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# Identification of bioactive constituents of chloroform fraction from *Annona muricata* leaf, its antioxidant activity and inhibitory potential against carbohydrate-hydrolyzing α-amylase and α-glucosidase activities linked to type II diabetes mellitus: *In vitro* study

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**ABSTRACT**

**Introduction:** Secondary metabolites from plants have been found to play an important role in the treatment of diabetes mellitus (DM) and its complications. Therefore, the purpose of this study was to identify the chemical components of *Annona muricata* leaf chloroform fraction (CF*Am*) and its *in vitro* antioxidant properties, as well as inhibitory activity against α-amylase and α-glucosidase enzymatic activities.

**Methods:** Gas chromatography–mass spectrometry (GC-MS) technique was engaged in the identification of phytochemical constituents. Antioxidant activities such as DPPH free radical scavenging ability, reducing power capacity, hydroxyl radical scavenging ability, singlet oxygen scavenging capacity, as well as α-amylase and α-glucosidase inhibition were carried out using standard *in vitro* methods.

**Results:** GC-MS analysis of CF*Am* revealed the presence of 23 phytochemicals, out of which 5 compounds had the highest % compositions (i.e., octadecanoic acid (20.35%), 2-propanone, 1-(4-hydroxy-3-methoxyphenyl) (12.04%), isocomene (22.60%), 9, 12, 15 octadecatrienoic acid, methyl ester (Z,Z,Z) (28. 98%), and quercetin, 5TMS derivative (18.28%)). Also, CF*Am* demonstrated a significant ($P<0.05$) inhibition against DPPH ($IC_{50}$= 26.33 ± 1.39 mg/mL), with OH free radical scavenging capacity (65.46 ± 1.39 mg/100 g), singlet oxygen scavenging capacity (55.24 ± 1.22 mg/100 g), and showed ferric reducing power (84.52 ± 2.84 mg/100 g). Also, CF*Am* exhibited a significant ($P<0.05$) inhibition against α-glucosidase ($IC_{50}$= 71.06 ±1.45 mg/mL) and α-amylase ($IC_{50}$= 73.88 ± 1.58 mg/mL) in a concentration-dependent manners.

**Conclusion:** The remarkable properties demonstrated by CF*Am*, which are essential for the management of DM, could probably be credited to the presence of the various identified phytonutrients.

*Implication for health policy/practice/research/medical education:*
Chloroform fraction from *Annona muricata* leaf revealed some important phytochemical constituents and demonstrated significant antidiabetic potentials that might be useful as an alternative therapy in the management of diabetes mellitus.

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## Introduction

Impairment in glucose metabolism causes physiological imbalance, resulting in hyperglycemia and eventually, diabetes mellitus (DM) (1,2). Diabetes is referred to as a complex metabolic disorder that is linked to a number of human diseases (3). It prevents the metabolism of macromolecules like proteins, lipids, and carbohydrates (4). DM has recently posed a significant threat to global healthcare system (5). However, according to a World Health Organization (WHO) report, approximately 22%

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of the global population is expected to be diabetic by 2050, up from the current 12% (6). DM is a major cause of cardiovascular disease and other co-morbidities. Cell dysfunction, on the other hand, is known to be a major factor in the progression from prediabetes to diabetes (7). Diabetes is divided into two types: type 1 (T1DM) and type 2 (T2DM) (8). T1DM (insulin-dependent DM) is caused by failure of the pancreatic-Langerhans islet cells to secrete insulin, whereas T2DM (non-insulin-dependent DM) is caused by a combination of insufficient insulin production from the β-cells and peripheral insulin resistance (9). Autoimmunity is thought to be the most important factor in the pathophysiology of T1DM. T2DM is reported to be the most common type of DM (10). Controlling postprandial elevated blood glucose levels is critical in the management of non-insulin-dependent diabetes (11,12). This may be accomplished by stimulating insulin secretion, inhibiting glucose transporters, or delaying starch digestibility by inhibiting the activities of starch-hydrolyzing enzymes such as pancreatic α-amylase and intestinal α-glucosidase (13).

