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Inhibitory effect of polyphenolic-rich extract from *Cola nitida* (Kolanut) seed on key enzyme linked to type 2 diabetes and Fe^{2+} induced lipid peroxidation in rat pancreas *in vitro*

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PEER REVIEW

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Comments

The most interesting aspect of the research work is the inhibitory effect of the phenolic-rich extract on α-amylase and α-glucosidase activity. The result elucidates how the inhibition of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) can be exploited in the management/prevention of oxidative stress associated pathologies. I therefore, recommend its publication.
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ABSTRACT

Objective: To investigate the inhibitory effect of phenolic-rich extracts from *Cola nitida* (*C. nitida*) seeds on key enzymes linked with type-2 diabetes and Fe^{2+} induced oxidative stress in rat pancreas.
Methods: The phenolic extract was prepared with 80% acetone (v/v). Subsequently, the antioxidant properties and inhibitory effect of the extract on α - amylase and α - glucosidase as well as on Fe^{2+} induced lipid peroxidation in rat pancreas were determined *in vitro*.
Results: The result revealed that *C. nitida* extract inhibited α-amylase (EC_{50}=0.34 mg/mL) and α-glucosidase (EC_{50}=0.32 mg/mL) activities as well as Fe^{2+} induced lipid peroxidation in rat pancreas in a dose dependent manner. In addition, the extract had high DPPH radical scavenging ability (EC_{50}=2.2 mg/mL) and reducing power (8.2 mg AAE/g). Characterization of the main phenolic compounds of the extract using gas chromatography analysis revealed catechin (6.6 mg/100 g), epicatechin (3.6 mg/100 g), apigenin (5.1 mg/100 g) and naringenin (3.6 mg/100 g) were the main compounds in the extract.
Conclusions: This antioxidant and enzyme inhibition could be some of the possible mechanism by which *C. nitida* is use in folklore for the management/treatment of type-2 diabetes. However, the enzyme inhibitory properties of the extract could be attributed to the presence of catechin, epicatechin, apigenin and naringenin.

KEYWORDS
α-Amylase, α-Glucosidase, Oxidative stress, Phenolic compounds

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency result in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves[1]. Increasing evidence in both experimental and clinical studies have shown the participation of oxidative stress in the development and progression of DM[2]. This is usually accompanied by increased production of free radicals or impaired antioxidant defenses[3]. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and

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the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance[2].

The growing number of diabetics, coupled with the harsh side effects of some synthetic drugs has led to the increasing search for alternatives, which are relatively cheap with minimal side effect. One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia[4]. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolysing enzymes α-glucosidase and α-amylase in the digestive tract. Inhibition of these enzymes delays carbohydrate digestion and prolongs overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise[4,5]. Many natural resources have been investigated with respect to the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine[5,6].

Polyphenolic compounds are widely distributed in edible plants and have been suggested to protect against a variety of diseases[7]. Recent investigations suggest that polyphenolic components of higher plants may act as antioxidants or via other mechanisms prevent disease processes[8]. Recent findings have also demonstrated that polyphenols cross intestinal barriers and are sufficiently absorbed to have the potential to exert biological effects[7]. Polyphenols are the most abundant antioxidants in human diets. They exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions[7,8]. They are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α-tocopherols and inhibiting oxidases[7,8]. They are also important for improving the sensory and nutritional qualities, in that they impart colors, flavors and tastes[8]. Polyphenols have been shown to have high antioxidant activity and certain therapeutic properties, including antidiabetic activity[9].

Kolanuts are the seed pods of various evergreen trees that are native to Africa. In West Africa and Sudan, they are popular masticatory agent, eaten as a principal stimulant to keep awake and withstand fatigue. Besides the fact that kolanuts contain caffeine and act as a stimulant and anti-depressant, they are also thought to reduce hunger, aid digestion, and work as an aphrodisiac[10]. In some parts of Africa, kolanuts are given as gifts to visitors entering a home, usually with some formal ceremony. Besides the ceremonial uses, many Africans consume kolanuts regularly, even daily, for the medicinal effects such as diabetes[10]. More recently, kolanut extracts have become popular in Europe and North America as a natural or alternative medicine for the treatment of diabetes[10]. Hence, this study sought to investigate the inhibitory effect of phenolic-rich extracts from *Cola nitida* (*C. nitida*) seeds on key enzymes linked with type-2 diabetes (α-amylase and α-glucosidase) and Fe^{2+} induced oxidative stress in rat pancreas *in vitro*.

