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# Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside-induced lipid peroxidation in rat pancreas by water-extractable phytochemicals from unripe pawpaw fruit (*Carica papaya*)

## Abstract

**Background:** Various parts of unripe pawpaw (*Carica papaya* Linn) fruit have been reportedly used for the management or treatment of diabetes mellitus in folklore medicine. Therefore, the present study sought to investigate the inhibitory effects of the aqueous extract of different parts of unripe pawpaw fruit on key enzymes linked to type 2 diabetes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and sodium nitroprusside (SNP)-induced lipid peroxidation in rat pancreas in vitro.

**Methods:** The aqueous extracts of the unripe pawpaw (*C. papaya*) fruit parts were prepared (1:20 w/v) and the ability of the extracts to inhibit  $\alpha$ -amylase,  $\alpha$ -glucosidase and SNP-induced lipid peroxidation in rat pancreas in vitro was investigated.

**Results:** The results revealed that all the extracts inhibited  $\alpha$ -amylase ( $IC_{50}$ =0.87–1.11 mg/mL),  $\alpha$ -glucosidase ( $IC_{50}$ =1.76–2.64 mg/mL) and SNP-induced lipid peroxidation ( $IC_{50}$ =1.99–2.42 mg/mL) in a dose-dependent manner. However, combination of the flesh, seed and peel in equal amounts had the highest inhibitory effect on  $\alpha$ -amylase and  $\alpha$ -glucosidase activities.

**Conclusions:** Strong inhibitory activities of the unripe pawpaw fruit against key enzymes linked to type 2 diabetes and SNP-induced lipid peroxidation in rat pancreas could be part of the mechanism by which unripe pawpaw is used in the management/prevention of diabetes mellitus in folk medicine. However, combining the unripe pawpaw fruit parts in equal amounts exhibited synergistic properties on  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities.

**Keywords:**  $\alpha$ -amylase;  $\alpha$ -glucosidase; diabetes; sodium nitroprusside; unripe pawpaw fruit.

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## 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 secrete insulin [1]. The prevalence of this disease is increasing annually and the number of diabetics is projected to rise above 300 million before 2025 [2, 3]. Increasing evidence in both experimental and clinical studies has shown the participation of oxidative stress in the development and progression of DM [4–6]. This is usually accompanied by increased production of free radicals [6, 7] or impaired antioxidant defenses [8]. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and 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 [9]. Previous studies indicate that hyperglycemia-induced vascular dysfunction may be triggered by reactive oxygen species (ROS) produced in the mitochondrial electron transport chain [10]. ROS such as the superoxide anion radical ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals (OH) are physiological metabolites formed as a result of respiration in aerobic

organisms. Uncontrolled generated ROS are very unstable and react rapidly with other substances including DNA, membrane lipids and proteins. In humans, this is believed to be involved with DM [11].

Starch is the major source of carbohydrates in most diets and plays a crucial role in the energy supply. The dietary carbohydrates are first broken down to monosaccharides by some gastrointestinal enzymes, as only monosaccharides can be absorbed from intestinal lumen [12].  $\alpha$ -Glucosidase and  $\alpha$ -amylase are the key enzymes involved in the digestion of carbohydrates [13].  $\alpha$ -Amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides that are ultimately converted into monosaccharides by  $\alpha$ -glucosidase. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia [14, 15]. The inhibition of enzymes involved in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet by delaying the process of carbohydrate hydrolysis and absorption [12, 16]. The control of postprandial hyperglycemia is an important strategy in the management of DM, especially type 2 diabetes, and reducing chronic complications associated with the disease [13, 14]. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes  $\alpha$ -glucosidase and  $\alpha$ -amylase in the digestive tract [14–18]. Inhibitors of these enzymes could cause delay in carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [19].

Increasing use of natural medicines as a result of consumers seeking complementary medicines and/or alternatives to prescribed drugs has provoked a great interest in research into medicinal plants. Some of these herbal products used to improve overall health and prevent and cure diseases are likely to act through a stimulation of receptor sites and the immune system, inhibit pathogenesis of disease condition or have a lethal effect on pathogens [20]. The discovery of bioactive compounds of plant origin offers an attractive approach to the control of infectious or noninfectious diseases. Hence, natural  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by ultimately slowing glucose release from starch.

The pawpaw plant (*Carica papaya*) is widespread throughout tropical Africa; it belongs to the group Caricaceae. *C. papaya* is cultivated for its fruits; it is favored by the people of the tropics as breakfast, as an ingredient in jellies and preserves, or cooked in various ways. The juice

makes a popular beverage; young leaves, shoots and fruits are cooked as a vegetable. In Nigeria, where the ripe fruit is cooked as soup with melon seeds and other spices, it is locally known as Ibepe, Gwanda and Okwere in Yoruba, Hausa and Igbo languages, respectively [21]. Phytochemical screening had shown *C. papaya* to contain alkaloids, nicotine, flavonols, tannins and terpenenes [22]. Different parts of the plant are employed in the treatment of different human and veterinary diseases and conditions, such as malaria, stomach ulcer, asthma, bronchitis and impotency, in the form of herbal tea, juice or infusion or even eaten raw [23]. Several independent animal studies have reported the antidiabetic effects of the unripe mature fruits of *C. papaya* [24–26]. Although some in vivo studies have been carried out on the antidiabetic properties of unripe pawpaw, there is limited information on the possible mechanism of action by which it exerts this therapeutic effect. Hence, this study seeks to evaluate the inhibitory effects of water-extractable phytochemicals from different parts of unripe pawpaw fruit (*C. papaya*) on some key enzymes linked with type 2 diabetes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and sodium nitroprusside (SNP)-induced lipid peroxidation in rat pancreas in vitro.

## Materials and methods

### Materials

#### Sample collection

Fresh unripe pawpaw fruit was obtained from a farmland at Obakekere, Akure, Ondo State, Nigeria. Authentication of the samples was carried out at the Department of Biology, Federal University of Technology, Akure, Nigeria. Ten adult male Wistar strain albino rats were purchased from the Animal Production and Health Department, Federal University of Technology, Akure, 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 [27].

#### Chemicals and reagents

Thiobarbituric acid (TBA), sulfanilamide, naphthyl ethylenediamine dihydrochloride and dinitrosalicylic acid (DNSA) were procured from Sigma-Aldrich, Inc. (St. Louis, MO, USA). *p*-Nitrophenyl- $\alpha$ -D-glucopyranoside was sourced from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Acetic acid,  $H_3PO_4$ , Tris-HCl buffer, sodium dodecyl sulfate (SDS) and  $FeSO_4$  were sourced from BDH Chemicals Ltd. (Poole, UK). The water used was glass distilled.

## Aqueous extract preparation

The unripe pawpaw fruit was thoroughly washed with water to remove any contaminant. Each of the unripe pawpaw fruit parts (seed, peel, flesh, flesh with peel and the combination of equal proportions of each part) was blended with distilled water (1:20 w/v), centrifuged and filtered to obtain a clear supernatant, which was then stored in a refrigerator for subsequent analysis [28].