Oxidative stress, defined as an imbalance between reactive oxygen species (ROS)/electrophiles (free radicals) and the antioxidant system, is known to disrupt cellular redox signaling and thus aggravate the severity of a variety of pathological conditions (14). However, evidence suggests that elevated systemic oxidative stress is closely linked to the etiology of diabetes (15). ROS are byproducts of normal cell metabolic activity that can be beneficial or harmful depending on their concentrations in the tissues (16). It is worth noting that free radicals are highly reactive and unstable, acting as oxidants or reductants when proliferated in the target tissue. Mitochondrial reactions are thought to be the primary sources of oxygen-derived free radicals within the cells (17). Sufficient research shows that DM patients have endothelial dysfunction, which invariably contributes to mitochondrial superoxide overproduction in endothelial cells. Nicotinamide dinucleotide phosphate (NADPH) oxidases, xanthine oxidases, cyclooxygenases, and, in some cases, endothelial NO synthases all play important roles in the generation of electrophiles/ROS (18). Furthermore, it has been reported that proliferated ROS directly affect post-translational modifications on biomolecules such as lipids, proteins, deoxyribonucleic acid (DNA), and low molecular weight antioxidant parameters (19). These modifications cause cellular dysfunction, which initiates the pathogenic milieu and the development of a number of chronic diseases (20). To maintain a healthy biological system, a balance of oxidation and antioxidation is always required.

Several interventions have been used to manage diabetes, one of which is the nutraceutical approach, which is currently of great scientific interest (21). There is mounting evidence that dietary antioxidants (polyphenols) increase plasma antioxidant capacity and protect cellular components from oxidative damage, thereby lowering the risk of a variety of oxidative stress-related diseases (22). Polyphenols (flavonoids and phenolics, for example) can accept an electron to form relatively stable phenoxyl radicals, disrupting ROS-induced chain autoxidation reactions in cell components (23). Natural plants are rich in these essential secondary metabolites, which have been linked to a variety of antioxidant activities (24). Antioxidants function as radical scavengers, hydrogen donors, electron donors, singlet oxygen quenchers, enzyme inhibitors, peroxide decomposers, and metal chelators (24).

Conversely, interactions of polyphenolics with carbohydrates have sparked considerable interest as anti-diabetic agents (25). They have an impact on starch digestion by inhibiting two key starch-hydrolyzing enzymes, α-amylase and α-glucosidase (26). Starch digestion is a central metabolic response that always occurs after a meal. Similarly, acarbose, voglibose, miglitol, and other starch-blockers are used effectively to control postprandial hyperglycemia in T2DM; however, the associated side effects have increased interest in alternative therapies.

*Annona muricata*, also known as soursop, *graviola*, and *guanabana*, is an evergreen plant found primarily in tropical and subtropical regions of the world (27,28). A variety of ethnopharmacological studies have indicated that various parts of *A. muricata* are used in local medicine. *A. muricata* fruit has also been shown in studies to reduce fever and improve milk secretion in lactating mothers, and it is widely used in folkloric medicine in some countries (29). Recent research has found that different solvent extractions of *A. muricata* have effective anti-diabetic properties (30). Therefore, antioxidant potentials, carbohydrate-hydrolyzing enzyme inhibitory activity, and potential bioactive components of *A. muricata* chloroform fraction were investigated in this study.

## Materials and Methods

### Chemicals used

Chemicals such as chloroform, n-hexane, ethyl acetate, n-butanol and methanol were procured from Merck Company (Darmstadt, Germany), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 3,5-dinitrosalicylic acid (DNSA), α-amylase (porcine, EC 3.2.1.1), 4-Nitrophenyl β-D-glucopyranoside (pNPG), and α-glucosidase (*Saccharomyces cerevisiae*, EC 3.2.1.20), acarbose, butylated hydroxytoluene (BHT) and gallic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade unless otherwise stated and were all prepared in all-glass ware.

### Sample collection

*Annona muricata* leaves were obtained from a garden at Okesha market, Ado-Ekiti, Ekiti State, Nigeria. Following that, Mr. Bolu Ajayi, a senior taxonomist at the Department

of Plant Biology, University of Ilorin, Nigeria, identified and documented the sample. The herbarium number: UILH/001/1106/2020 was provided from the database, along with a voucher specimen deposited appropriately at the University herbarium.