2. Materials and methods

2.1. Materials

2.1.1. Sample collection

Kolanut (*C. nitida*) seeds were purchased in the local market in Ibadan metropolis and authenticated at the Department of Botany, University of Ibadan, Oyo State, Nigeria. Five adult male wistar strain albino rats were purchased from the animal colony, Department of Biochemistry, University of Ilorin, Nigeria and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on commercial diet and water. The handling of animals was carried out in accordance with the recommended international standard[11]. All the chemicals were of analytical grade while the water was glass distilled.

2.1.2. Extraction of phenolic extract

The seeds were peeled and chopped into small chips and allowed to dry at room temperature on the laboratory bench. Dried chips were blended into powdery form. The extraction of the phenolic extract was carried out according to the method reported by Oboh *et al*[12]. About 10 g of the powdered seed was extracted with 100 mL of 80% acetone and was filtered (Whatman No. 2) under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 45 °C until about 90% of the filtrate had been evaporated. The phenolic extract was frozen and stored for subsequent analysis.

2.2. Method

2.2.1. α-amylase inhibition assay

Briefly, appropriate dilutions (0 - 200 μL) of the extracts and 500 μL of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) containing porcine pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated at 25 °C for 10 min. Then, 500 μL of 1% starch solution in 0.02 mol/L sodium phosphate buffer

(pH 6.9 with 0.006 mol/L NaCl) was added to each tube. The reaction mixtures was incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm. The α-amylase inhibitory activity was expressed as percentage inhibition[13].

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{Samples})/Abs_{Control}] \times 100$$

2.2.2. α-glucosidase inhibition assay

Appropriate dilutions of the extracts (0 - 200 μL) and 100 μL of α-glucosidase (EC 3.2.1.20) solution (1.0 U/mL) in 0.1 mol/L phosphate buffer (pH 6.9) were incubated at 25 °C for 10 min. Then, 50 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/L phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25 °C for 5 min, before reading the absorbance at 405 nm in the spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition[14].

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{Samples})/Abs_{Control}] \times 100$$

2.2.3. Lipid peroxidation assay

2.2.3.1. Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anaesthesia and the pancreas was rapidly excised, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10 w/v) with about 10-up-and -down strokes at approximately 1200 rev/min in a teflon glass homogenizer respectively. The homogenate was centrifuged for 10 min at 3000 xg to yield a pellet that was discarded, and a low-speed supernatant (S1) was kept for lipid peroxidation assay[15].

2.2.3.2. Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*[16], briefly 100 μL S1 fraction was mixed with a reaction mixture containing 30 μL of 0.1 mol/L pH 7.4 Tris-HCl buffer, extract (0-100 μL) and 30 μL of 250 μM freshly prepared FeSO4. The volume was made up to 300 μL by water before incubation at 37 °C for 1 h. The colour reaction was developed by adding 300 μL 8.1% sodium dodecyl sulphate to the reaction mixture containing S1, this was subsequently followed by the addition of 600 μL of acetic acid/HCl (pH 3.4) mixture and 600 μl 0.8% thiobarbituric acid. This mixture was incubated at 100 °C for 1 h. Thiobarbituric acid reactive species produced were measured at 532 nm and the absorbance was compared with that of standard curve using malondialdehyde (MDA).

2.2.4. 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH free radical was evaluated as described by Gyamfi *et al*[17]. Briefly, appropriate dilution of the extract (0-1 mL) was mixed with 1 mL, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The percentage DPPH free radical scavenging ability was subsequently calculated.

2.2.5. Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu[18]. A volume of 2.5 mL aliquot was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 1000 g for 10 min. About 5 mL of the supernatant was mixed with an equal volume of water and 1 mL 0.1% ferric chloride. The absorbance was measured at 700 nm. The reducing power was subsequently calculated as ascorbic acid equivalent.

