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Evaluation of antioxidant, antidiabetic and bioactive compounds in polyphenol-rich stem bark extract and fractions of *Averrhoa carambola*

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Averrhoa carambola is employed traditionally in Nigeria to treat diabetes, vomiting, cough, chickenpox, ringworm, aphthous stomatitis, high blood pressure, eczema, diarrhea, and kidney dysfunction. This study investigated the bioactive compounds, antioxidant and antidiabetic activities of the stem bark of *Averrhoa carambola* using standard methods. The antioxidant properties were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) assay and the metal chelating (MC) activity. The phytochemical analysis of the extract indicated the presence of flavonoids, saponins, tannins, and cardiac glycosides. The total phenolic and flavonoid content ranged from 4.46 ± 0.19 to 51.25 ± 0.52 mg GAE/g and from 441.00 ± 5.98 to 2285.00 ± 11.13 mg QE/g, respectively. Antioxidant study revealed IC_{50} values for DPPH ranging from 26.47 ± 0.38 to 29.64 ± 0.25 µg/mL, FRAP from 26.51 ± 0.26 to 51.30 ± 0.34 µg/mL, and MC from 69.53 ± 0.66 to 360.66 ± 2.55 µg/mL. The acute toxicity test of *Averrhoa carambola* stem bark extract showed a mean lethal dose (LD_{50}) of 3872.98 mg/kg. The extract's effect on α-amylase and α-glucosidase enzymes in rats, at doses of 155, 310 and 620 mg/kg, showed a significant reduction in blood glucose levels ($p < 0.05$). Gas chromatography-mass spectrometry (GC-MS) analysis of the ethyl acetate fraction indicated several compounds, including 5-(2-Amino-phenyl)-[1,3,4]thiadiazo-2-yl-p-tolyl-amine, 2-hydroxymethoxybenzaldehyde, tert-butyldimethylsilyl ether, phenol, 4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy, thiazolo[3,2-a]benzimidazole-3(2H)-one, 2-(2-fluorobenzylideno)-7,8-dimethyl, squalene, phenol, 2-methoxy-4-(2-propenyl)-, acetate, 2-(2,5-dimethoxy-4-propylphenyl) ethanamine, isoquinoline, 1,2,3,4-tetrahydro-8-amino-2-methyl-4-phenyl-, and phenol, 2,6-dimethoxy-4-(2-propenyl)-. This research suggests that *Averrhoa carambola* stem bark possesses antioxidative activity and inhibits the enzymes α-amylase and α-glucosidase in rats, probably due to the presence of its bioactive constituents.

Keywords: *Averrhoa carambola, stem bark extract, antioxidant activity, diabetes, bioactive compounds*

Introduction

Free radicals are highly reactive and unstable chemical species with unpaired electrons, produced in the human body during normal cellular processes such as enzymatic activities (e.g., xanthine oxidase), phagocytosis, inflammation, and physical exercise (Zujko & Witkowska, 2023; Gulcin, 2020). Environmental pollutants, smoking, drugs, industrial effluents, and ozone have also contributed to the production of free radicals. A significant drawback of these species is their ability to disrupt cellular processes by oxidizing biomolecules, impairing cell functions, and potentially causing cell death through the destruction of DNA, RNA, lipids, and carbohydrates (Pisoschi & Pop, 2015; Mutha et al., 2021). If the

cell is unable to repair the damage caused by these species, it may lead to further deterioration of the system, resulting in oxidative stress (Jomova et al., 2023; Muscolo et al., 2024; Yang et al., 2024). Natural enzymatic antioxidants, along with non-enzymatic remedies such as superoxide dismutase, glutathione peroxidase, glutathione, uric acid, melatonin, metal-binding proteins, and polyamines, have been identified (Jaydeokar et al., 2012; Neha et al., 2019). In addition, various synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) are used in the food and medical industries. However, these synthetic compounds raise concerns regarding health risks, availability, and bioavailability (Wojcik et al., 2010; Gulcin, 2020). The current focus is on finding natural antioxidants due to their lower toxicity, better solubility, and greater accessibility. Food rich in phenolics are considered a primary dietary antioxidant (Mutha et al., 2021; Wanjala Wafula et al., 2023). As a result, researchers are screening a variety of vegetables, fruits, and spices for bioactive compounds with antioxidant properties (Gulcin, 2020). In this regard, phenolic acids, flavonoids, tannins, ligands, and terpenes have become key targets. Flavonoids, including flavanols, flavanonols, anthocyanidins, and isoflavonoids, are not only effective in reducing oxidative stress but also modulate enzyme activity to prevent hyperglycemia by inhibiting specific enzymes (de Sousa et al., 2004). Studies have shown that consuming foods rich in phenolic compounds can consistently inhibit starch-hydrolyzing enzymes, such as α-amylase, from breaking down carbohydrates into monosaccharides, as well as preventing the further degradation of α-glucosidase. This process delays glucose absorption in the small intestine and prevent postprandial glucose spikes (Hanamura et al., 2005; Thilagam et al., 2013). Managing postprandial hyperglycemia is an effective approach to controlling early-stage diabetes by inhibiting α-glucosidase and α-amylase, enzymes that slow glucose absorption and reduce blood glucose spikes after meals (Park and Han, 2015; Hiyoshi et al., 2017; Morais et al., 2020). Common antidiabetic drugs, including acarbose, voglibose, and miglitol, are used for this purpose, although these medications show side effects such as flatulence, stomach discomfort, and allergic reactions (Shehadeh et al., 2021). Plant extracts have been found to inhibit α-amylase and α-glucosidase activities, likely due to the presence of bioactive compounds (Bnouham et al., 2006; Proença et al., 2017; Yang et al., 2019; Unuofin and Lebelo, 2020). These bioactive compounds have demonstrated antidiabetic effects in various ways, including targeting various human proteins and enzymes (Bogle and Mendes, 2015; Salehi et al., 2019; Egbuna et al., 2021). *Averrhoa carambola* is a perennial tree in the Oxalidaceae family. It often grows to a height of 3–15 meters and produces star-shaped fruits that are green when unripe and turn yellow-golden when mature. These fruits are rich in minerals and natural antioxidants such as magnesium, potassium, iron, carotenoids, and vitamin C (Khoo et al., 2010; Khoo et al., 2017; Zainudin et al., 2014; Vargas-Madriz et al., 2021), as well as fiber and low-calorie content (Patil et al., 2010; Muthu et al., 2016). Traditionally, the plant in employed to treat diabetes, arthralgia, vomiting, cough, chickenpox, and ringworm (Lakmal et al., 2021; Luan et al., 2021). A decoction of its leaves and fruits is reported to treat fevers, aphthous stomatitis, angina, high blood pressure, eczema, diarrhea, and kidney dysfunction (Gowrishankar et al., 2018; Yang et al., 2020).