### Preparation of plant extract

*Annona muricata* fresh leaves were dried in a laboratory oven called a Uniscope SM9053 from Surgifriend Medicals in England at a temperature of 40°C. Using an electric blender (Crown Star Blender CS-242B, Trident (H.K) Ltd, China), the dried leaves (120 g) were ground into a powder. The extracted powder was then continuously shaken in 1200 mL of 95% methanol for 72 hours at room temperature. The filtrate from this extraction was then concentrated in a water bath at 40°C after being filtered with Whatman No. 1 filter paper.

### Solvent partition-fractionation method

According to the method of Goulart et al, the methanol leaf extract of *A. muricata* (25 g) was successively extracted with the solvents of increasing polarity (i.e., hexane, ethyl acetate, n-butanol, and methanol) (31). To separate the extract into an n-hexane fraction and a methanol portion, 2 × 200 mL each of n-hexane and methanol were used. The resulting methanol portion was subsequently partitioned into a chloroform fraction and a methanol portion using chloroform (2 × 200 mL). The crude extract and chloroform fraction yields were 11.89 and 3.44 g, respectively. The chloroform fraction was thereafter used for the bioassays as well as the GC-MS analysis for available bioactive components.

### *In vitro* antioxidant bioassays

#### *DPPH free radical scavenging activity assay*

DPPH free radical scavenging activity of CF*Am* was carried out according to the method described by Gyamfi et al (32). A properly diluted portion (1 mL) was combined with an equal volume of a methanol-based 0.4 mM DPPH solution. After 30 minutes of dark incubation, the mixture was measured at 516 nm for absorbance. By using BHT as a standard, the DPPH free radical scavenging activity was reported as a percentage (%) control inhibition.

#### *Ferric-reducing power (FRAP) assay*

The FRAP of CF*Am* was carried out using the method of Pulido et al (33). The sample (2.5 mL of the fraction) was combined with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 200 mM phosphate buffer (pH 6.6). 2.5 mL of 10% trichloroacetic acid (TCA) was added after the solution had been incubated for 20 minutes at 50°C in a water bath. The resulting solution was then centrifuged at 3000 rpm for 10 minutes. Following that, 5 mL of the filtrate were combined with 1 mL of 0.1% $FeCl_3$ and an equal volume of distilled water. At 700 nm, the mixture was read, and the results were reported as mg per 100 g of dried material using gallic acid as the reference.

#### *Hydroxyl (OH) radical scavenging activity assay*

The OH radical scavenging ability of CF*Am* was determined according to the method of Klein et al (34). The fraction (0.2 mL) was combined with 1 mL 0.13% ferrous ammonium sulfate in 0.26% EDTA, 0.5 mL 0.018% EDTA, and 1 mL 0.85% in 0.1 mol/L phosphate buffered saline pH 7.4 dimethyl sulfoxide (DMSO) solution. 1 mL of trichloroacetic acid was added to end the reaction (TCA). The above mixture was then mixed with 3 mL of Nash reagent, composed of 7.5 g of ammonium acetate, 0.3 mL of glacial acetic acid, 0.2 mL of acetylacetone, and 100 mL of distilled water. After 15 minutes of incubation at room temperature, the absorbance values were measured at 412 nm using a UV-visible spectrophotometer. The capacity to scavenge hydroxyl radicals was calculated as mg per 100 g of the dried sample using gallic acid as the reference.

#### *Singlet oxygen scavenging activity assay*

The production of singlet oxygen (1 $O_2$) was determined by monitoring N, N-dimethyl-4-nitrosoaniline (pNDA) bleaching using a previously reported spectrophotometric method (35). The formation of singlet oxygen by the interaction of NaOCl with $H_2O_2$ was seen to bleach pNDA. The reaction mixture contained different sample concentrations (0.0-200.0 g/mL), 45 mM phosphate buffer (pH 7.1), 50 mM NaOCl, 50 mM $H_2O_2$, 50 mM histidine, and 10 M pNDA in a final combined volume of (2 mL). pNDA absorbance was measured at 440 nm during the reaction mixture's 40 minutes incubation at 30°C. The amount of scavenging activity per 100 grams of dry material was calculated using gallic acid as the reference.