## $\alpha$ -Amylase inhibition assay

The  $\alpha$ -amylase inhibitory activity was determined according to the method of Worthington [29]. The aqueous extract dilution (500  $\mu$ L) and 500  $\mu$ L of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) containing hog pancreatic  $\alpha$ -amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated at 25°C for 10 min. Then, 500  $\mu$ L of 1% starch solution in 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) was added to the reaction mixture. Thereafter, the reaction mixture was incubated at 25°C for 10 min and stopped with 1.0 mL of DNSA. The mixture was then 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 was measured at 540 nm in a Jenway UV-visible spectrophotometer (model 6305; Jenway, Barloworld Scientific, Dunmow, UK). The reference sample included all other reagents and the enzyme with the exception of the test sample. The  $\alpha$ -amylase inhibitory activity was then expressed as percentage inhibition.

$$\text{Inhibition (\%)} = (1 - B/A) \times 100 \quad (1)$$

where  $A$  is the absorbance of control (without the test samples) and  $B$  is the absorbance of the reaction mixture (with the test samples).

## $\alpha$ -Glucosidase inhibition assay

The  $\alpha$ -glucosidase inhibitory activity was determined according to the method of Apostolidis et al. [30]. An appropriate dilution of the aqueous extracts (0–50  $\mu$ L) and 100  $\mu$ L of  $\alpha$ -glucosidase solution was incubated at 25°C for 10 min. Thereafter, 50  $\mu$ L of 5 mmol/L *p*-nitrophenyl- $\alpha$ -D-glucopyranoside solution in 0.1 mol/L phosphate buffer (pH 6.9) was added. The reaction mixture was then incubated at 25°C for 5 min before the absorbance at 405 nm was read in the Jenway UV-visible spectrophotometer. The reference sample included all other reagents and the enzyme with the exception of the test sample. The  $\alpha$ -glucosidase inhibitory activity was then expressed as percentage inhibition calculated using Eq. (1).

## Inhibition of Fe<sup>2+</sup>-induced pancreatic lipid peroxidation

### Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anesthesia, and the pancreas was rapidly isolated and 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 rpm in a Teflon glass homogenizer. The homogenates were centrifuged for 10 min at 3000 *g* to yield a pellet that was discarded, and a low-speed supernatant (S1) was kept for lipid peroxidation assay [31].

### Lipid peroxidation and TBA reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa et al. [32]. Briefly, 100  $\mu$ L of S1 fraction was mixed with a reaction mixture containing 30  $\mu$ L of 0.1 M Tris-HCl buffer (pH 7.4), extract (0–100  $\mu$ L) and 30  $\mu$ L of 5 mM freshly prepared SNP. The volume was made up to 300  $\mu$ L with water before incubation at 37°C for 1 h. The color reaction was developed by adding 300  $\mu$ L of 8.1% SDS to the reaction mixture containing S1; this was subsequently followed by the addition of 500  $\mu$ L of acetic acid/HCl (pH 3.4) mixture and 500  $\mu$ L 0.8% TBA. This mixture was incubated at 100°C for 1 h. TBA reactive species produced were measured at 532 nm in the Jenway UV-visible spectrophotometer, and the absorbance was compared with that of a standard curve using malondialdehyde (MDA).

### Nitric oxide radical scavenging assay

The scavenging effect of the aqueous extract of different parts of unripe pawpaw on nitric oxide (NO) radical was measured according to the method of Mercocci et al. [33]. An amount of 100–400  $\mu$ L of the aqueous extract was added in test tubes to 1 mL of SNP solution (25 mM) and the tubes were incubated at 37°C for 2 h. An aliquot (0.5 mL) of the incubating mixture was removed and diluted with 0.3 mL of Griess reagent (1% sulfanilamide in 5% H<sub>3</sub>PO<sub>4</sub> and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank. Results were expressed as percentage radical-scavenging activity.

## Characterization 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. [34]. The phenolic compounds were extracted from each sample as described by Kelley et al. After extraction, the purified phenolic extracts (1  $\mu$ L:10:1 split) were analyzed for composition by comparison with phenolic standards (Aldrich Chemical Co., Milwaukee, WI, USA) and a co-chromatography with standards on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Corp., Palo Alto, CA, USA) equipped with a derivatized, nonpacked injection liner and an Rtx-5MS (5% diphenyl-95% dimethyl polysiloxane) capillary column (30-m length, 0.25-mm column inside diameter, 0.25- $\mu$ m film thickness) and detected with a flame ionization detector (FID). The following conditions were employed for separation; injector temperature, 230°C; temperature ramp, 80°C for 5 min then ramped to 250°C at 30°C/min; and a detector temperature of 320°C.

## Characterization of alkaloid constituent using GC analysis

The characterization of the alkaloid constituent of the samples was carried out using the method reported by Ngounou et al. [35]. The alkaloid compounds were extracted from each sample as described by a modified method of Ngounou et al. Five grams of the pulverized sample was macerated in 25 mL of hexane for approximately 72 h. The extract was filtered, and the residue was air-dried and later treated with 10% aqueous  $\text{NH}_3$  and macerated in  $\text{CHCl}_3$  for 24 h. After the filtration and evaporation at reduced pressure, the resultant crude extract was treated with 5% aqueous HCl of approximately 7.5 mL. The aqueous phase was made alkaline with aqueous  $\text{NH}_3$  and extracted thrice with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  fraction was washed with water, and the concentrated extract was dried of water by using anhydrous sodium sulfate before GC analysis. A DB-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  column dimensions) was used for the analysis and detected with an FID. The following conditions were employed for alkaloid separation; injector temperature, 230°C; temperature ramp, 80°C for 5 min then ramped to 250°C at 30°C/min; and a detector temperature of 320°C.

## Determination of $\text{IC}_{50}$

In order to determine the  $\text{IC}_{50}$  (the concentration of extracts required to inhibit 50% of the enzyme activity) values, the percentage of enzyme inhibition of the unripe pawpaw extracts was plotted against the extracts at various concentrations (0.5–2.0 mg/mL) for  $\alpha$ -amylase and  $\alpha$ -glucosidase. The  $\text{IC}_{50}$  was then calculated using nonlinear regression analysis.

## Data analysis

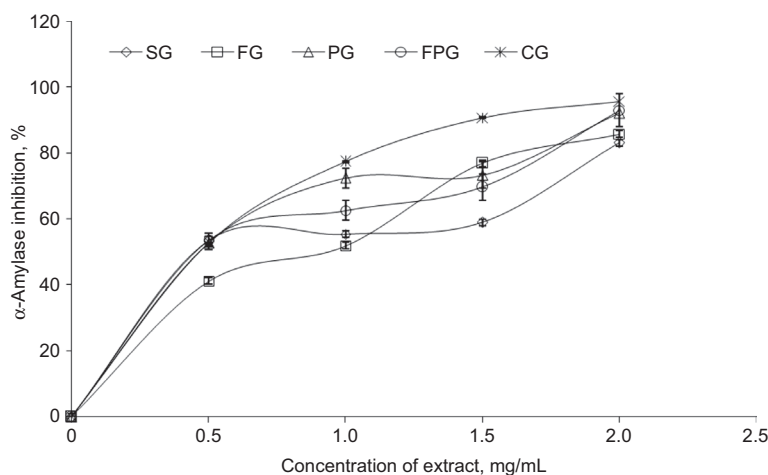
The results of the three replicate experiments were pooled and expressed as mean  $\pm$  standard deviation (SD). Analysis of variance and the least significant difference test were carried out [36].

