Global Scientific JOURNALS

GSJ: Volume 9, Issue 7, July 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

IN VITRO ANTIOXIDATIVE ANTICHOLINESTERASE INHIBITORY POTENTIAL OF ETHANOLIC EXTRACT AND ENZYME DIGESTED UNRIPE PLANTAIN (MUSA PARASIDIACA) ON ENZYMES LINKED TO TYPE-2 DIABETES

[1]Gbolagade Abiola Monsurat ,[2]Olu Isreal Oyewole , [3]Salawu Sule Ola and [4]Omowumi O Adewale

[1]Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[2]Department of Biochemistry, Osun State University, Osogbo, Osun State, Nigeria.
[3]Department of Biochemistry, Federal University of Technology Akure, Akure, Ondo State, Nigeria.
[4]Department of Biochemistry, Osun State University, Osogbo, Osun State, Nigeria

ABSTRACT

The dried plantain pulps were crushed and milled into flour (Raw flour), while a portion of its flour was reconstituted in boiling water to form a thick paste known locally in Western Nigeria as 'amala'. This was dried and milled into 'Amala' flour. The ethanol extracts and *in vitro* digest of raw and cooked flour were prepared (1 g/40 mL). The phenolic contents (TPC), total flavonoid contents (TFC), ferric reduce antioxidant power (FRAP) and radical (DPPH, ABTS, NO and OH radical scavenging ability) were analyzed. Also, their inhibition action against lipid peroxidation, cholinesterase, α-amylase and α-glucosidase were determined. The results of the TPC were higher in *in vitro* digests (DPLT, 13.11 mg/g; DCKDPLT, 5.87 mg/g) compared to the ethanol extracts (PLT, 3.51 mg/g; CKDPLT, 1.61 mg/g). This result also showed that raw plantain flour had higher phenolic contents than their respective cooked flour. Similar trend was observed in the result of TFC, FRAP, DPPH, ABTS, NO and OH radical scavenging activities. The *in vitro* digested raw flour had higher inhibitory action against iron (ll) sulphate and sodium nitropruside-induced lipid peroxidation in rat's brain and liver homogenates. Furthermore, the *in vitro* digested cooked flour had higher α-amylase and α-glucosidase inhibitory activities than raw flour. These suggest their potential use in the management of type-2 diabetes and oxidative mediated diseases.

KEYWORDS; Diabetes

Anticholinesterase, Antioxidative, IN VITRO

ENZYMATIC DIGESTION, Unripe plantain

(MUSA PARASIDIACA)

1. INTRODUCTION

Plantain, a tropical fruit with high calorie, provides exceptional nutrition in different forms. Potassium, an important component of cell and body fluids, supports muscles and nerves. It controls heart beat and blood pressure. Plantain is low on glycemic index. Consequently, it results in moderate impact on sugar level in blood (Adebayo, A. and Tenkouano, A. (2019)).

Diabetes mellitus is a heterogeneous metabolic syndrome with several different causes characterized by chronic hyperglycemia with partial or total lack of insulin secretion and a reduced sensitivity to the hormone in peripheral tissues. There

It is a very good source of vitamin-B6 (pyridoxine). Pyridoxine is an important B-complex vitamin. It plays a supportive role in the treatment of neuritis and anemia. Moreover, it helps to decrease homocystine (one of the causative factors in coronary artery disease (CHD) and stroke episodes) level inside body. Plantain is widely used in Indian system of medicine for the treatment of diabetes mellitus (Adebayo, A. T. and Tenkouano, A. (2019))

are two major forms of diabetes: type 1 diabetes (formerly called insulin-dependent or juvenile-onset diabetes), and type 2 (formerly called non-insulin-dependent or adult-onset) diabetes Brand-Miller, J. (2018).

2. MATERIALS AND METHODS

2.1 MATERIALS

2-Deoxy-D-ribose,2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt, 2,2-Diphenyl-1-picrylhydrazyl, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), ferrous sulphate, potassium dichromate ($K_2Cr_2O_7$), Ferric chloride ($FeCl_3$), Methanol, Folin-Ciocalteu's phenol reagent, sodium bicarbonate, aluminum chloride, potassium acetate, sodium phosphate dibasic, sodium phosphate monobasic, potassium

ferricyanide, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid(Trolox), sodium nitroprusside, sulphanilamide, N-(1-Naphthyl ethyldiaminedihydrochloride (NED), orthophosphoric acid, hydrochloric acid, sulphuric acid, chloroform, tannic acid, sodium carbonate, aluminum chloride, quercetin, ascorbic acid, glacial acetic acid, sodium azide, adrenaline, GSH, xanthine oxidase, xanthine, acetylcholine iodide and butyrylcholine iodide were obtained from Sigma chemical company, USA.