Pharmacologically, the leaves and fruit extracts exhibit properties such as antiulcer, antidiabetic, anti-inflammatory, anti-hypertensive, anti-obesity, antimicrobial, hepatoprotective, neuroprotective, and anticancer effects (Manda et al., 2012; Luan et al., 2021; Beas-Guzman et al., 2024). Bioactive compounds such as isovitexin, carambolaflavones A and B, apigenin 6-C-(200-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside, dihydrochalcone C-glycosides, xanthones, and fatty acids have been identified from the plant extracts (Luan et al., 2021). A recent study found that methanolic extracts of the leaves showed no acute or chronic toxicity in male or female rats at doses up to 5,000 mg/kg (Saghir et al., 2022). Additionally, the leaf fractions were shown to have anti-hyperlipidemic effects, reducing total cholesterol, triglycerides, and the atherogenic index in a poloxamer-407-induced rat model (Abduh et al., 2023). While other parts of the plant have been studied for their medicinal properties, research on the phytochemistry and pharmacology of the stem bark extracts remains limited. Information on the antioxidant and antidiabetic activity of the stem bark extract is scarce. The ability of plant extracts to inhibit alpha-amylase and alpha-glucosidase is commonly used to assess their antidiabetic potential. This study, therefore, aims to investigate the antioxidant activity of *A. carambola* stem bark extract and its fractions, as well as their inhibitory effects on alpha-amylase and alpha-glucosidase in rats, to explore their potential medicinal benefits.

Materials and Methods

Materials

The materials used included *Averrhoa carambola* powdered stem bark extract, oral gastric gavage, a weighing balance, gloves, scissors, glucometer with fine test strips, distilled water, a stirrer, beakers, and a 1 ml syringe. Acarbose (the standard drug), starch, sucrose, and maltose were obtained from Aldrich Sigma, USA.

Plant collection and identification

Averrhoa carambola fresh stem bark was harvested from a farmland in Nung Ukim Ikono in Uyo Local Government Area of Akwa Ibom State, Nigeria in March 2024. The plant identification and authentication were conducted at the

Department of Botany and Ecological Study, University of Uyo, Uyo, Nigeria. Specimen voucher (ID: UUH4541) was deposited at the Herbarium of the Faculty of Biological Sciences of University of Uyo, Nigeria.

Plant Extraction

Averrhoa carambola fresh stem bark was washed, shade-dried for 14 days and ground into powder form using a lab mill. Five hundred and twenty-nine grammes (529.7 g) of the powdered stem bark was macerated with 70% ethanol for 72 hours, filtered, and concentrated under vacuum to obtained the ethanol crude extract. The weight and the percentage yield of the extract were then calculated.

Partitioning of Extract

The crude extract (11.3 g) was dissolved in 100 mL of distilled water and partitioned successively with n-hexane, dichloromethane, ethyl acetate, and butanol using a separatory funnel (Pyrex England) to obtain the hexane, dichloromethane, ethyl acetate, butanol, and aqueous fractions, respectively. The fractions were concentrated *in vacuo* to dryness. The weights of the extract and fractions were noted and the percentage yield calculated.

Animals

Albino Wistar rats (120-135 g), of both sexes, housed at the animal facility of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria, were employed. The rats were kept in standard cages and provided with a standard pelleted diet (Guinea feed) and water *ad libitum*.

Acute Toxicity Test (ATT)

An acute toxicity test was performed to establish the median lethal dose (LD_{50}) of *Averrhoa carambola* stem bark, following the Lorke (1983) method. The test aimed to assess the safety of the extract using Swiss albino mice, as described by Ubulom et al. (2017).

Phytochemical Screening

Preliminary phytochemical analysis for the detection of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, and anthraquinones were conducted according to standard methods (Ouandaogo et al., 2023).

Total Phenolic Content (TPC)

The total phenolic content was measured spectrophotometrically according to a standard procedure (Kim et al., 2003). Briefly, 0.5 mL of the sample (1 mg/mL in methanol) was combined with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7% Na_2CO_3. The mixture was vortexed for 15 seconds and incubated at 40 °C for 30 minutes in the dark to allow colour development. The absorbance was then measured using a UV-Vis spectrophotometer (Model: Techmel and Techmel USA) at 765 nm. A calibration curve was created with gallic acid solutions ranging from 10 to 100 µg/mL. The total phenolic content was calculated by comparing the sample's absorbance to the standard curve, with results expressed as milligrams of gallic acid equivalent per gram of dry weight.

Total Flavonoids Content (TFC)

The total flavonoid content was determined using a prescribed method (Subhashini et al., 2010). In brief, a 1 mg/mL extract solution was mixed with 200 µL of distilled water, then 150 µL of a 5% sodium nitrite ($NaNO_2$) solution. The mixture was allowed to incubate for 5 minutes before adding 150 µL of a 10% aluminium trichloride ($AlCl_3 \cdot 6H_2O$) solution. Six minutes later, 2 mL of 1M NaOH was added, and the absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was calculated and expressed as milligrams of quercetin (QE) equivalent per gram of dry weight.

***In vitro* antioxidant analysis**

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The DPPH activity was assessed following a standard methodology (Shekhar & Anju, 2014). To DPPH (1mL, 0.1 Mm) was added the extract's solution (3 mL) and the resulting solution was left to stir for 1 minute. The mixture was incubated

for 30 minutes (without light) and the spectrophotometer reading was recorded at 517 nm. The procedure was repeated using ascorbic acid as substrate. The percentage of DPPH radical scavenging activity was calculated using the following equation.

$$\text{DPPH percentage scavenging effect} = \frac{[(Ao - As)]}{[Ao]} \times 100\ \%$$

Where Ao is the absorbance of the control reaction, and As is the absorbance of the standard

Ferric Reducing Antioxidant Power (FRAP) Assay

The method of Ali et al. (2020) was adopted. Different concentrations (µg/mL) of the extract were added to sodium phosphate buffer (1 mL, 200 mM; pH 6.6) and 1 mL of 0.69 mL potassium ferricyanide ($K_3[Fe(CN)_6]$). Incubation of the resulting mixture took place at 50°C within 20 minutes. Next, 1 mL of 10% trichloroacetic acid was prepared and dissolved in distilled water (50 mL). The mixture was then centrifuged at 650 rpm for 10 minutes. The supernatant (4 mL) was combined with 4 mL of deionized water and 0.8 mL of 0.1% ferric chloride anhydrous ($FeCl_3$), and the absorbance was recorded with a UV/Vis spectrophotometer at 700 nm. Similar steps were conducted with different concentrations of ascorbic acid. A higher absorbance indicates a greater reducing power.