### *In vitro* enzyme inhibitory activity assays

#### *α-Glucosidase (EC 3.2.1.20) inhibitory activity assay*

The α-glucosidase inhibitory activity of CF*Am* was determined according to the method described by Ademiluyi and Oboh (36), with slight modifications. In brief, 250 µL of *A. muricata* leaf fraction at various concentrations (1-5 mg/mL) was incubated with 500 L of 1.0 U/mL α-glucosidase solution in 100 mMol/L phosphate buffer (pH 6.8) for 15 minutes at 37°C. The mixture was then incubated at 37°C for 20 minutes with 250 µL of pNPG solution (5 mMol/L) in 100 mMol/L phosphate buffer (pH 6.8). At 405 nm, the absorbance of the released p-nitrophenol was measured, and the inhibitory activity was expressed as a percentage of control inhibition.

#### *α-Amylase (EC 3.2.1.1) inhibitory assay*

The α-amylase inhibitory activity of CF*Am* was determined according to the method described by Shai et al (37), with slight modifications. At 37°C, a volume of 250 µL of chloroform fraction of *A. muricata* leaves at various concentrations (1-5 mg/mL) was incubated with 500 µL of porcine pancreatic amylase (2 U/mL) in 100 mMol/L

phosphate buffer (pH 6.8) for 20 minutes. The reaction mixture was then treated with 250 µL of 1% starch dissolved in 100 mMol/L phosphate buffer (pH 6.8) and incubated at 37°C for 1 hour. The color-forming reagent (DNSA, 1 mL) was then added and the mixture was boiled for 10 minutes. The resulting mixture's absorbance was measured at 540 nm, and the inhibitory activity was expressed as a percentage of control inhibition.

### Determination of $IC_{50}$

The CF*Am* concentration required to cause 50% inhibition ($IC_{50}$) was calculated using a linear regression curve generated from a plot of the percentage inhibition caused by the extracts versus different concentrations (g/mL) of the extract used.

### GC-MS analysis

#### *Gas chromatography*

GC-MS analysis of CF*Am* was carried out using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), interfaced to a mass spectrometer (GC-MS), equipped with a straight deactivated 2 mm direct injector liner and a 15 m Alltech EC-5 column (250 µ I.D., 0.25 µ film thickness). For sample introduction, a split injection was used with a split ratio of 10:1. The oven temperature program was set to begin at 35°C and hold for 2 minutes before ramping to 300°C and holding for 5 minutes. The flow rate of the helium carrier gas was set to 2 mL/min (constant flow mode). All analyses were performed using a JEOL GCmate II benchtop double-focusing magnetic sector mass spectrometer in electron ionization (EI) mode with TSS-20001 software. Low-resolution mass spectra were collected at a resolving power of 1000 (20% height definition) and scanned from m/z 25 to m/z 700 in 0.3 seconds with a 0.2-second inter-scan delay. High-resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

### Identification of bioactive components

The interpretation of mass-spectrum GC-MS data was carried out using the National Institute of Standards and Technology (NIST) database, which contained over 62 000 patterns. The unknown components were identified by comparing their recorded spectra to the data bank mass spectra of NIST library V 11 provided by the instrument software.

### Data analyses

All data were analyzed using the one-way ANOVA test (with the Statistical Package for the Social Sciences (SPSS) Evaluation version 16.0, SPSS Inc., Chicago, IL, USA), followed by the Duncan multiple range test as needed. The GraphPad Prism 8.5 program was used for graphical analysis (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $P<0.05$, and results were presented as the mean of three triplicate determinations plus standard deviation (n = 3).

## Results

### *In vitro* antioxidant activity of CF*Am*

Figure 1 depicts CF*Am* ability to scavenge DPPH free radicals. The results show that CF*Am* has a significant ($P<0.05$) inhibitory activity against DPPH free radicals ($IC_{50}$ = 26.331.39 mg/mL) in a concentration-dependent manner across all concentrations tested. This inhibitory activity outperformed BHT ($IC_{50}$ = 22.44 1.58 mg/mL) (Table 1).