2.2.7. Determination of total phenol content

The total phenol content of the extracts (free and bound) was determined using the method reported by Singleton *et al*[19]. Appropriate dilution of the extracts was oxidized with 2.5 mL 10% Folin-Ciocalteau's reagent (v/v) and neutralized with 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

2.2.8. Determination of total flavonoid content

The total flavonoid content of both extracts were determined using a slightly modified method reported by Meda *et al*[20]. Briefly 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 μL 10% $AlCl_3$, 50 μL 1 mol/L potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid content calculated quercetin equivalent. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

2.2.9. Characterisation of phenolic constituent using gas chromatography (GC) analysis

The qualitative-quantitative analysis of the phenolic compounds of the samples was carried out using the method reported by Kelley *et al*[21]. The purified phenolic extracts (1 uL:10:1 splitless) was analyzed for composition by comparison with authentic standards (Aldrich, Milwaukee, WI) and with cochromatography with standards on a Hewlett-Packard 6890 GC (Hewlett-Packard Corp., Palo Alto, CA) equipped with a derivatized, nonpacked injection liner, a Rtx-5MS (5% diphenyl-95% dimethyl polysiloxane) capillary column (30 m length, 0.25 mm column *id.*, 0.25 μm film thickness), and detected with a flame ionization detector. The following conditions were employed for phenolics separation: injector temp. of 250 °C; temp. ramp, 80 °C for 2 min then ramped to 280 °C at 30 °C min^{-1} and a detector temp. of 320 °C.

2.3. Data analysis

Results of three replicate readings were pooled and expressed as mean±standard deviation. One way analysis of variance was used to treat difference between means while Duncan multiple test was used for the *post hoc* analysis. Significance was accepted at $P \leq 0.05$[22].

3. Results

First, the ability of the phenolic extracts from kolanut seed to inhibit α-amylase activity *in vitro* was investigated and the result is presented in Figure 1. The result revealed that *C. nitida* (EC_{50} = 0.34 mg/mL) seed inhibited α-amylase in a dose-dependent manner (in the range of 0 - 0.4 mg/mL).

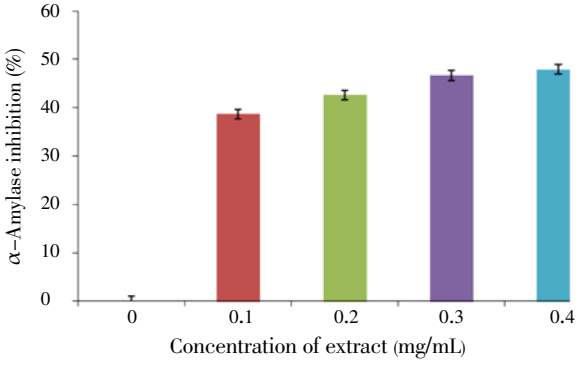

Figure 1. α-Amylase inhibitory activity of phenolic extract of kolanut (*C. nitida*) seed.

Furthermore, the interaction of the phenolic extracts from *C. nitida* seed with α-glucosidase *in vitro* was investigated and the result is presented in Figure 2. The result revealed that the phenolic extract (EC_{50}=0.32 mg/mL) exhibited a dose-dependent inhibition of α-glucosidase (0 - 0.4 mg/mL).

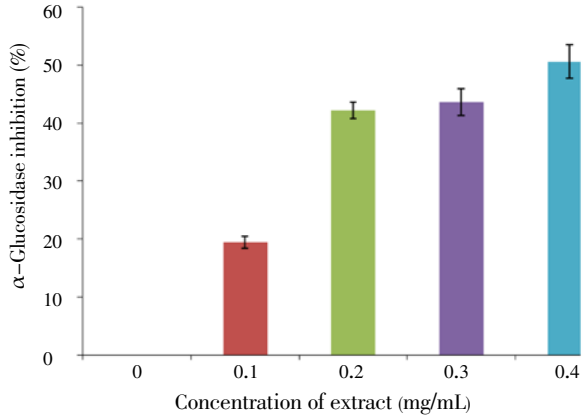

Figure 2. α-Glucosidase inhibitory activity of phenolic extract of kolanut (*C. nitida*) seed.

In addition, the ability of the phenolic extract to inhibit Fe^{2+} induced lipid peroxidation was investigated. The result as presented in Figure 3 revealed Fe^{2+} caused a significant ($P<0.05$) increase in the MDA content of the rat pancreas (123.0%) when compared with the basal pancreas homogenate (100%). However, the phenolic extract from *C. nitida* seed (EC_{50}=1.2 mg/mL) inhibited MDA production in rat's pancreas in a dose-dependent manner (0.83-1.67 mg/mL).

Also, the DPPH radical scavenging ability of the extract was determined, as shown in Figure 4, the phenolic extract was able to scavenge the DPPH radical in a dose dependent manner (0-2.52 mg/mL).