Metal Chelating Activity

The metal chelating activity of the samples was determined following a previous method (Köksal et al., 2009) with minor modifications. A methanol solution of the extract (0.5 mL) and ascorbic acid (0.5 mL) at various concentrations (20–100 µ/mL) was mixed with methanol (3 mL), iron (II) chloride tetrahydrate $FeCl_2 \cdot 4H_2O$ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL). The mixture was incubated in the dark for 10 minutes. The control which served as the blank was also prepared but without the extract. The metal chelating activity was determined by measuring the absorbance at 562 nm using a UV/Vis spectrophotometer.

$$\text{The metal chelating activity (\%)} = (1 - \text{absorbance of sample/absorbance of control}) \times 100\%$$

GC/MS Experiment

A SHIMADZU GCMS-QP2010SE equipped with a 30 m column, 0.25 mm diameter, and 0.25 µm thickness was used for sample analysis. Helium was employed as the carrier gas at a flow rate of 1 mL/min, and the sample injection volume was 1 µL with a split ratio of 10:1. The oven temperature was initially set at 60 °C and increased by 5 °C/min to 180 °C, followed by a ramp of 20 °C/min to 250 °C. The ion source temperature was set to 230 °C, and the ionization voltage was maintained at 70 eV. Data interpretation was performed using the National Institute of Standards and Technology (NIST) library (Kadhim et al., 2016).

Alpha-amylase inhibitory study using starch as substrate

Thirty Wistar rats were separated into six groups, each consisting of five rats. All rats were fasted for 18 hours, and blood glucose levels were measured at 0 minutes before any treatment. Group I, serving as the normal control, was given distilled water (10 mL/kg). Group II rats were orally administered starch at a dose of 2 g/kg body weight. In Group III, rats received both starch (2 g/kg) and the standard drug, acarbose, at a dose of 100 mg/kg. Groups IV, V, and VI were given starch (2 g/kg) along with *Averrhoa carambola* stem bark extract at doses of 155, 310, and 620 mg/kg, respectively. All treatments were administered orally, and blood glucose levels were observed at 60, 120, and 180 minutes (Gidado et al., 2019).

Sucrase inhibitory study (using sucrose as substrate)

A total of thirty Wistar rats were allocated into six groups, with five rats in each group. All rats were fasted for 18 hours, and blood glucose levels were measured at 0 minutes before any treatment. Group I, the normal control, received distilled water (10 mL/kg). Group II rats were given sucrose orally at a dose of 2 g/kg body weight, along with distilled water (10 mL/kg) as the vehicle. In Group III, the rats received both sucrose (2 g/kg) and the standard drug acarbose (100 mg/kg). Groups IV, V, and VI were treated with sucrose (2 g/kg) and *Averrhoa carambola* stem bark extract at doses of 155, 310, and 620 mg/kg, respectively. All treatments were administered orally, and blood glucose levels were measured at 60, 120, and 180 minutes (Gidado et al., 2019).

Maltase inhibitory study (using maltose as substrate)

A total of thirty Wistar rats were allocated into six groups, each containing five rats. All the rats were fasted for 18 hours, and their fasting blood glucose levels were measured at the baseline (0 minutes) before any treatments. Group I served as

the normal control and was given distilled water (10 mL/kg). Group II rats received an oral dose of maltose at 2 g/kg body weight, with distilled water (10 mL/kg) as the vehicle. In Group III, rats were given both maltose (2 g/kg) and the standard drug acarbose (100 mg/kg). Groups IV, V, and VI received maltose (2 g/kg) along with *Averrhoa carambola* stem bark extract at doses of 155, 310, and 620 mg/kg, respectively. All treatments were administered orally, and blood glucose levels were checked at 60, 120, and 180 minutes (Gidado et al., 2019).

Blood Glucose Determination

Blood drops from the tips of rats' tails were placed on test strips, and glucose concentration was measured using a glucometer (fine test) following the manufacturer's instructions. The glucometer works using an electrochemical detection system with the following principle; the biosensor system makes use of disposable dry reagent strip utilizing the glucose oxidase method. Each disposable reagent strip contains an electrode coated with glucose oxidase, which reacts with glucose in the blood sample when applied to the strip's membrane, producing gluconic acid. During the reaction, in which electric current is generated, an electrochemical intermediary transfers electron to the electrode surface. This electrode sensor measures the current produced when the enzyme converts glucose to gluconic acid. The amount of current is directly proportional to the glucose concentration in the blood sample, providing an accurate reading of blood glucose levels (WHO, 2011).

Statistical Analysis

Data collected in this study were analysed using one-way ANOVA, thereafter, Tukey-Kramer multiple comparison test, with Instat Graphpad software (San Diego, USA) was assessed. A p-value of < 0.05 was deemed statistically significant, while a $p < 0.001$ was considered highly significant.

Results

Plant Extraction

Extraction with ethanol (70%) afforded ninety-six (96 g) grammes of ethanol crude extract (18% yield). Fractionation of crude extract (11.30 g) afforded beige, pale-green, green, dark brown and brown-coloured extracts. Their percentage yields are as follows: n-hexane (3.4%), dichloromethane (2.9%), ethyl acetate (17.6%), butanol (26.5%) and aqueous (49.5%), as presented in Table 1.

Table 1. Extraction yields of *A. carambola* stem bark

Extract/ fraction	Colour	Amount (g)	Percentage yield (%)
Extract	Brown	96.00	18.00
n-HEF	Beige	0.39	3.40
DCMF	Green	0.33	2.90
EAEF	Pale green	2.00	17.60
BUTF	Brown	3.00	26.50
AQF	Brown	5.60	49.50

n-HEF: hexane fraction, DCMF: dichloromethane fraction; EAEF: ethyl acetate fraction; BUTF: butanol fraction; AQF: aqueous fraction

Preliminary phytochemical analysis

Phytochemical screening of the stem bark ethanol extract of *Averrhoa carambola* indicated the presence of flavonoid, saponins, tannins and cardiac glycosides while alkaloids and anthraquinones were not detected. Generally, extracts from different parts of *Averrhoa carambola* have notable constitution of saponins, flavonoids, alkaloids, tannins, and pyrogallic steroids (Luan et al., 2021).