Figure 2 shows ferric reducing antioxidant power (FRAP), hydroxyl radical (OH) scavenging activity, and singlet oxygen scavenging abilities of CF*Am*. Results indicate that CF*Am* had a considerable ferric reducing power (84.52 ± 2.84 mg/100 g), OH free radical scavenging (65.46 ± 1.39 mg/100 g), and singlet oxygen (55.24 ± 1.22 mg/100 g) inhibitory activities. However, the results were favorably compared with gallic acid that showed relatively higher ferric reducing power (125.81 ± 0.39 mg/100 g), OH free radical (90.13 ± 0.31 mg/100 g), and singlet oxygen (75.80 ± 1.8 mg/100 g) inhibitory activities.

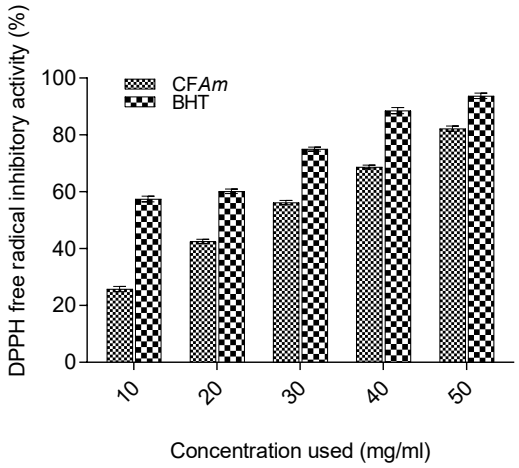


**Figure 1.** DPPH free radical scavenging ability of chloroform fraction of *Annona muricata* leaf. Abbreviations: DPPH: 2, 2-diphenyl-1-picrylhydrazyl; CF*Am*: chloroform fraction of *Annona muricata*; BHT: butylated hydroxytoluene.

**Table 1.** $IC_{50}$ values

| Parameters | $IC_{50}$ (mg/mL) | | |
|---|---|---|---|
| | DPPH | α-Amylase | α-Glucosidase |
| CF*Am* | 26.33±1.39 | 73.88 ±1.58 | 71.06 ±1.45 |
| Acarbose | - | 43.25±1.84 | 44.39 ±1.82 |
| BHT | 22.44 ±1.58 | - | - |

Abbreviations: DPPH: 2, 2-diphenyl-1-picrylhydrazyl; CF*Am*: Chloroform fraction of *Annona muricata*; BHT: Butylated hydroxytoluene.
Results are represented as mean of triplicate determinations ± SD (n=3).

### *In vitro* enzyme inhibitory activity of CF*Am*

Figure 3 represents the inhibitory potential of CF*Am* against the α-glucosidase carbohydrate-hydrolyzing activity. The results indicate that CF*Am* significantly ($P<0.05$) inhibited α-glucosidase carbohydrate-hydrolyzing activity ($IC_{50}=71.06\pm1.45$ mg/mL) (Table 1) in a concentration-dependent trend at different concentrations that were considered (0-100 mg/mL). The observation was compared with acarbose ($IC_{50}=44.39\pm1.82$ mg/mL; however, a significant ($P<0.05$) difference was indicated.

Figure 4 represents the inhibitory potential of CF*Am* against α-amylase carbohydrate-hydrolyzing activity. The results indicate that CF*Am* significantly ($P<0.05$) inhibited α-amylase carbohydrate -hydrolyzing activity ($IC_{50}=73.88\pm1.58$ mg/mL) (Table 1) in a concentration-dependent trend at different concentrations that were considered (0-100 mg/mL). The observation was compared with acarbose ($IC_{50}=43.25\pm1.84$ mg/mL); however, a significant ($P<0.05$) difference was indicated.