2.1.1 Sample Collection

Freshly harvested unripe plantains were bought from National Horticultural Research Institute in Ibadan, Oyo state, Nigeria. The raw plantains (unripe) were identified and authenticated in the Department of Crop, Soil and Pest Management, the Federal University of Technology, Akure, Nigeria.

2.2 METHODS

2.2.1 Sample Treatment and Preparation

The raw plantains were peeled and washed, and divided into two portions. A portion was cooked while the other portion is left uncooked. Both the processed and the unprocessed samples were milled into plantain flour. Flour from each portion was also divided into two portions, ethanolic extraction was carried out on the first portion while *in vitro* enzyme digestion was carried out on the other portion.

2.3 EXTRACTION OF THE SAMPLE

The extraction steps were carried out by soaking 2 g of each of the powdered plantain sample in 40ml of 96% ethanol for 24 hours, after which the supernatant was filtered with filter paper no.42 and stored in an amber bottle. This process was repeated by the addition of another 40ml of 96% ethanol to the residue for another 24 hours and the supernatant pulled together. The filtrate was stored at -4^0c.

2.4 PHYTOCHEMICAL SCREENING

Phytochemical screening involves performing simple chemical tests on the sample for the purpose of detecting different phytochemicals present. Chemical test was carried out on the ethanolic extract to identify the constituents using standard procedures as described by Afoakwa, E. (2015). with slight modification.

absorbance was taken immediately. 1mL of distilled water and 1mL of 10mM DTNB was used as blank. The procedure was repeated using 8mM buyytrlcholine iodide as substrate. The results were expressed in µmol min^{-1}mg $protein^{-1}$ using a molar extinction coefficient $13.6\times10^{3}M^{-1}cm^{-1}$

RESULTS

The high inhibitory effect of digested and ethanoilic extract (cooked and uncooked) from unripe plantains on key enzymes linked to type 2 diabetes could make unripe plantains cheap and good source of nutraceutical for the management of diabetes. Higher inhibitory action (against cholinesterase, α- amylase and α-glucosidase) and antioxidant activities were recorded for the *in vitro* digested sample. It could therefore be concluded that simulated gastro intestinal model is better suitable for measuring nutrients bioavailability *in vitro* rather than mere organic solvent extraction.

Table 1: Phytochemical screening of the ethanolic extract of *musa paradisiaca*.

Sample	saponin	tannin	alkaloid	phlobatanin	salkonski	keller-killani	anthraquinone	steroid	terpenoid	flavonoid
PLT	++	-	+	-	++	+	-	+	+	+
CKDPLT	+	-	+	-	+	+	-	-	-	+

Key; PLT= Uncooked plantain flour, CKDPLT= Cooked plantain flour, ++= strongly present, += present, -= absent.

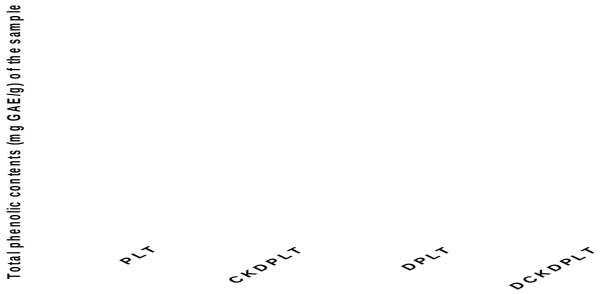

Figure 1: Total phenolic content (mg tannic acid equivalent/g of sample) of unripe plantain. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