Total phenolic and flavonoid content

The total phenolic content in the extract and fractions ranged from 4.46 mg GAE/g to 51.25 mg GAE/g. From the gallic acid calibration curve expression ($y = 0.0112x + 0.256$), we observed that hexane fraction exhibited the lowest phenolic content (4.46 ± 0.19 mg GAE/g) while the ethyl acetate fraction had the highest phenolic content (51.25 ± 0.52 mg GAE/g). The total flavonoid content ranged from 441.00 ± 5.98 mg QE/g to 2285.00 ± 11.13 mg QE/g. The trend was as follows: hexane fraction (441.00 ± 5.98 mg QE/g) < dichloromethane fraction (576.00 ± 6.24 mg QE/g) < extract (639.00

± 8.66 mg QE/g) < aqueous fraction (700.00 ± 7.55 mg QE/g) < butanol fraction (1101.00 ± 10.17 mg QE/g) < ethyl acetate fraction (2285.00 ± 11.13 mg QE/g) (Table 2).

Table 2. TPC, TFC, DPPH, FRAP and MC values of *A. carambola* extract and fractions

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC_{50} (µg/mL)	FRAP IC_{50} (µg/mL)	MC IC_{50} (µg/mL)
Extract	38.21± 0.34	639.00 ± 8.66	26.47 ± 0.38	34.42 ± 0.29	69.53 ± 0.66
n-HEF	4.46 ± 0.19	441.00 ± 5.98	28.59 ± 0.29	51.30 ± 0.34	360.66 ± 2.55
DCMF	20.89 ± 0.21	576.00 ± 6.24	29.64 ± 0.25	43.09 ± 0.32	74.55 ± 0.43
EAEF	51.25 ± 0.52	2285.00 ± 11.13	27.88 ± 0.31	26.51 ± 0.26	122.83 ± 0.87
BUTF	45.53 ± 0.42	1101.00 ± 10.17	27.54 ± 0.30	29.99 ± 0.22	138.15 ± 0.59
AQF	21.16 ± 0.28	700.00 ± 7.55	27.54 ± 0.24	28.46 ± 0.18	205.63 ± 1.35
AA	-	-	20.25 ± 0.21	38.21 ± 0.26	76.55 ± 0.64

TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: 2,2-Diphenyl-1-picrylhydrazyl radical activity; FRAP: Ferric reducing antioxidant power; MC Metal chelating activity; n-HEF: hexane fraction, DCMF: dichloromethane fraction; EAEF: ethyl acetate fraction; BUTF: butanol fraction; AQF: aqueous fraction; AA: ascorbic acid.

Antioxidant analysis

The 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), ferric reducing antioxidant power (FRAP) and metal chelating (MC) assay were evaluated. Extract and fractions showed a concentration-dependent attitude in all the analysed models.

The 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) activity

For the DPPH analysis, at 40 µg/mL, the percentage inhibition (%) values were 75.27, 73.84, 73.12, 75.27, 74.19, 76.34, and 80.65% for the extract, hexane fraction, dichloromethane fraction, ethyl acetate fraction, butanol fraction, aqueous fraction, and ascorbic acid, respectively. However, at a higher concentration (100 µg/ mL), the values were: 77.06, 75.63, 75.99, 77.06, 77.88, 78.13 and 81.36% for extract, hexane, dichloromethane, ethylcetate, butanol, aqueous and ascorbic acid, respectively. The IC_{50} values were derived from the plot of the concentration (µg/mL) against percentage inhibition and were as follows: extract (26.47 ± 0.38 µg/mL), hexane (28.59 ± 0.29 µg/mL), dichloromethane (29.64 ± 0.25 µg/mL), ethyl acetate (27.88 ± 0.31 µg/mL), butanol (27.54 ± 0.30 µg/mL), aqueous (27.54 ± 0.24 µg/mL) and ascorbic acid (20.25 ± 0.21 µg/mL) (Figure 1).

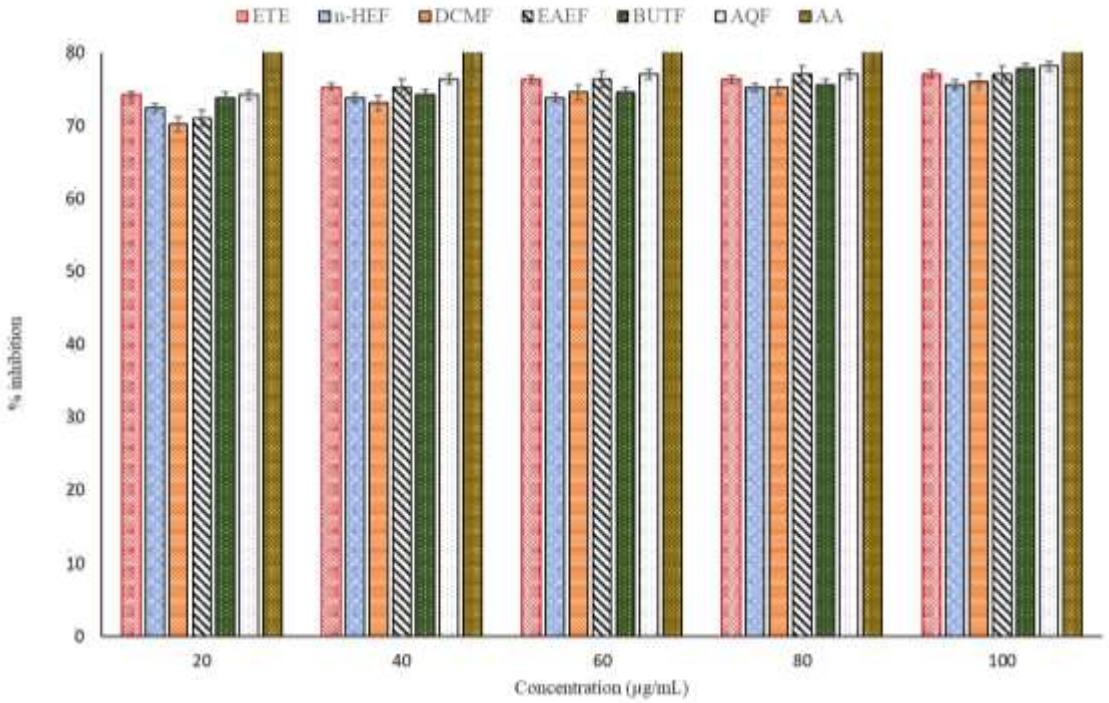


Figure 1. DPPH radical scavenging activity of *A. carambola* stem bark extract and fractions. ETE = Ethanol extract; n-HEF = Hexane fraction; DCMF = Dichloromethane fraction; EAEF = Ethyl acetate fraction; BUTF = Butanol fraction; AQF = Aqueous fraction; AA = Ascorbic acid.