### GC-MS analysis of CF*Am*

Figure 5 represents the GC-MS spectra of the identified bioactive compounds in CF*Am*. The analysis shows the presence of 23 compounds available in CF*Am*. However, the peaks indicate the presence of different compounds (Table 2) such as decane, 3,7 dimethyl- (0.76%), phenol (1.29%), 2-methylresorcinol, acetate (6.00%), 2,4-dimethyl-6-tert-butylphenol (0.68%), decane, 2-methyl (5.6%), octadecanoic acid (20.35%), n-heneicosane (6.33%), n-hexadecanoic acid (2.00%), 2-propanone, 1-(4-hydroxy-3-methoxyphenyl) (12.04%), isocomene (22.60%), eugenol (7.45%), 1-nonadecene (1.72%), β-sitosterol (2.96%), hexadecanoic acid ethyl ester (1.54%), 9, 12, 15 octadecatrienoic acid, methyl ester (Z,Z,Z) (28. 98%), 1,2-benzenedicarboxylic acid, bis(2-methyl propyl) ester (6.37%), octadecanoic acid, ethyl ester (6.51%), palmitoyl chloride (0.81%), decyl sulfide (0.58%), bis-(2-ethylhexyl) phthalate (0.26%), 2-pentadecanone,6,10,14-trimethyl (1.01%), 4,7,10,13,16,19-docosahexaenoic acid, methyl ester, (all-Z) (0.62%), and quercetin, 5TMS derivative (18.28%).

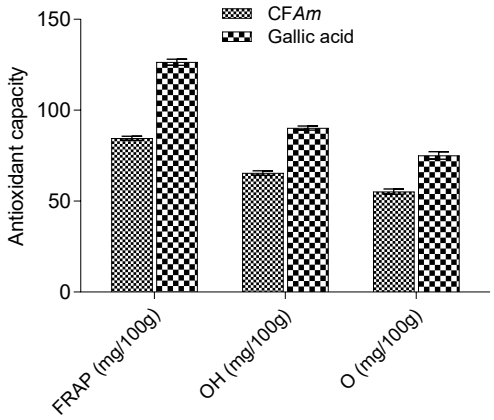


**Figure 2.** Ferric reducing antioxidant power (FRAP), hydroxyl radical (OH) scavenging activity and singlet oxygen scavenging abilities of chloroform fraction of *Annona muricata* (CF*Am*) leaf.

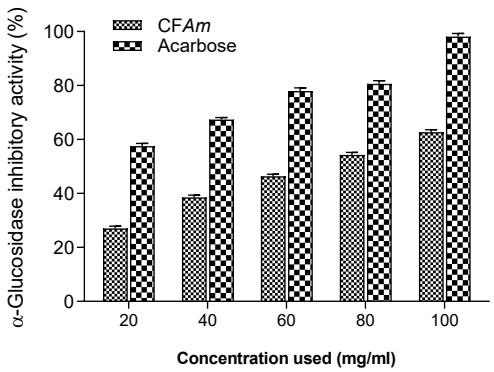


**Figure 3.** Inhibitory potential of chloroform fraction of *Annona muricata* (CF*Am*) leaf against α-glucosidase carbohydrate-hydrolyzing activity.

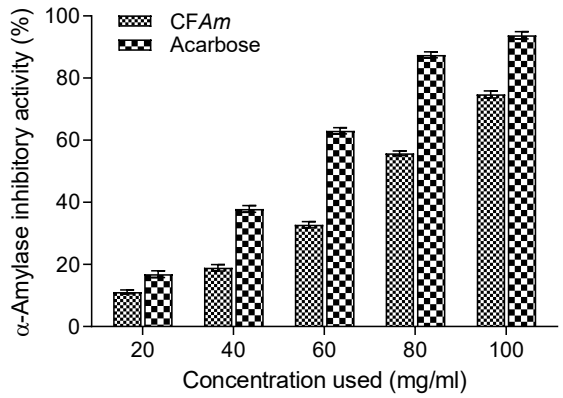


**Figure 4.** Inhibitory potential of chloroform fraction of *Annona muricata* (CF*Am*) leaf against α-amylase carbohydrate-hydrolyzing activity.