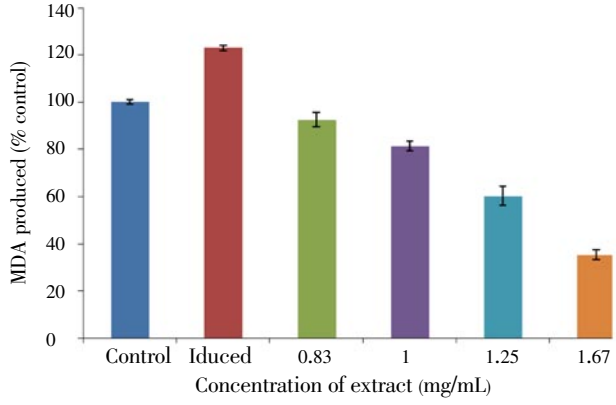

Figure 3. Inhibition of Fe^{2+} induced lipid peroxidation in rat's pancreas by phenolic extract of kolanut (*C. nitida*) seed.
Control=lipid peroxidation without $FeSO_4$ as prooxidants and no kolanut seed extract; Induced=lipid peroxidation with $FeSO_4$ as prooxidants and no kolanut seed extract.

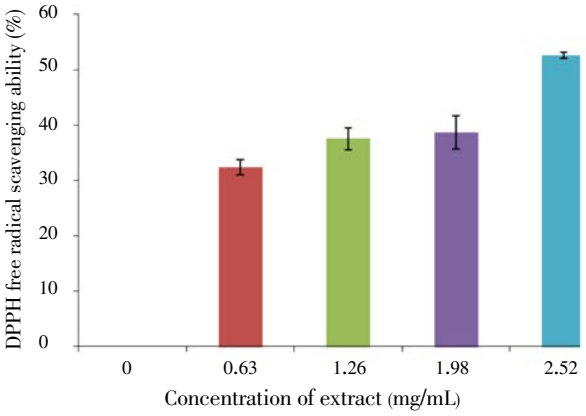

Figure 4. DPPH free radical scavenging ability of phenolic extract of kolanut (*C. nitida*) seed.

Table 1 revealed the total phenol (57.7 mg/g), flavonoid (6.3 mg/g) content and ferric reducing antioxidant property (FRAP) (8.2 mg AAE/g) of *C. nitida* seed. Further characterization of phenolic constituents in the extract using GC was investigated. The result as presented in Table 2 and Figure 5 revealed that the main constituent of the polyphenol-rich extract of *C. nitida* seed are catechin (6.6 mg/100 g), epicatechin (3.6 mg/100 g), apigenin (5.1 mg/100 g) and Naringenin (3.6 mg/100 g).

Table 1

The total phenol, flavonoid content and FRAP of *C. nitida* seed.

Samples	*C. nitida* seed
Total phenol (mg/g)	57.7±2.7
Total flavonoid (mg/g)	6.3±1.9
FRAP (mg AAE/g)	8.2±1.2

Values represent mean±standard deviation of triplicate experiments.

Table 2

The main phenolic constituents of *C. nitida* seed (mg/100 g).

Sample	*C. nitida* extract
Catechin	6.6±0.02
Epicatechin	3.6±0.01
Apigenin	5.1±0.01
Narigenin	3.6±0.03

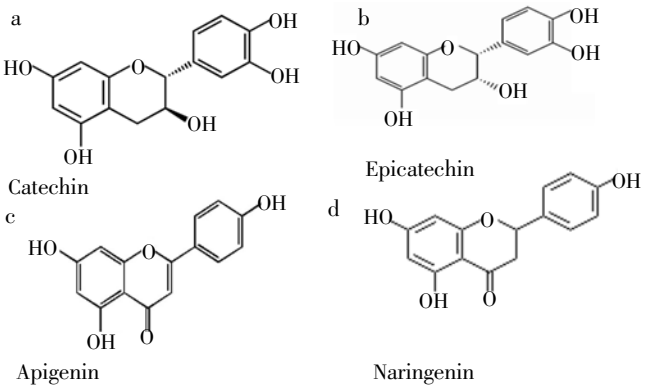

Figure 5. The structure of the main phenolic constituents of *C. nitida* seed.