## Results

The inhibitory effect of the aqueous extract of the different parts of unripe pawpaw fruit on  $\alpha$ -amylase activity is presented in Figure 1. The result revealed that all the extracts inhibited  $\alpha$ -amylase in a dose-dependent manner (0–2.0 mg/mL); however, as revealed by the  $\text{IC}_{50}$  values (Table 1), the combination of seed, flesh and peel of unripe pawpaw extract in equal proportions ( $\text{IC}_{50}$ =0.87 mg/mL) had the highest inhibitory effect followed by the peel ( $\text{IC}_{50}$ =0.96 mg/mL), whereas the seed ( $\text{IC}_{50}$ =1.11 mg/mL) had the least effect.

The inhibitory effect of the aqueous extract of the different parts of unripe pawpaw fruit on  $\alpha$ -glucosidase is presented in Figure 2. The result revealed that all the extracts inhibited  $\alpha$ -glucosidase in a dose-dependent manner (0–2.0 mg/mL); however, the combination of the seed, flesh and peel of unripe pawpaw extract in equal proportions ( $\text{IC}_{50}$ =1.76 mg/mL) had the highest inhibitory effect followed by the flesh with peel ( $\text{IC}_{50}$ =2.13 mg/mL), whereas the peel ( $\text{IC}_{50}$ =2.64 mg/mL) had the least, when taking into account the  $\text{IC}_{50}$  values of the aqueous extracts (Table 1).

The protective ability of the extracts against SNP-induced lipid peroxidation in cultured rat pancreas is presented in Figure 3. Incubation of the rat pancreas in the presence of SNP also caused a significant ( $p < 0.05$ ) increase in the pancreas MDA content (122.2%). However, the flesh with peel ( $\text{IC}_{50}$ =1.99 mg/mL) had the highest inhibitory effect on SNP-induced lipid peroxidation in rat pancreas followed by flesh ( $\text{IC}_{50}$ =2.21 mg/mL) and peel ( $\text{IC}_{50}$ =2.23 mg/mL), whereas the seed ( $\text{IC}_{50}$ =2.37 mg/mL)



**Figure 1**  $\alpha$ -Amylase inhibition of aqueous extract of different parts of unripe pawpaw fruit (*C. papaya*).

SG, seed of unripe pawpaw extract; FG, flesh of unripe pawpaw extract; PG, peel of unripe pawpaw extract; FPG, flesh with peel of unripe pawpaw extract; CG, combination of equal proportions of seed, flesh and peel of unripe pawpaw extract.

**Table 1**  $IC_{50}$  value of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity and SNP-induced lipid peroxidation in rat pancreas of the different parts of unripe pawpaw fruit.

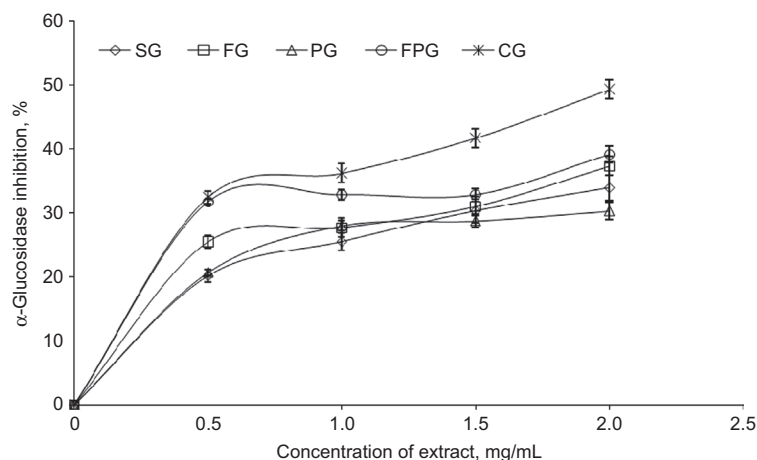
Sample	$IC_{50}$ , mg/mL		
	$\alpha$ -Amylase inhibitory activity	$\alpha$ -Glucosidase inhibitory activity	SNP-induced lipid peroxidation in rat pancreas
SG	1.11±0.05	2.52±0.07	2.42±0.05
FG	1.04±0.05	2.32±0.10	2.21±0.05
PG	0.96±0.02	2.64±0.10	2.23±0.05
FPG	0.99±0.03	2.13±0.05	1.99±0.03
CG	0.87±0.01	1.76±0.04	2.37±0.06

SG, seed of unripe pawpaw extract; FG, flesh of unripe pawpaw extract; PG, peel of unripe pawpaw extract; FPG, flesh with peel of unripe pawpaw extract; CG, combination of equal proportions of seed, flesh and peel of unripe pawpaw extract.

had the least, when taking into account the  $IC_{50}$  values of the aqueous extracts (Table 1).

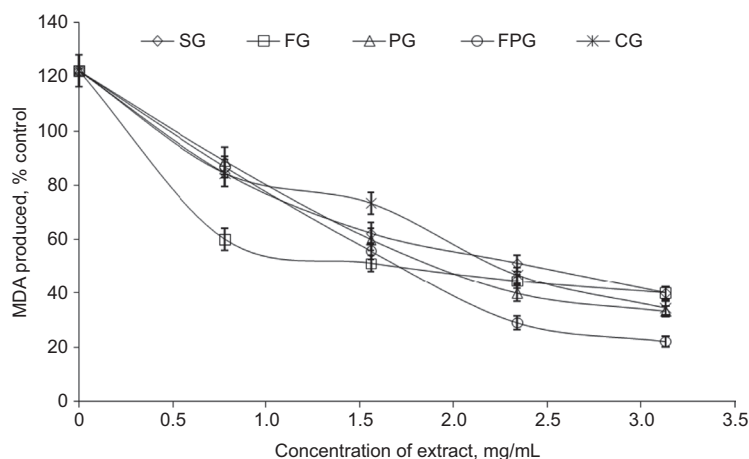
The NO radical scavenging ability of the aqueous extract from different parts of unripe pawpaw fruit is presented in Figure 4. The result revealed that all the extracts scavenged NO radical in a dose-dependent pattern (0–7.5 mg/mL). However, the flesh with peel (7.7–52.5%) had the highest NO radical scavenging ability, whereas the seed of the unripe pawpaw (1.5–12.5%) had the least.