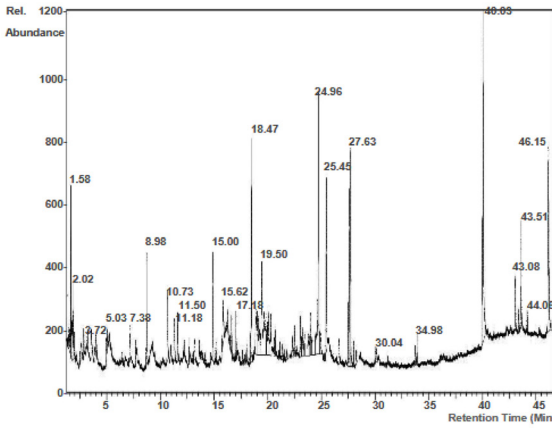


**Figure 5**. Gas chromatography–mass spectrometry (GC-MS) spectra of the identified bioactive compounds in of chloroform fraction of *Annona muricata* (CF*Am*) leaf.

**Table 2.** Chemical compounds identified in chloroform fraction of *Annona muricata* (CFAm)

| RT | Compound detected | Molecular formula | MW | Peak area (%) | % Composition | m/z |
|---|---|---|---|---|---|---|
| 1.56 | Decane, 3,7 dimethyl- | $C_5H_{10}O$ | 86 | 5.89 | 0.76 | 43, 57, 86 |
| 2.02 | Phenol | $C_6H_6O$ | 94 | 3.38 | 1.29 | 39, 66, 94 |
| 3.72 | 2-Methylresorcinol, acetate | $C_2H_4O_2$ | 166 | 1.82 | 6.00 | 42,124, 166 |
| 5.03 | 2,4-Dimethyl-6-tert-butylphenol | $C_{12}H_{18}O$ | 178 | 1.91 | 0.68 | 91, 135, 178 |
| 7.38 | Decane, 2-methyl | $C_{11}H_{24}$ | 156 | 2.08 | 5.6 | 43, 57, 156 |
| 8.98 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 4.16 | 20.35 | 43, 73, 284 |
| 10.73 | n-Heneicosane | $C_{21}H_{44}$ | 296 | 2.77 | 6.33 | 43, 57, 296 |
| 11.50 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 2.60 | 2.00 | 43, 73, 256 |
| 15.00 | 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl) | $C_{10}H_{12}O_3$ | 180 | 4.24 | 12.04 | 43, 137, 180 |
| 15.62 | Isocomene | $C_{15}H_{24}$ | 204 | 2.82 | 22.60 | 43, 57, 204 |
| 17.18 | Eugenol | $C_{10}H_{12}O_2$ | 164 | 2.34 | 7.45 | 39, 77, 164 |
| 18.47 | 1-Nonadecene | $C_{19}H_{38}$ | 266 | 7.28 | 1.72 | 43, 57, 266 |
| 19.50 | β-Sitosterol | $C_{29}H_{50}O$ | 414 | 3.81 | 2.96 | 43, 107, 414 |
| 24.96 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 8.32 | 1.54 | 43, 88, 284 |
| 25.45 | 9, 12, 15 Octadecatrienoic acid, methyl ester, (Z,Z,Z) | $C_{19}H_{32}O_2$ | 292 | 6.50 | 28.98 | 67, 79, 292 |
| 27.63 | 1,2-benzenedicarboxylic acid, bis(2-methyl propyl) ester | $C_{16}H_{22}O_4$ | 278 | 6.93 | 6.37 | 57, 149, 278 |
| 30.04 | Octadecanoic acid, ethyl ester | $C_{20}H_{40}O_2$ | 312 | 1.39 | 6.51 | 88, 101, 312 |
| 34.98 | Palmitoyl chloride | $C_{16}H_{31}O$ | 274 | 1.73 | 0.81 | 55, 98, 274 |
| 40.03 | Decyl sulfide | $C_{20}H_{42}S$ | 314 | 10.39 | 0.58 | 43, 173, 314 |
| 43.08 | Bis-(2-Ethylhexyl)phthalate | $C_{24}H_{38}O_4$ | 390 | 3.47 | 0.26 | 57, 149, 390 |
| 43.51 | 2-Pentadecanone,6,10,14-trimethyl | $C_{18}H_{36}O$ | 268 | 4.85 | 1.01 | 43, 58, 268 |
| 44.06 | 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z) | $C_{23}H_{34}O_2$ | 342 | 2.34 | 0.62 | 79, 91, 342 |
| 46.15 | Quercetin, 5TMS derivative | $C_{30}H_{50}O_7Si_5$ | 663 | 6.93 | 18.28 | 73, 559, 663 |

RT, Retention time; MW, Molacular weight; m/z, Mass divided by charge number.