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power was assessed from a plot of the mean absorbance reading against the concentrations (µg/mL). From the graph (Figure 2), absorbance reading at concentration of 40 µg/mL, were as follows: extract (0.677), hexane (0.567), dichloromethane (0.625), ethyl acetate (0.743), butanol (0.692), aqueous (0.746) and ascorbic acid (0.64). Higher absorbance reading was recorded at maximum concentration (100 µg/mL) for the extract (0.871), hexane (0.639), dichloromethane (0.774), ethyl acetate (0.966), butanol (0.854), aqueous (0.912), ascorbic acid (0.820). Values for the IC_{50} were: extract (34.42 ± 0.29 µg/mL), hexane (51.30 ± 0.34 µg/mL), dichloromethane (43.09 ± 0.32 µg/mL), ethyl acetate (26.51 ± 0.26 µg/mL), butanol (29.99 ± 0.22 µg/mL), aqueous (28.46 ± 0.18µg/mL), and ascorbic acid (38.21 ± 0.26 µg/mL) (Figure 2).

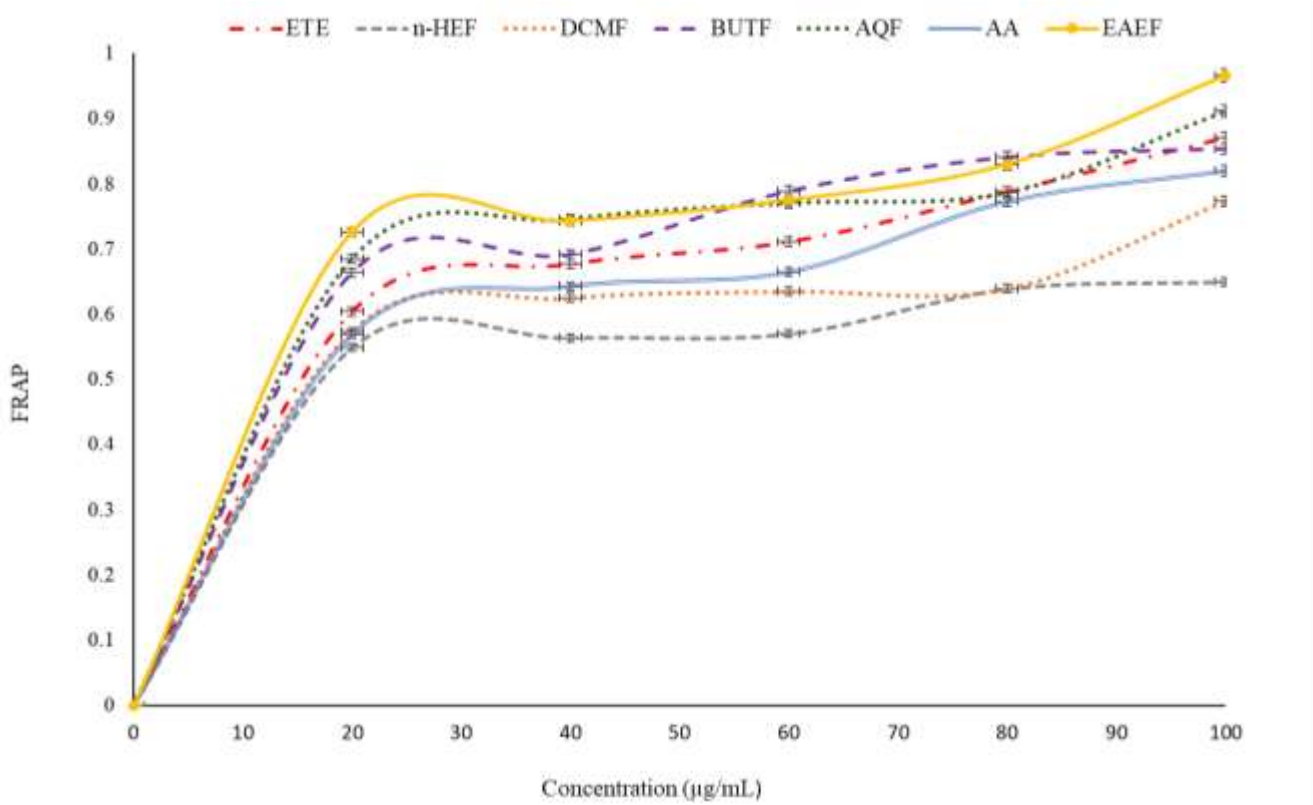


Figure 2. Ferric reducing antioxidant property of *A. carambola* stem bark extract and fractions. ETE = Ethanol extract; n-HEF = Hexane fraction; DCMF = Dichloromethane fraction; EAEF = Ethyl acetate fraction; BUTF = Butanol fraction; AQF = Aqueous fraction; AA = Ascorbic acid.

Metal chelating (MC) assay

The metal chelating antioxidant capacity was evaluated with the extract and all the fractions. The result showed increasing chelation at higher extract concentration. At concentration of 40 µg/ mL we observed the following chelation (%); extract (7.4%), hexane (6.1%), dichloromethane (16.6%), ethyl acetate (29.4%), butanol (15.3%) and ascorbic acid (45.4%), respectively. At concentration of 100 µg/ mL, the percentage metal chelation was extract (19.6%), hexane (12.9%), dichloromethane (25.8%), ethyl acetate (33.8%), butanol (24.5%), and ascorbic acid (52.1%), respectively. For the aqueous fraction, it was observed that chelation seemed to be better at higher concentration so that there was an increase chelation between 80 µg/mL and 100 µg/mL from 11.7% to 24.5%. All other fractions maintain steady rise in the metal chelation; the ethyl acetate fraction exhibited the highest chelation of all the fractions. Ascorbic acid was the control and showed chelation in the ranged of 44.20 to 52.10 µg/mL, which were greater than values for ethyl acetate fraction (Figure 3). Observed IC_{50} values ranged between 69.53 ± 0.66 and 360.66 ± 2.55 µg/mL; the values were: extract (69.53 ± 0.66 µg/mL), hexane (360.66 ± 2.55 µg/mL), dichloromethane (74.55 ± 0.43 µg/mL), ethyl acetate (122.83 ± 0.87 µg/mL), butanol (138.15 ± 0.59 µg/mL), aqueous (205.63 ± 1.35 µg/mL), and ascorbic acid (76.55 ± 0.64 µg/mL).