The main phenolic constituents of the unripe pawpaw parts are flesh [sinapinic acid (4.81 mg/100 g), 2-phenyl-6<sup>l</sup>-O- $\beta$ -D-glucoside (4.37 mg/100 g) and *o*-coumaric acid (1.72 mg/100 g)], seed [epicatechin (27.09 mg/100 g), quercetin (18.24 mg/100 g) and luteolin (15.09 mg/100 g)], peel [epicatechin (18.86 mg/100



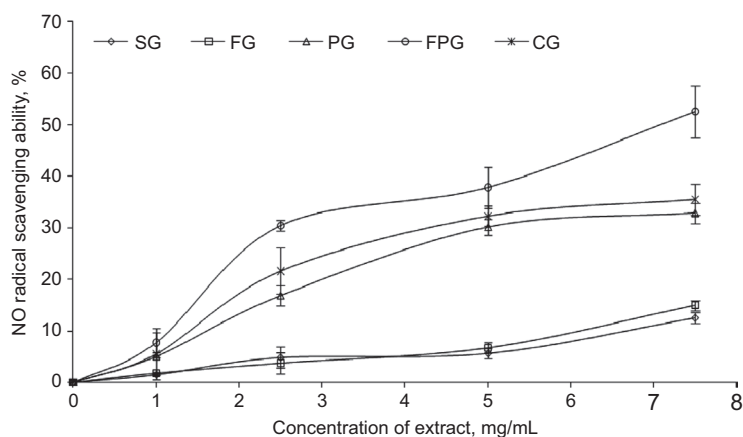
**Figure 2**  $\alpha$ -Glucosidase inhibition of aqueous extract of different parts of unripe pawpaw fruit (*C. papaya*).

SG, seed of unripe pawpaw extract; FG, flesh of unripe pawpaw extract; PG, peel of unripe pawpaw extract; FPG, flesh with peel of unripe pawpaw extract; CG, combination of equal proportions of seed, flesh and peel of unripe pawpaw extract.



**Figure 3** SNP-induced lipid peroxidation in rat pancreas by aqueous extract of different parts of unripe pawpaw fruit (*C. papaya*).

SG, seed of unripe pawpaw extract; FG, flesh of unripe pawpaw extract; PG, peel of unripe pawpaw extract; FPG, flesh with peel of unripe pawpaw extract; CG, combination of equal proportions of seed, flesh and peel of unripe pawpaw extract.



**Figure 4** NO radical scavenging ability of aqueous extract of different parts of unripe pawpaw fruit (*C. papaya*).

SG, seed of unripe pawpaw extract; FG, flesh of unripe pawpaw extract; PG, peel of unripe pawpaw extract; FPG, flesh with peel of unripe pawpaw extract; CG, combination of equal proportions of seed, flesh and peel of unripe pawpaw extract.

g), ferulic acid (15.40 mg/100 g), kaempferol (13.98 mg/100 g), quercetin (13.37 mg/100 g) and caffeic acid (13.35 mg/100 g) as shown in Table 2 and Figure 5A–E. Furthermore, characterization of the extract using standard alkaloid compounds revealed that carpaine is the main alkaloid constituent of the different parts of unripe pawpaw, with the peel (792.9 mg/100 g) having the highest amount followed by the seed (72.3 mg/100 g), whereas the flesh (17.7 mg/100 g) had the least (Table 3 and Figure 6A–E).

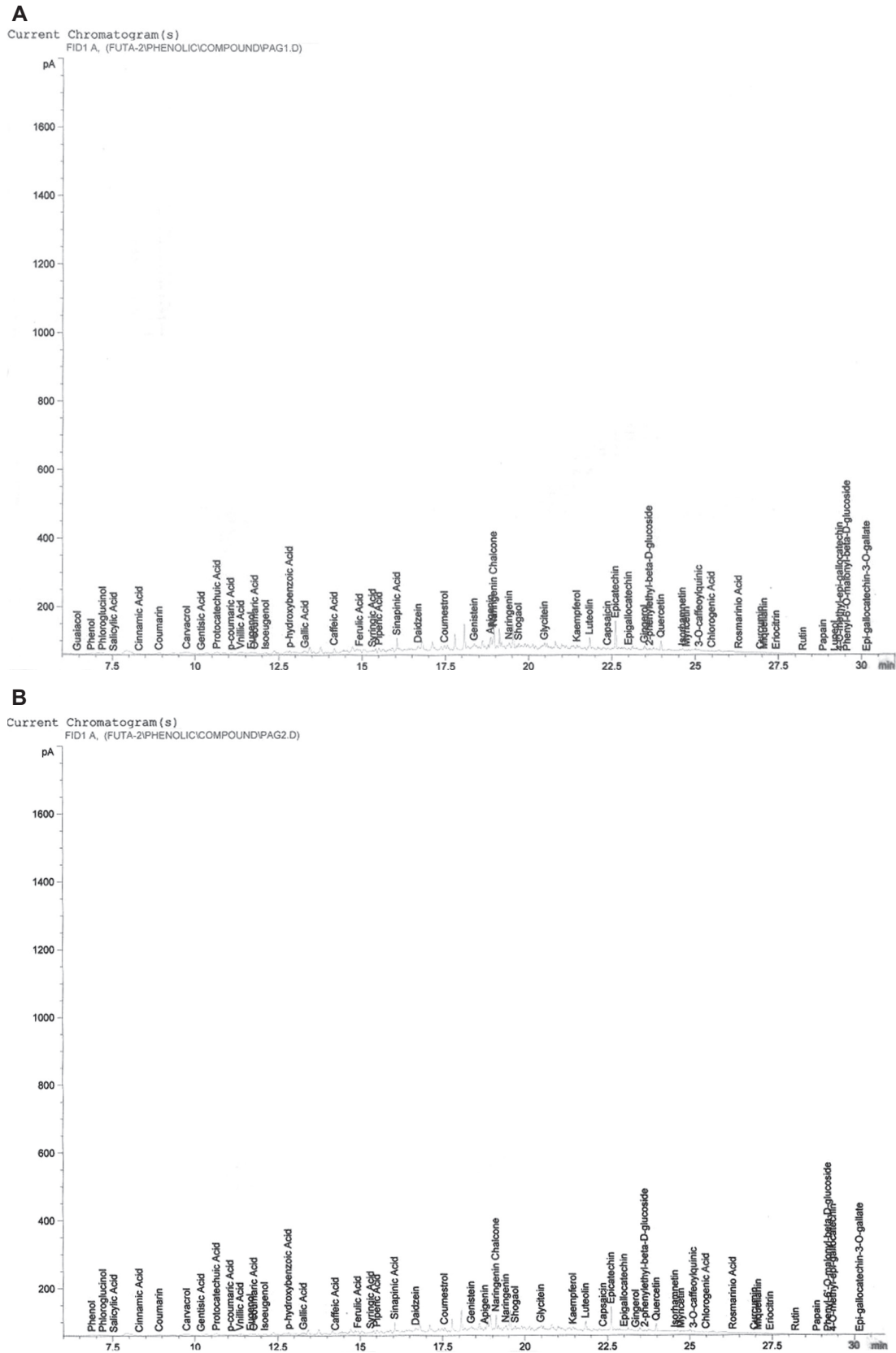
## Discussion

Control of postprandial plasma glucose levels is critical in the early treatment of DM and in reducing chronic vascular complications [37]. Inhibitors of saccharide-hydrolyzing enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type 2 DM [15]. Inhibition of these enzymes delay carbohydrate digestion and prolong