## Discussion

Epidemiological studies show that the global burden of diabetes is increasing (8,38). As a result, evidence-based reports have indicated that polyphenol-rich foods play critical roles in the development of T2DM (13). Plant bioactive molecules such as phenolics, flavonoids, carotenoids, and alkaloids have been linked to a variety of therapeutic activities in biological systems, including antioxidant, antifungal, anti-inflammatory, antiallergenic, and antidiabetic properties (39). The ability of these compounds to donate H-atoms (H+) and then stabilize/neutralize unstable electrophiles has been identified as critical in their antioxidative/reductive activities. Thus, a compound's reducing capacity serves as an indicator of its antioxidant property (40).

In this study, CF*Am* demonstrated a significant antioxidant activity by inhibiting DPPH, OH, and $H_2O_2$ free radicals (Figures 1 and 2). DPPH free radical accepts an electron or hydrogen ion ($H^+$) to become a stable diamagnetic molecule, while OH radical is believed to be generated via Fenton reaction from the $H_2O_2$ (1). Hydroxyl radicals are the primary active oxygen species responsible for lipid peroxidation and massive biological damage (41). The FRAP assay, on the other hand, is commonly used to assess the total antioxidant activity of plant extracts by reducing $Fe^{3+}$ to $Fe^{2+}$ in the presence of antioxidants (42). Free radicals are constantly produced and cause extensive damage to normal cell tissues and biomolecules, contributing to the onset of T2DM (43). The antioxidant activities of CF*Am* are most likely due to the presence of various phytonutrients (Figure 5 and Table 2), which have been shown to prevent chain initiation, peroxide decomposition, and radical proliferation, all of which are important in the pathophysiology of T2DM (44). Similarly, inhibition of α-amylases and α-glucosidases activities has been identified as an effective strategy for controlling the postprandial glycemic index in T2DM (45). Dietary carbohydrates are broken down into oligosaccharides and disaccharides, which are then converted into reducing sugars by the enzymes (46). In a recent study, it has been indicated that plant bioactive phytonutrients with anti-oxidation properties may act as inhibitors of pancreatic

α-amylase and intestinal α-glucosidase activities (47). This property has only been linked to the redox potential of their hydroxyl groups, which act as inhibitors of these enzymes (48). The CF*Am* inhibited activities of these carbohydrate-hydrolyzing enzymes in this study (Figures 3 and 4). This observation perhaps suggests the antidiabetic potential of this plant fraction, which could be credited to the list of available phytochemical constituents that were revealed by GC-MS technique (Figure 5 and Table 2), comprising flavonoids, esters, aldehydes, phenolics, and ketones (49). The report of our findings could probably justify the folkloric usage of this plant in the management of DM and some other ailments.

## Conclusion

In this study, the CF*Am* exhibited the inhibition of some biological activities that have been implicated in the management of T2DM. The CF*Am* demonstrated DPPH inhibition, OH and $1O_2$ radicals scavenging abilities, as well as ferric reducing ability. Similarly, significant inhibitory activities were demonstrated against α-amylase and α-glucosidase via the *in vitro* model adopted. Nonetheless, various activities revealed by CF*Am* could be credited to the available phytochemical components identified by GC-MS technique.

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## Authors' contributions

OOR conceptualized the research; AOB prepared drafted the manuscript; OOR & AOB reviewed the literature, analyzed the data, and prepared the draft; OOR & AOB conducted the research and analyzed the data. Both authors read and approved the final report.

## Conflict of interests

No conflict of interest was declared by the authors.

## Ethical considerations

Ethics approval was obtained from the Afe Babalola University ethical committee (ethical code: 249 ABUAD/ACA/458). All experiments carried out on the plant (*A. muricata*) were performed in accordance with the international guidelines and regulations for standard practice.

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