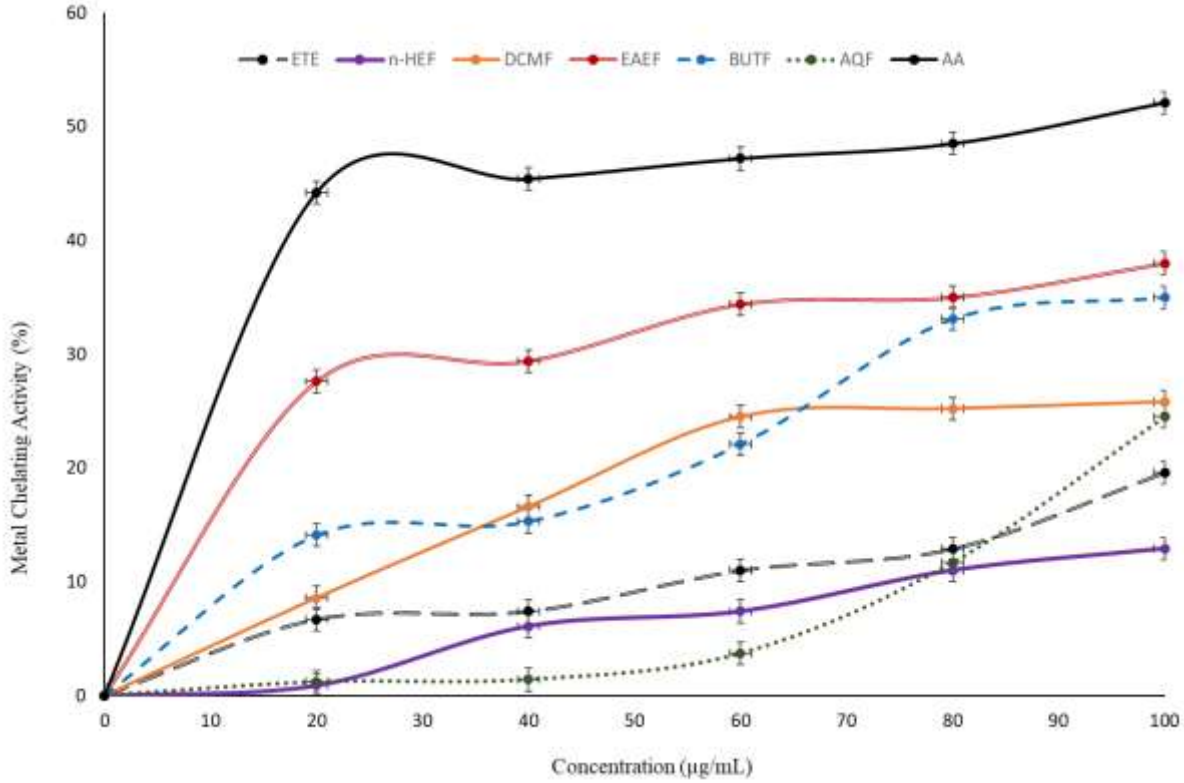


Figure 3. Metal chelating activity of *A. carambola* stem bark extract and fractions. ETE = Ethanol extract; n-HEF = Hexane fraction; DCMF = Dichloromethane fraction; EAEF = Ethyl acetate fraction; BUTF = Butanol fraction; AQF = Aqueous fraction; AA = Ascorbic acid.

Result of acute toxicity test

The median lethal dose (LD_{50}) of ethanol extract of *Averrhoa carambola* stem bark which was determined using the method of Lorke (1983) and was found to be 3872.98 mg/kg.

Alpha amylase and alpha-glucosidase inhibitory study

Administration of starch (2 g/kg) to fasted rats led to varying increases in blood glucose levels after one hour. The observed increases were as follows: starch (40.31%), *Averrhoa carambola* stembark extract-treated groups (0 - 20.39%), the low-dose group showed no increase, and the acarbose-treated group (11.06%). These increases returned to normal within 120 minutes, with only the group receiving the middle dose of the extract (310 mg/kg) showing a slight rise of 1.71%. By 180 minutes, all extract-treated groups had their blood glucose levels normalized, and this effect was maintained throughout the study. Additionally, when starch was co-administered with acarbose, it significantly inhibited the rise in blood glucose levels (Table 3).

Table 3. Effect of ethanol stem bark extract of *A. carambola* on blood glucose levels in rats after oral administration of starch load

Treatment	Dose	Blood glucose level mg/dl in minutes			
	mg/kg	0 min	60 min	120 min	180 min
Control (Normal saline)	-	86.00 ±11.53	87.66 ±7.62(1.93)	91.0 ±7.50(5.81)	80.00 ± 6.02
Starch		80.00 ± 4.54	112.25 ± 4.73(40.31)	92.50 ± 1.70(15.62)	87.25 ± 6.52(9.06)
Acarbose	100	72.33 ± 2.69	80.33±7.21(11.06)	74.0 ± 1.00(2.30)	72.33 ± 8.68
Extract	155	98.66 ± 1.45	91.33 ± 5.60	88.33 ± 6.33[a]	87.33 ± 4.97[a]
	310	97.33 ± 2.33	102.33 ± 2.60(5.13)	99.0 ± 7.50[b](1.71)	87.0 ± 4.04[a]
	620	86.66 ±11.86	104.33 ± 2.84(20.39)	86.0 ± 2.51[a]	78.0 ± 5.77

Data is expressed as mean ± SEM, significant at [a]$p < 0.05$, [b]$p < 0.01$, when compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Administration of sucrose (2 g/kg) afforded a 39.08% increase in blood glucose level one-hour post-administration of the sucrose in the control group. Blood glucose rise of 23.79 - 35.59 % were also recorded in groups treated with 155 - 620 mg/kg of *Averrhoa carambola* stem extract and no increment for acarbose treated group. At 120 min, the group treated with the middle and high doses (310 and 620 mg/kg) had the BGL reduced to normal, while low dose (155 mg/kg) group had BGL increment of 11.44%. At 180 minutes, groups treated with the extract had their blood glucose levels reduced normalised (Table 4).