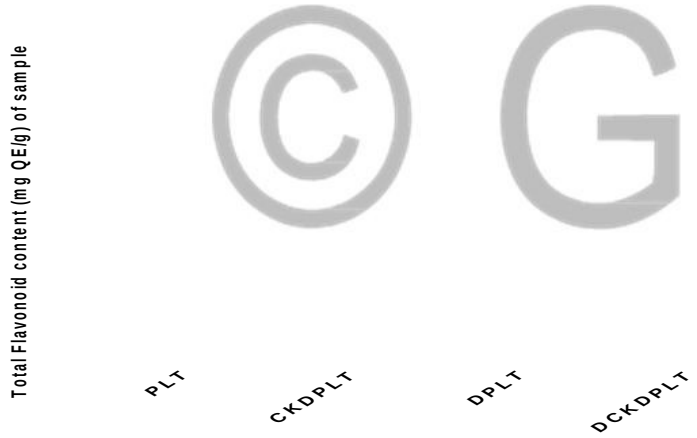

Figure 2: Total flavonoid content (mg quercitin equivalent/g of sample) of unripe plantain. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

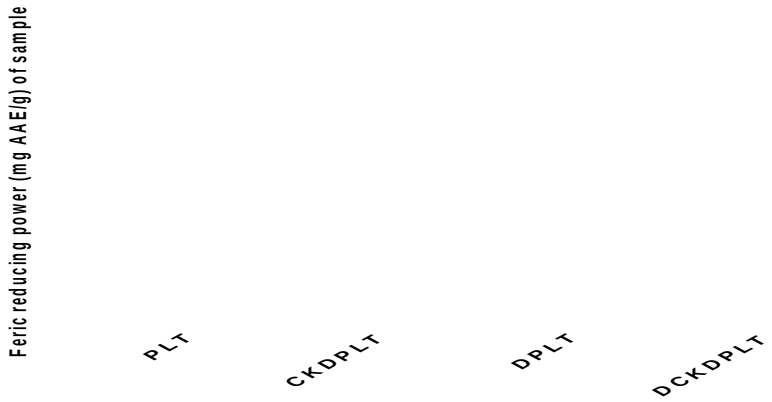

Figure 3: Ferric reducing antioxidant power (mg ascorbic acid equivalent/g of sample) of unripe plantain. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

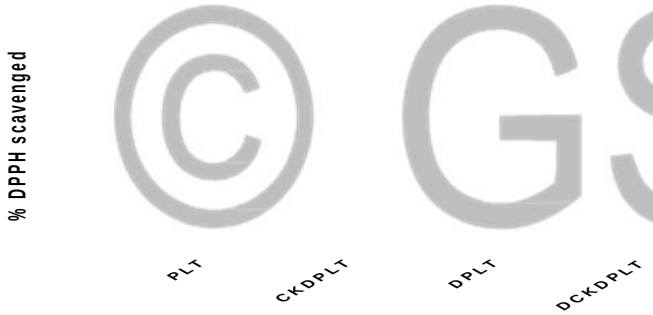

Figure 4:DPPH˙ Radical Scavenging activity of unripe plantain. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

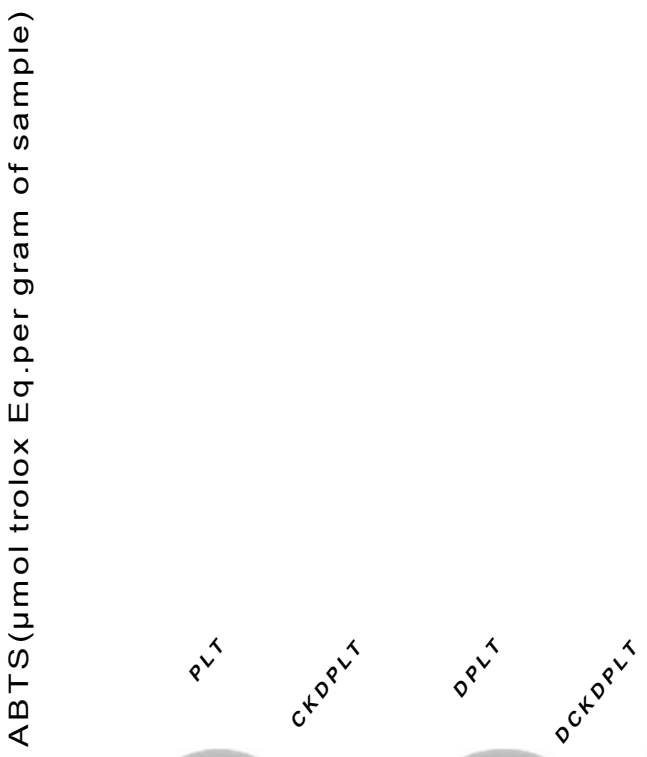

Figure 5: $ABTS^{\cdot+}$ Radical Scavenging Activities (µmol Trolox equivalent/g of sample) of unripe plantain. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic

extract,DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

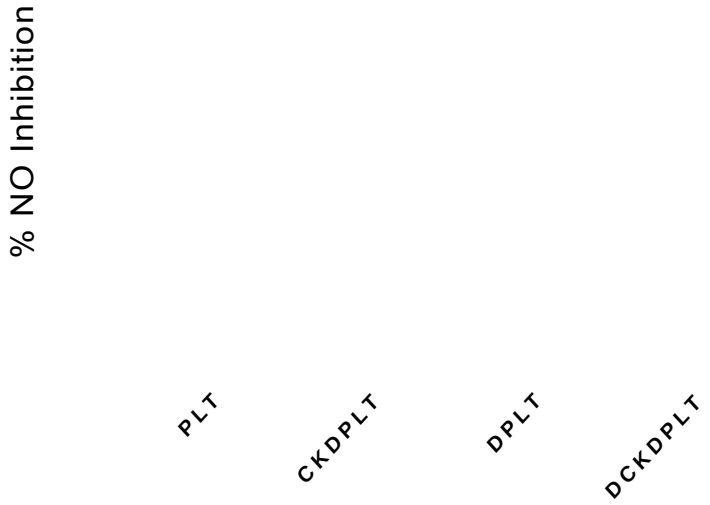

Figure 6: % Nitric oxide scavenging ability of processed plantain (coo and uncooked). Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

Table 2: The inhibitory effects of Plantain on iron induced lipid peroxidation in rat's brain and liver homogenate.

	Fe^{2+} induced Lipid Peroxidation in the Brain				Fe^{2+} induced Lipid Peroxidation in the Liver			
Conc. (mg/ml)	**PLT**	**CKDPLT**	**DPLT**	**DCKDPLT**	**PLT**	**CKDPLT**	**DPLT**	**DCKDPLT**
25	41.7±0.00	34.1±0.07	40. 2±0.02	43.4±0.03	35.0±0.21	16.2±0.00	30.6±0.14	42.0±0.00
50	53.2±0.01	46.3±0.02	57.3±0.00	51.1±0.14	41.9±0.07	24.7±0.35	54.5±0.14	48.1±0.02
75	71.0±0.01	61.6±0.05	71.6±0.02	70.1±0.10	60.4±0.12	34.1±0.00	64.6±0.08	64.3±0.00
100	79.4±0.00	74.0±0.03	83.8±0.00	81.8±0.26	68.1±0.70	47.3±0.41	78.5±0.70	77.5±0.49

The inhibitory effects of Plantain on iron induced lipid peroxidation in rat's brain and liver homogenate. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

Table 3: The inhibitory effects of Plantain on iron induced lipid peroxidation in rat's brain and liver homogenate.

	SNP induced Lipid Peroxidation in the Brain				SNP induced Lipid Peroxidation in the Liver		
Conc. (mg/ml)	**PLT**	**CKDPLT**	**DPLT**	**DCKDPLT**	**PLT**	**CKDPLT**	**DPLT**

DCKDPLT

25	8.11±0.41	5.73±0.00	14.9±0.00	12.8±0.01	26.1±0.34	22.9±0.00	27.8±0.01	24.6±0.40
50	18.3±0.28	12.2±0.14	26.5±0.07	20.5±0.28	30.7±0.04	29.7±0.47	46.5±0.01	28.6±0.03
75	21.6±0.35	17.9±0.04	28.0±0.00	26.5±0.35	46.6±0.28	39.9±0.02	49.6± 0.03	46.6±0.00
100	32.8±0.70	27.7±0.02	40.4±0.01	38.6±0.70	51.3±0.78	46.1±0.47	57.3± 0.01	51.3±0.20

The inhibitory effects of Plantain on iron induced lipid peroxidation in rat's brain and liver homogenate. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain

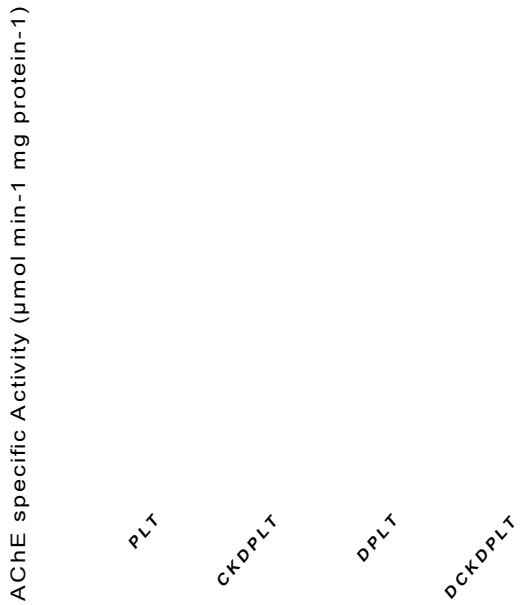

Figure 7: Effect of unripe plantain flour (cooked and uncooked) samples on brain acetylcholinesterase activity. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

Figure 8: Effect of unripe plantain flour (cooked and uncooked) samples on brain butrylcholinesterase activity. Values are given as mean ± SE of independent experiments

performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain

Table 4

ConcMg/ml	ACABOSE	PLT	CKDPLT	DPLT	DCKDPLT
25	`72.7±0.01	7.51±0.00	14.4±0.25	12.7±0.46	35.2±0.00
50	75.8± 0.50	7.44±0.01	41.0±0.00	37.6± 0.03	52.4±0.02
75	80.2± 0.46	18.5±0.69	60.6± 0.40	50.7±0.18	65.5±0.15
100	86.4±0.17	32.6±0.03	64.3±0.00	76.1±0.34	79.9±0.15

The inhibitory effect of Plantain (*Musa parasidiaca*) on α-amylase.Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked plantain flour ethanolic extract, CKDPLT= Cooked plantain flour ethanolic extract, DPLT= Digested uncooked plantain, DCKDPLT= Digested cooked plantain.

Table 5

Concmg/ml	ACABOSE	PLT	CKDPLT	DPLT	DCKDPLT
25	72.7±0.50	1.46±0.01	16.4±0.11	13.4±0.07	40.5±0.26
50	75.8±0.10	16.3±0.40	21.6± 0.16	23.3±0.20	44.2±0.23
75	80.2±0.46	16.4±0.30	46.6±0.00	50.0±0.06	61.4±0.25
100	86.5±0.17	27.5±0.01	59.7±0.18	69.6±0.01	74.5±0.15

The inhibitory effect of Plantain (*Musa parasidiaca*) on α-glucosidase. Values are given as mean ± SE of independent experiments performed in triplicate. PLT= Uncooked Plantain ethanolic extract; CKDPLT= Cooked Plantain ethanolic extract; DPLT= Digested Plantain; DCKDPLT= Digested Cooked Plantain

3. DISCUSSION

The result of the phytochemical screening of the *Musa parasidiaca* in this study showed the presence of saponins, alkaloids, salkonski, keller-killani and flavonoid with steroid and terpenoids present in uncooked plantain and tannin, phlobatanin, anthraquinone absent in the both processed and unprocessed plantain Björk I. and Liljeberg H. (2018).

The result obtained from of inhibitory action of unripe plantain flour (cooked and uncooked) on AChE and BuChE activity revealed that *in vitro* enzyme digested and ethanolic extracts of plantain samples possessed appreciable potential of inhibiting AChE and BuChE activity *in vitro* (Figure 7 and 8). The *in vitro* digested samples displayed higher inhibitory action compared to the ethanolic extracts. Highest % inhibitory action against AChE and BuChE activity was recorded for digested raw plantain flour, followed by digested cooked plantain flour, while the least % inhibition was observed in cooked plantain flour extract.

The interaction of *in vitro* digest and ethanolic extract of unripe plantain flour (cooked and uncooked) with α-amylase, and α-glucosidase are presented in Table 4 and 5 respectively; both digested and ethanol extracts inhibited the enzymes in a dose-dependent manner. However, digested samples showed higher inhibitory action than ethanol extracts. Unlike result of antioxidant indices, cooked flour had higher inhibitory activity than the raw flour in both digested and ethanolic extract.

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