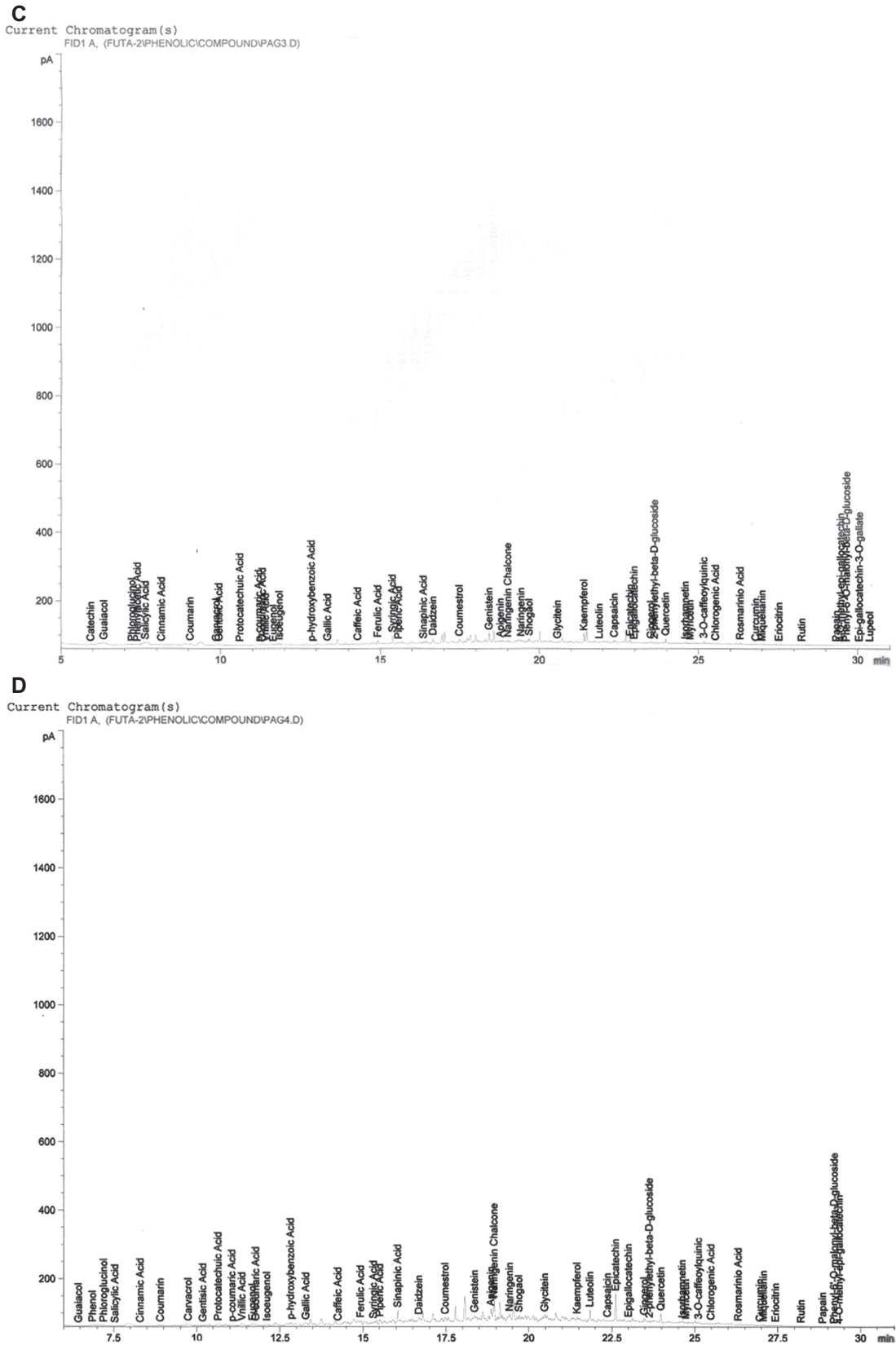
**Table 2** The main phenolic constituents in different parts of unripe pawpaw fruit (*C. papaya*).

Name of compound	FG, mg/100 g	SG, mg/100 g	PG, mg/100 g	FPG, mg/100 g	CG, mg/100 g
Coumarin	$1.78 \times 10^{-1}$	$9.20 \times 10^{-4}$	9.00	8.43	7.98
<i>p</i> -Coumaric acid	1.52	$6.32 \times 10^{-4}$	9.68	10.97	11.78
<i>o</i> -Coumaric acid	1.73	$6.61 \times 10^{-4}$	5.85	7.59	8.94
Vanillic acid	3.64	$1.37 \times 10^{-4}$	$1.67 \times 10^{-5}$	4.16	4.87
Caffeic acid	1.25	$1.58 \times 10^{-5}$	13.35	15.09	18.99
Ferulic acid	$6.14 \times 10^{-1}$	$2.48 \times 10^{-4}$	15.40	17.03	16.77
Syringic acid	1.63	$4.26 \times 10^{-4}$	$7.08 \times 10^{-4}$	1.79	2.15
Sinapinic acid	4.81	$1.71 \times 10^{-4}$	$7.12 \times 10^{-5}$	4.63	5.60
Genistein	$1.64 \times 10^{-2}$	3.84	1.56	1.77	2.12
Apigenin	$2.38 \times 10^{-2}$	11.89	13.96	14.50	15.31
Naringenin	$2.93 \times 10^{-3}$	$2.39 \times 10^{-3}$	11.82	13.07	15.73
Kaempferol	$4.31 \times 10^{-2}$	$1.58 \times 10^{-4}$	18.86	20.48	24.52
Luteolin	$2.77 \times 10^{-2}$	15.10	7.77	8.66	8.03
Epicatechin	$3.26 \times 10^{-4}$	27.09	18.86	20.48	24.52
Epigallocatechin	$9.13 \times 10^{-4}$	7.39	8.33	9.96	11.81
Quercetin	$1.07 \times 10^{-4}$	18.24	13.37	14.78	17.42
2-Phenylethyl- $\beta$ -D-glucoside	4.38	8.93	4.33	4.31	5.14
Phenyl-6'- <i>O</i> -maloyl- $\beta$ -D-glucoside	$1.28 \times 10^{-4}$	$7.59 \times 10^{-5}$	8.13	8.58	7.67

SG, seed of unripe pawpaw extract; FG, flesh of unripe pawpaw extract; PG, peel of unripe pawpaw extract; FPG, flesh with peel of unripe pawpaw extract; CG, combination of equal proportions of seed, flesh and peel of unripe pawpaw extract.