Table 4. Effect of ethanol stem bark extract of *A. carambola* on blood glucose levels in rats after oral administration of sucrose load

Treatment	Dose	Blood glucose level mg/dl in minutes			
	mg/kg	0 min	60 min	120 min	180 min
Control (Normal saline)	-	86.00 ± 11.53	87.66 ± 7.62(1.93)	91.00 ± 7.50(5.81)	80.00 ± 6.02
Sucrose		81.00 ± 4.50	112.66 ± 1.45[a](39.08)	97.33 ± 1.63(20.16)	94.15 ± 4.81(16.23)
Acarbose	100	90.33 ± 2.48	82.00 ± 6.00	71.66 ± 3.75	78.00 ± 3.78
Extract	155	78.66 ± 6.74	106.66 ± 8.96(35.59)	87.66 ± 0.88(11.44)	73.00 ± 3.05
	310	91.33 ± 2.18	113.0 ± 6.02(23.72)	82.66 ± 2.38	78.66 ± 3.84
	620	88.33 ± 1.20	114.0 ± 1.73(29.06)	79.66 ± 5.84	87.00 ± 6.02

Data is expressed as mean ± SEM, significant at [a]$p < 0.05$, [b]$p < 0.01$, when compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Administration of maltose (2 g/kg) to fasted rats caused varying percentages of increase in blood glucose levels of the treated animals after one hour. The percentages were maltose (44.95%), and acarbose-treated group (0.77%). Only the group treated with the middle dose of *A. carambola* stembark extract (310 mg/kg) had BGL of 3.58%, while other groups (155 and 620 mg/kg) had no rise in BGL. These increases were reduced to normal after 120 min and sustained throughout the duration of the study. Also, co-administration of the maltose with acarbose prominently inhibited the rise in the blood glucose concentrations (Table 5).

Table 5. Effect of ethanol stem bark extract of *A. carambola* on blood glucose levels in rats after oral administration of maltose load

Treatment	Dose	Blood glucose level mg/dl in minutes			
	mg/kg	0 min	60 min	120 min	180 min
Control (Normal saline)	-	86.00 ±11.53	87.66 ± 7.62(1.93)	91.00 ± 7.50(5.81)	80.00 ± 6.02
Maltose		86.75 ± 2.52	125.75 ± 1.65(44.95)	99.50 ± 2.90(12.75)	88.00 ± 1.68(1.44)
Acarbose	100	85.34 ± 1.36	86.00 ± 2.20(0.77)	84.26 ± 1.14[a]	82.28 ± 2.26
Extract	155	95.33 ± 2.33	92.33 ± 5.89	86.66 ± 6.64	75.33 ± 4.84
	310	93.00 ± 3.51	96.33 ± 2.33(3.58)	85.66 ± 6.74	91.33 ± 6.88
	620	89.66 ± 9.83	88.33 ± 16.34	83.33 ± 10.83	84.66 ± 9.73

Data is expressed as mean ± SEM, significant at [a]$p < 0.05$, [b]$p < 0.01$, when compared to control (n=6). Values in parenthesis are percentage increases in blood glucose concentrations compared to 0 min in the same group.

Gas chromatography-mass spectrometry (GC-MS)

The results of the GC-MS analysis revealed a total of eleven compounds in the ethyl acetate fraction (Table 6). These compounds and their peak area (%) were: 5-(2-Amino-phenyl)-[1,3,4]thiadiazo-2-yl]-p-tolyl-amine (0.38%); 2-hydroxymethoxybenzaldehyde, tert-butyldimethylsilyl ether (6.63%), phenol,4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy (13.24%), thiazolo[3,2-a]35enzimidazole-3(2H)-one,2-(2-fluorobenzylideno)-7,8-dimethyl- (17.9%), squalene (0.21%), phenol 2-methoxy-4-(2-propenyl)-, 2-(2,5-dimethoxy-4-propylphenyl) ethanamine (2.48%), isoquinoline,1,2,3,4-tetrahydro-8-amino-2-methyl-4-phenyl- (25.85%), benzene butyric acid, 2,3-dimethoxy- (1.33%), phenol, 2,6-dimethoxy-4-(2-propenyl)- (26.89%), phenol, 2-(1-methyl-2-buthenyl)-4-methoxy- (5.06%) (Figure 5).

Table 6. GC-MS Analysis of stem bark ethyl acetate fraction of *Averrhoa carambola*

Peak	Compound	MF	RT (min)	PA (%)
1.	5-(2-Amino-phenyl)-[1,3,4] thiadiazol-2-yl]-p-tolyl-amine	$C_{15}H1_4N_4S$	18.084	0.36
2.	2-Hydroxymethoxybenzaldehyde, tert- butyldimethylsilyl ether	$C_{14}H_{22}O_3Si$	18.981	6.63
3.	Phenol,4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy	$C_{20}H_{22}O_4$	19.414	11.55
4.	Thiazolo[3,2-a]36enzimidazole-3(2H)-one,2-(2-fluorobenzylideno)-7,8-dimethyl-	$C_{18}H_{13}FN_2OS$	19.676	17.59
5.	Phenol,4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy	$C_{20}H_{22}O_4$	19.907	1.43
6.	Squalene	$C_{30}H_{50}$	20.085	0.21
7.	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	20.276	0.35
8.	Phenol,4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy	$C_{20}H_{22}O_4$	21.523	0.26
9.	2-(2,5-dimethoxy-4-propylphenyl) ethanamine	$C_{13}H_{21}NO_2$	21.928	2.48
10.	Isoquinoline, 1,2,3,4-tetrahydro-8-amino-2-methyl-4-phenyl-	$C_{16}H_{18}N_2$	22.611	25.85
11.	Benzene butyric acid, 2,3-dimethoxy-	$C_{12}H_{16}O_4$	23.225	1.33
12.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{10}H_{12}O_2$	23.849	1.87
13.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{10}H_{12}O_2$	24.682	14.00
14.	Phenol, 2-(1-methyl-2-buthenyl)-4-methoxy-	$C_{12}H_{16}O_2$	25.088	5.06
15.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{10}H_{12}O_2$	25.583	11.02

MF: Molecular formular; RT: Retention time; PA: Peak area.