4. Discussion

Management of the blood glucose level is a critical strategy in the control of diabetes complications. Inhibitors of saccharide hydrolysing enzymes (α-amylase and α-glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type-2 DM. Inhibition of these enzymes delays carbohydrate digestion and prolongs overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise[4]. The inhibition of α-amylase by the phenolic extract could be attributed to its high flavonoid content (Table 1), and the presence of flavonoids like apigenin, naringenin and catechins (Table 2, Figure 5). Previous research has shown that phenolic compounds could inhibit α-amylase; however flavonoid compounds are stronger α-amylase inhibitors[5-7]. Nevertheless, the inhibitory action of the *C. nitida* extract on α-amylase activity agreed with some earlier reports where plant phytochemicals from *Telfairia occidentalis* inhibited saliva α-amylase activity and inhibition of pancreatic α-amylase activity by red and white ginger[23,24], grapefruits and orange peels[25,26]; pepper and bitter leaf[27,28].

The inhibition of α-glucosidase by the phenolic extract could be attributed to the presence of flavonoids like apigenin, naringenin and catechins which have been shown to be good inhibitor of α-glucosidase[29]. The inhibition of intestinal α-glucosidase activities by the phenolic extract agreed with recent reports on the inhibitory potentials of commonly used medicinal plants, herbs and spices in Latin America against key enzymes relevant to hyperglycemia and common constituents from some traditional Chinese medicine used for DM[5,30]. It is worth noting that the extracts inhibited α-glucosidase activities than α-amylase. This agrees with previous reports that plant phytochemicals are mild inhibitors of α-amylase and strong inhibitors of α-glucosidase activities[23-28].

The finding that Fe^{2+} caused a significant increase in the MDA content of the pancreas agreed with earlier report where Fe^{2+} was shown to be a potent initiator of lipid peroxidation[12]. The increased lipid peroxidation in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyze one-electron transfer reactions that generate reactive oxygen species, such as the reactive OH, which is formed from H_2O_2 through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favors the propagation of lipid oxidation[12]. In the pancreas, Fe accumulates in acinar cells and in the islets of langerhans, thereby resulting in the destruction of β-cells associated with DM[31,32]. Therefore, possible depletion of iron could decrease oxidative stress throughout the whole body[33]. The ability of the phenolic extract

of *C. nitida* to inhibit Fe^{2+} induced lipid peroxidation in the pancreas is presented in Figure 3. Incubation of the pancreas in the presence of Fe^{2+} caused a significant ($P<0.05$) increase in the MDA content of the rat pancreas when compared with the basal pancreas homogenate. However, the phenolic extract from *C. nitida* seed inhibited MDA production in rat's pancreas in a dose-dependent manner. The possible mechanism through which the phenolic extract inhibits lipid peroxidation in the pancreas could be attributed to the presence of flavonoids which have been reported to be chelator of divalent cation thereby preventing them from catalyzing the initiation of lipid peroxidation[32-34].

Antioxidants carry out their protective role on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body[33,34]. The phenolic-rich extract was able to scavenge DPPH radical in a dose dependent manner. The DPPH radical scavenging ability of the phenolic extract could be attributed to the hydrogen donating ability of the hydroxyl groups of the flavonoids. However, as revealed by the EC_{50} (Table 3), the DPPH radical scavenging ability of the phenolic-rich extract was higher than that of *Hyptis suaveolens* leaf[35].

Table 3

EC_{50} values of α-amylase and α-glucosidase inhibitory activity, DPPH radical scavenging ability, Fe^{2+} chelating ability and inhibition of Fe^{2+} induced lipid peroxidation in pancreas by phenolic extract of *C. nitida* seed.

Samples	*C. nitida* seed
α-Amylase inhibitory activity (mg/mL)	0.34±0.01
α-Glucosidase inhibitory activity (mg/mL)	0.32±0.00
Inhibition of Fe^{2+} induced lipid peroxidation in pancreas (mg/mL)	1.20±0.02
Fe^{2+} chelating ability (mg/mL)	0.46±0.01
DPPH radical scavenging ability (mg/mL)	2.20±0.30

Values represent mean±standard deviation of triplicate experiments.

Reducing power is a potent antioxidation defence mechanism; the two mechanisms that are available to affect this reducing power are by electron transfer and hydrogen atom transfer[36]. This is because the ferric to ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the FRAP assay will express the corresponding concentration of electron-donating antioxidants[36]. Phenolic compound had been reported to have a higher reducing power than classical antioxidants such as BHA, BHT, tocopherol and trolox[36]. The high reducing power of the *C. nitida* polyphenol-rich extracts will be of immense advantage in neutralizing free radicals generated in hyperglycaemic condition associated with diabetes, thus slowing down the development of diabetic complications arising from oxidative stress.