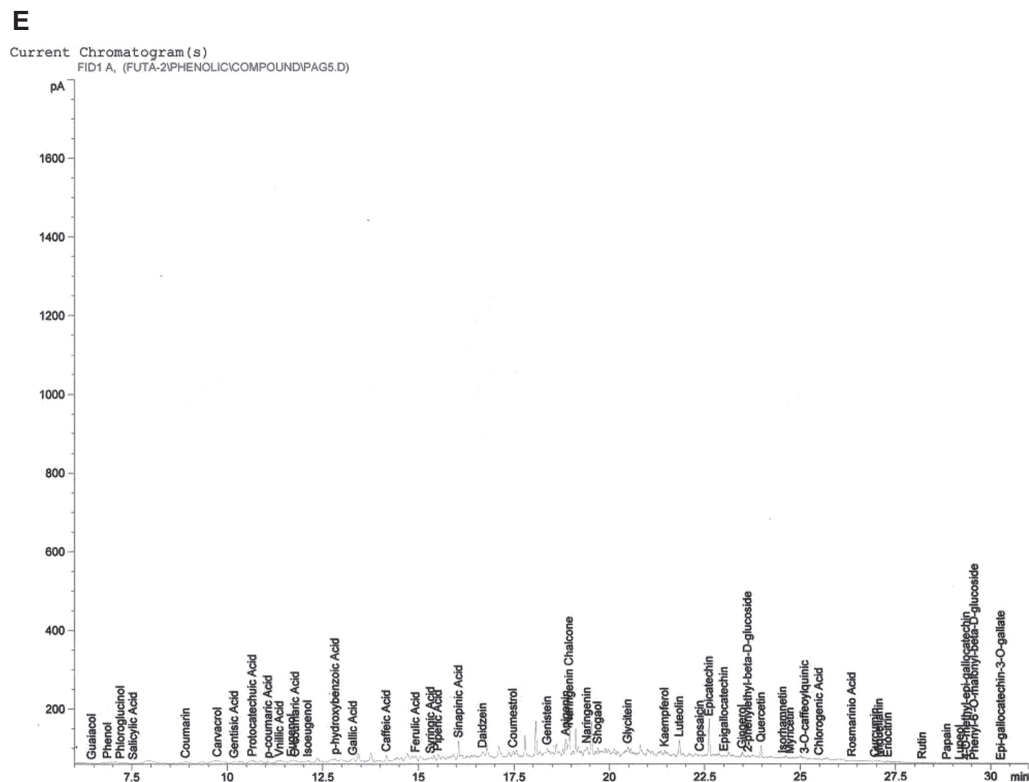


(Figure 5 Continued)



(Figure 5 Continued)





**Figure 5** Chromatographic trace of phenolic constituent in flesh (A), seed (B), peel (C), flesh with peel (D) and combination of flesh, seed and peel in equal amounts (E) of unripe pawpaw by GC with FID. The main phenolic constituent is shown in Table 2.

**Table 3** The main alkaloid constituent in different parts of unripe pawpaw fruit (*C. papaya*).

Sample	Carpaine, mg/100 g
SG	72.3±0.04 <sup>a</sup>
FG	17.7±0.04 <sup>b</sup>
PG	792.9±0.10 <sup>c</sup>
FPG	33.9±0.00 <sup>d</sup>
CG	25.2±0.01 <sup>e</sup>

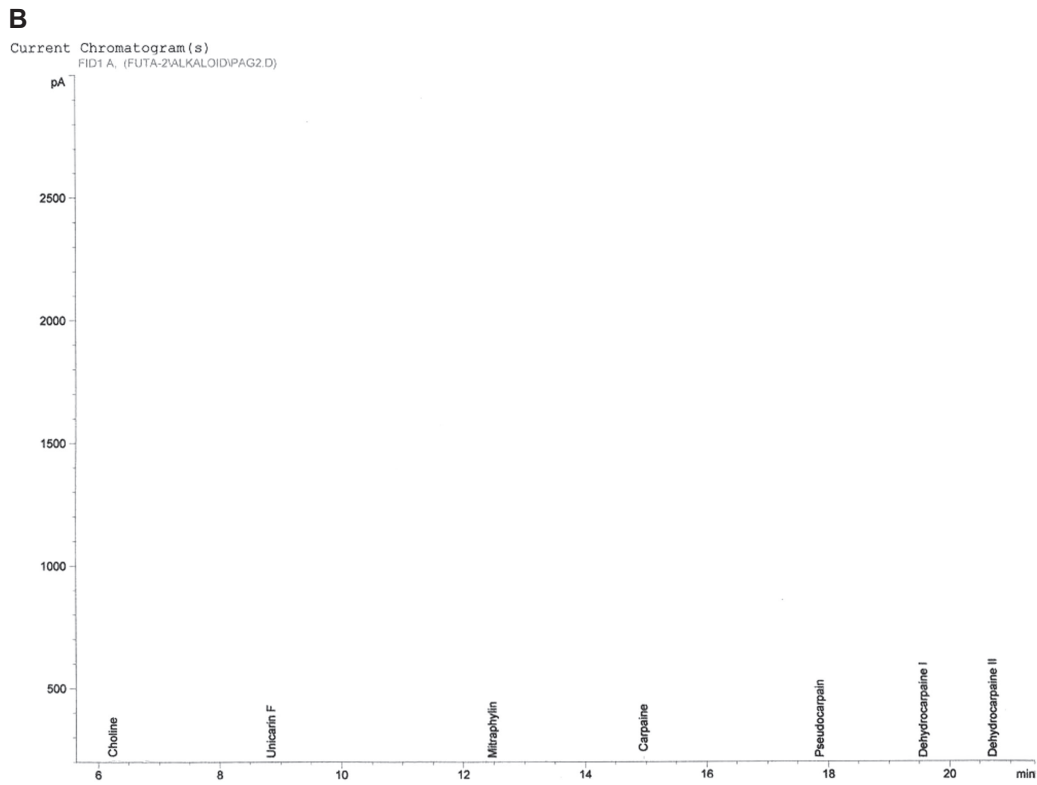
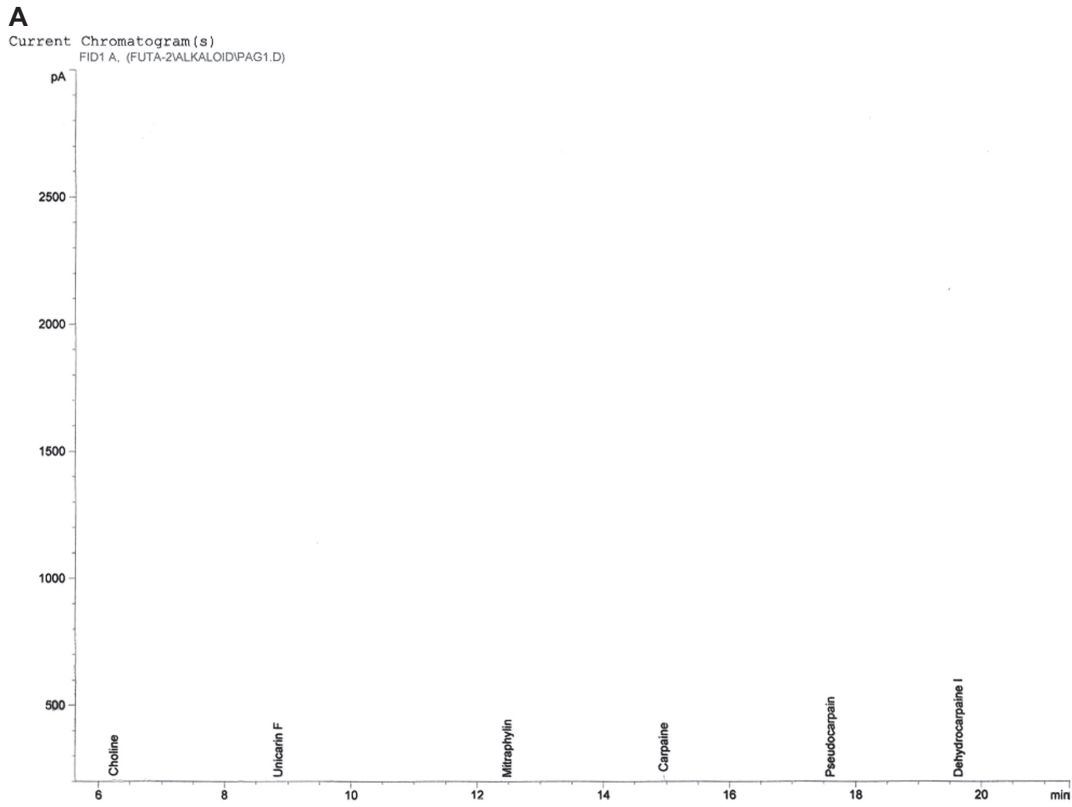
Values represent mean±SD of triplicate readings. Values with the same superscript are not significantly (p<0.05) different. For abbreviations, see the footnote to Table 1.

overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise [12]. It is of note that the combination of the unripe pawpaw parts exhibited a synergistic effect on the  $\alpha$ -amylase inhibitory activity when compared to individual parts of the unripe pawpaw fruit extracts. The inhibition of  $\alpha$ -amylase by the aqueous extract of the different parts of unripe pawpaw fruit agree with some earlier reports on green and black tea [38], pepper [39], ginger varieties [16], jute leaf [40], soybean [41] and shaddock peels [42]. Therefore, these could explain part of the reason for their use in the management of DM.

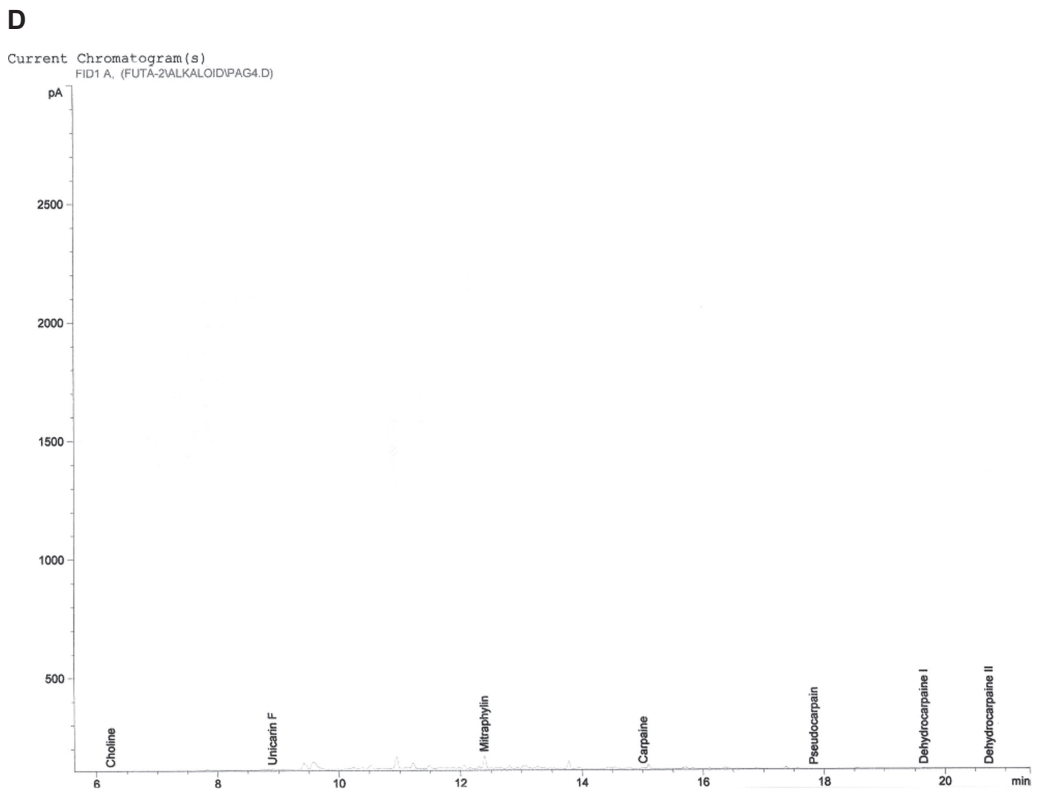
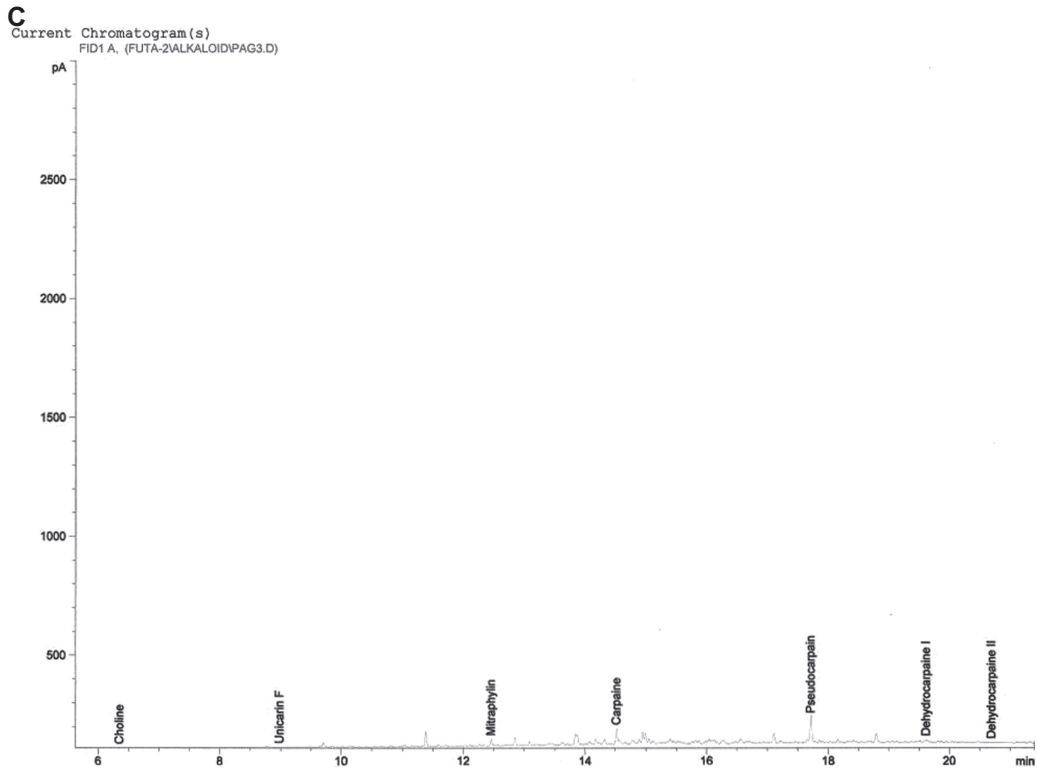
Furthermore, the inhibitory effect of the aqueous extract of the different parts of unripe pawpaw fruit on  $\alpha$ -glucosidase as presented in Figure 2 revealed that the combination of the seed, flesh and peel of unripe pawpaw extract in equal proportions had the highest inhibitory effect followed by the flesh with peel, whereas the peel of the unripe pawpaw fruit had the least. This is in agreement with the  $\alpha$ -amylase inhibitory activities of the unripe pawpaw fruit part extracts (Figure 1). The inhibition of  $\alpha$ -glucosidase activity slows down the breakdown of disaccharide to simple glucose and by so doing reduces the amount of glucose absorbed in the blood [12].

