \geq ٠,٠٥$). ومع ذلك ، فإن دو– ي (IC50= ٣٥,٠٢±١,٢٢) و دو– ه (IC50= ٣١,٢٨±١,٢٣ميكروجم / مل) (IC50= ٢٥,٩٧±٠,٩٦ ميكروجم / مل) كان لهما أفضل نشاط مثبط ضد الالفا جلوكوزيداز نسبيًا بعد أكاربوز (٢٥,٩٧ ± ٠,٩٦ ميكروجم/ مل). يمكن ربط الإمكانات المثبطة لـ دو–ي بوجود فينولات و فلافونويدات. لذلك ، يمكن لجزء أسيتات الإيثيل الذي تم الحصول عليه من المستخلص الإيثانولي لنبات دانيليا اوليفيري أن يثبط بشكل فعال الإنزيمات الرئيسية المرتبطة بداء السكري من النوع الثاني (الالفا اميلز و الالفا جلوكوزيداز). مزيد من الدراسات الأخرى مطلوبة لفصل المركبات الموجودة في النبات و المعالجة لمرض السكري.