The antioxidant properties of plant foods have been attributed mainly due to their phenolic content[8]. The total phenol and flavonoid contents of the polyphenol extract of *C. nitida* seed is presented in Table 1. However, the values obtained were higher than that reported for kolanut and bitter kola[37]; this difference can be attributed to geographical location and mode of storage of these plant foods. The phenolic content of the *C. nitida* seed as presented in Table 1 is high compared with the phenolic content of many plant foods such as fruits[8], vegetables and peppers[23,27]. Nevertheless, the total flavonoid, reported as quercetin equivalent was lower than that reported for some commonly consumed green leafy vegetables in Nigeria[23]. Flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health they have been reported to have antiviral, antiallergic, antiplatelet, antiinflammatory, antitumor and antioxidant activities[5-7]. However, further characterization of the extract with GC in comparison with standard phenolic compounds revealed that the main constituent of the polyphenol-rich extract of *C. nitida* seed are catechin, epicatechin, apigenin and naringenin (Table 2). The structures of the polyphenols identified in the extract are shown in Figure 5. Flavonoids have been reported to stimulate Ca^{2+} uptaken from isolated islet cells thus suggesting it to be effective even in type-2 diabetes[5-7]. Flavonoids such as catechin, epicatechin, apigenin and naringenin have been identified to be good inhibitors of α-amylase and α-glucosidase activity[5-7]. The chemical structures such as the unsaturated C ring, 3-OH, 4-CO, the linkage of the B ring at the 3 position and the hydroxyl substitution on the B ring of flavonoids have been reported to be responsible for α-amylase and α-glucosidase activity[5-7]. Therefore, the presence of phenolic constituents such as; catechin, epicatechin, apigenin and naringenin may have contributed immensely to the medicinal properties of the *C. nitida* seed. Reports have shown that flavonoids could prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes[5-7]. These phytochemicals may have been responsible for the inhibition of α-amylase and α-glucosidase activity as well as the high antioxidant properties of *C. nitida* seed extract.

5. Conclusion

In conclusion, *C. nitida* seed exhibited antioxidant properties and inhibited α-amylase and α-glucosidase (key enzyme linked to type-2 diabetes) activities as well as Fe^{2+} induced lipid peroxidation in rat pancreas *in vitro*. This antioxidant and enzyme inhibition could be some of the possible mechanism by which *C. nitida* is used in folklore for the management/

treatment of type-2 diabetes. However, these characteristics could be attributed to the contributions of phenolic constituents such as: catechin, epicatechin, apigenin and naringenin.

Conflict of interest statement

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

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Comments

Background

Due to the growing number of diabetics, coupled with the harsh side effects of some synthetic drugs have led to the increasing search for alternatives, which are relatively cheap with minimal side effect, this study investigate the inhibitory effect of phenolic-rich extracts from *C. nitida* seeds on key enzymes linked with type-2 diabetes and Fe^{2+} induced oxidative stress in rat pancreas *in vitro*.

Research frontiers

This work is real. It was able to provide some possible mechanism by which *C. nitida* is used in folklore for the management/treatment of type-2 diabetes. The data was well represented. It is innovative. Pharmaceutical companies can bank on the information to improve their means of managing DM type-2.

Related reports

The inhibitory action of the *C. nitida* extract on α-amylase and α-glucosidase activities agreed with some earlier reports where plant phytochemicals from *Telfairia occidentalis* inhibited saliva α-amylase activity (Oboh *et al.*, 2012) and also it agrees with Oboh *et al.* (2010) where red and white ginger inhibited α-amylase and α-glucosidase activity.

Innovations and breakthroughs

This research work is very innovative as it unravels the anti-diabetic mechanism of action of *C. nitida* (kolanut) seeds through the inhibition of key enzymes linked with type-2 diabetes (α-amylase and α-glucosidase), thereby making them a very good and cheap nutraceuticals for management/treatment of type-2 diabetes.

Applications

The result elucidates how the inhibition of prooxidant-induced malondialdehyde production and carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) can be exploited in the management/prevention of oxidative stress associated pathologies. In this research manuscript, type-2 diabetes was emphasized.

Peer review

The most interesting aspect of the research work is the inhibitory effect of the phenolic-rich extract on α-amylase and α-glucosidase activity. The result elucidates how the inhibition of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) can be exploited in the management/prevention of oxidative stress associated pathologies. I therefore, recommend its publication.

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