Free radicals are involved in the case of diabetes, and they also play a role in some of the complications seen in long-term treatment of diabetes. Drugs such as alloxan and streptozotocin are used to induce diabetes in animals by different mechanisms of action, but both result in the production of ROS [43]. In humans, the white cell production of ROS mediates the immune destruction of the  $\beta$  cells [43]; therefore, scavengers of free radicals are effective in preventing diabetes [43].

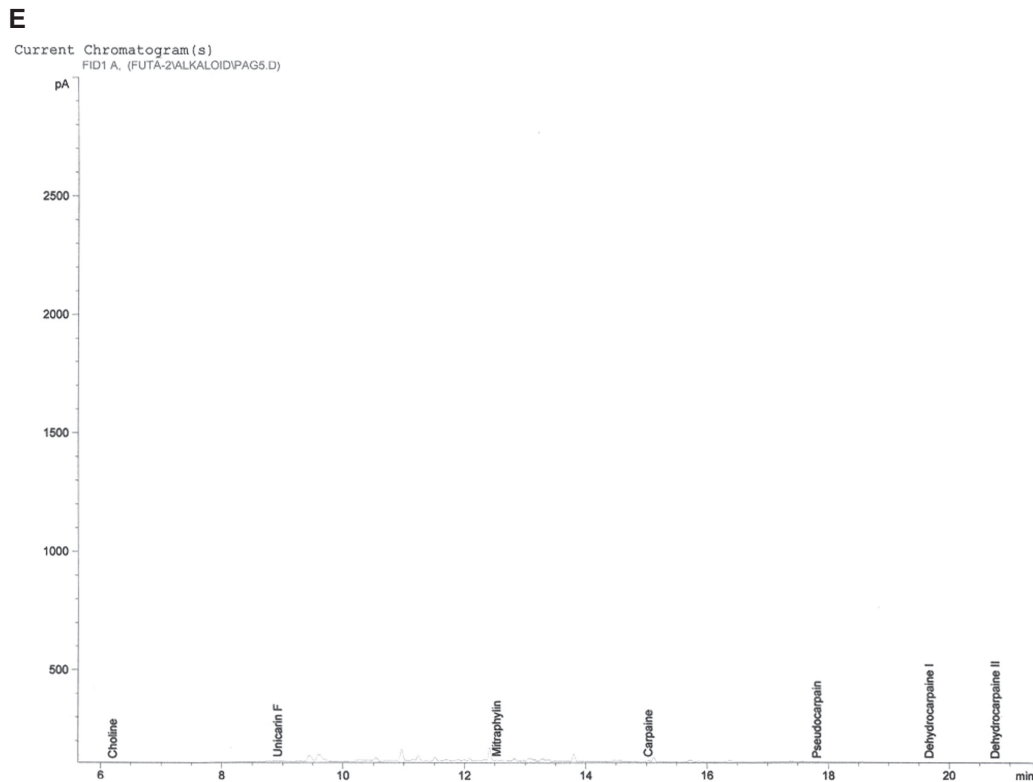
SNP, a component of antihypertensive drugs, causes cytotoxicity through the release of cyanide and/or NO. NO is a universal neuronal messenger in the central nervous system and acts independently; it may also cause



(Figure 6 Continued)



(Figure 6 Continued)



**Figure 6** Chromatographic trace of alkaloid constituent in flesh (A), seed (B), peel (C), flesh with peel (D) and combination of flesh, seed and peel in equal amounts (E) of unripe pawpaw by GC with FID. The main alkaloid constituent is shown in Table 3.

neuronal damage in cooperation with other ROS, forming peroxynitrite (ONOO<sup>-</sup>), a potent oxidizing and nitrating species [44, 45]. Peroxynitrite can impair most cellular components, including proteins, DNA and phospholipid membranes [46, 47]. The mechanisms that account for cytotoxicity elicited by peroxynitrite are multiple; among them is the induction of DNA strand breaks. In this context, DNA single-strand breakage initially triggered by peroxynitrite may lead to cell death [48]. Moreover, this study revealed that extracts of the different parts of unripe pawpaw fruit were able to protect the pancreas against SNP-induced lipid peroxidation. However, extract of the flesh with peel had the highest inhibitory effect on MDA production, whereas the seed had the least inhibitory effect (Table 1). The higher inhibitory ability of the aqueous extract of the flesh with peel could be attributed to its high NO radical scavenging ability as observed in Figure 4. Studies have shown that plant extracts with peroxynitrite-scavenging activity are able to protect against peroxynitrite mediated DNA damage [49].

NO plays an important role as a physiological messenger [50]. NO is a free radical with a short half-life (<30 s). Although NO acts independently, it may also cause tissue damage in cooperation with other ROS such as superoxide radical to form peroxynitrite radical [51]. The NO radical

scavenging ability of the aqueous extract of different parts of unripe pawpaw fruit is presented in Figure 4. The results revealed that the flesh with peel had the highest NO radical scavenging ability and the seed had the least. This result is in agreement with the SNP-induced lipid peroxidation in rat pancreas in vitro (Figure 3).

The antioxidant properties of plant foods have been linked to the presence of an array of important phenolic and nonphenolic phytochemicals including phenolic acids, flavonoids and alkaloids [52–55]. However, characterization of the extract with GC revealed that the major constituents of the flesh, seed and peel extract of unripe pawpaw are sinapinic acid, 2-phenyl-6'-*O*- $\beta$ -D-glucoside, *o*-coumaric acid, epicatechin, quercetin, luteolin, ferulic acid, kaempferol and caffeic acid (Table 2 and Figure 5A–E). Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing  $\alpha$ -tocopherol radicals and inhibiting oxidases [56]. Further characterization of the extract using standard alkaloid compounds revealed that carpaine is the main alkaloid constituent of the different parts of unripe pawpaw with the peel having the highest amount followed by the seed,

whereas the flesh had the least (Table 3 and Figure 6A–E). This result is in agreement with a recent work by Krishna et al. [57], where carpaine is the main alkaloid constituent of *C. papaya*. In vitro and in vivo studies have shown that natural plant alkaloid isolated from the Chinese herb, *Coptis chinensis* (Huanglian), has glucose-lowering effect and is also used in the treatment of diarrhea [58].

## Conclusions

The inhibition of key enzymes linked to type 2 diabetes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and lipid peroxidation by the water-extractable phytochemicals could be part of the mechanism through which unripe pawpaw fruit is used in the management/prevention of DM in folk medicine. Nevertheless, combination of the unripe pawpaw fruit parts in equal amounts exhibited synergistic effects on inhibition

of  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. However, these characteristics may be due to phenolic constituents such as sinapinic acid, 2-phenyl-6'-*O*- $\beta$ -D-glucoside, *o*-coumaric acid, epicatechin, quercetin, luteolin, ferulic acid, kaempferol and caffeic acid, which may be exhibiting either an additive or a synergistic effect with the nonphenolic constituent such as carpaine.

## Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article.

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