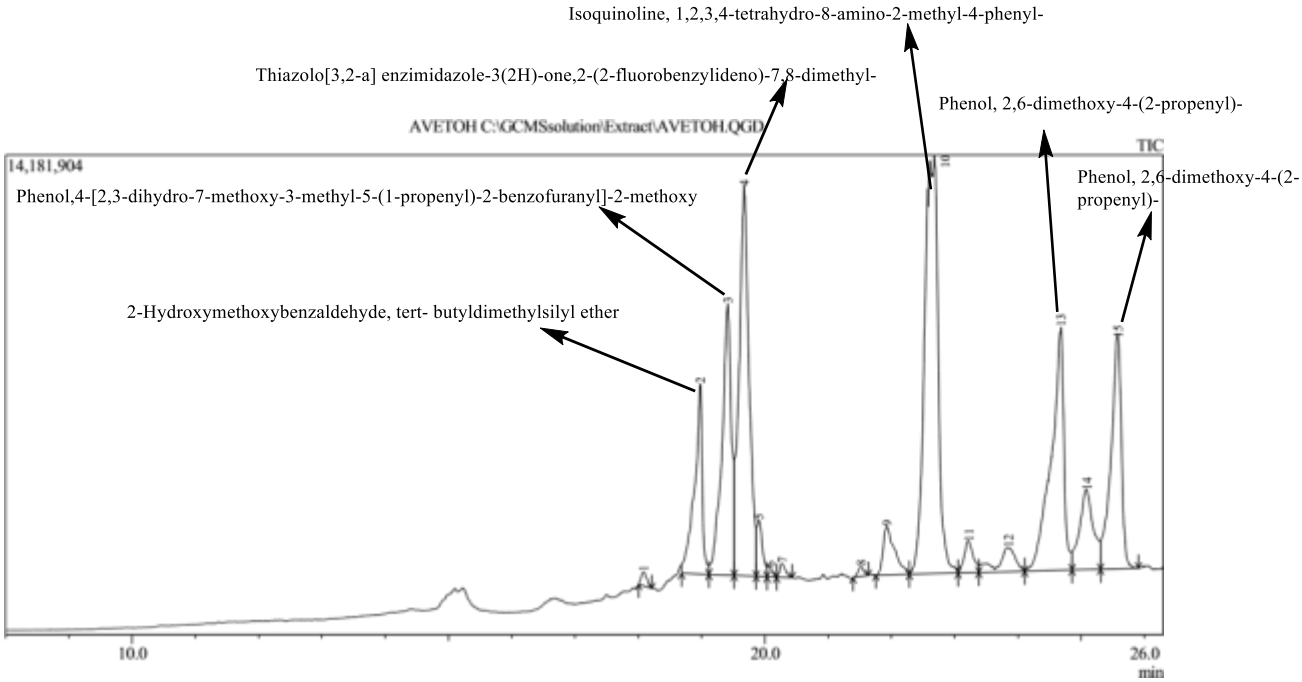


Figure 4. GC-MS Chromatogram of *A. carambola* stem bark ethyl acetate fraction

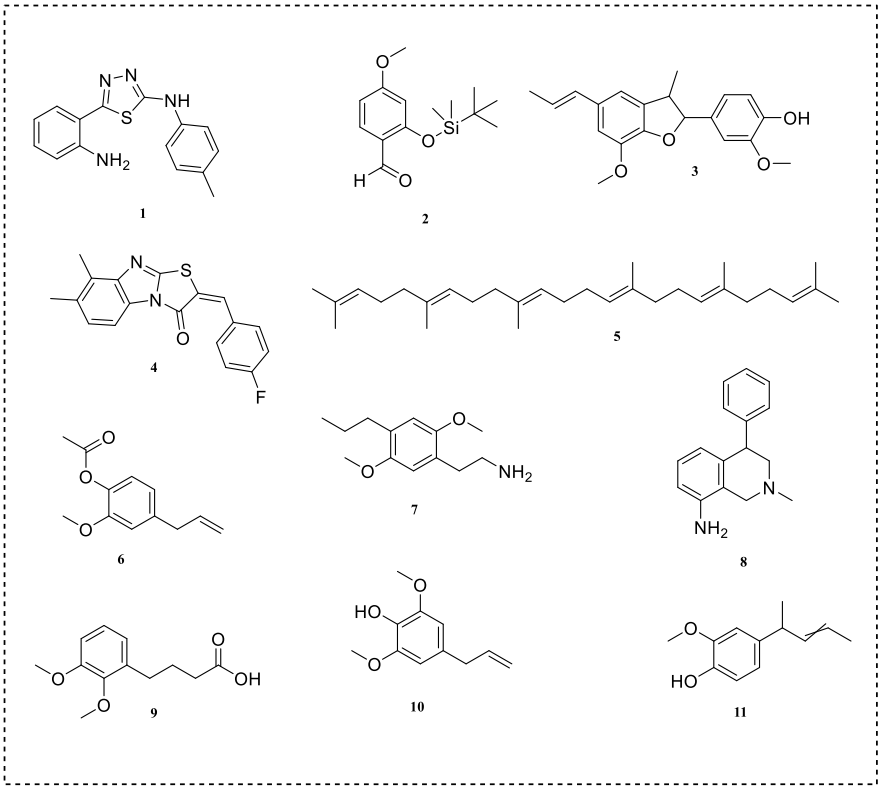


Figure 5. Bioactive compounds from GC-MS analysis of *A. carambola* stem bark ethyl acetate fraction

Compound 1: 5-(2-Amino-phenyl)-[1,3,4]thiadiazo-2-yl]-p-tolyl-amine;
Compound 2: 2-hydroxymethoxybenzaldehyde, tert-butyldimethylsilyl ether; **Compound 3:** Phenol,4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy; **Compound 4:** Thiazolo[3,2-a]37enzimidazole-3(2H)-one,2-(2-fluorobenzylideno)-7,8-dimethyl-; **Compound 5:** Squalene; **Compound 6:** Phenol 2-methoxy-4-(2-propenyl)-, acetate; **Compound 7:** 2-(2,5-dimethoxy-4-propylphenyl)ethanamine; **Compound 8:** Isoquinoline,1,2,3,4-tetrahydro-8-amino-2-methyl-4-phenyl-; **Compound 9:** Benzene butyric acid, 2,3-dimethoxy-; **Compound 10:** Phenol, 2,6-dimethoxy-4-(2-propenyl)-; **Compound 11:** Phenol, 2-(1-methyl-2-buthenyl)-4-methoxy.

Discussion

The study investigated the phytochemical, antioxidant and antidiabetic properties of the stem bark of *Averrhoa carambola*. Preliminary phytochemical analysis revealed that phenols, flavonoids, saponins and glycosides were the predominant constituents in the stem bark ethanol extract. Among the extracts, the ethyl acetate and butanol fractions had the highest phenolic and flavonoid content, while the hexane fraction contained the least. Previous studies have also reported high content of phenolic and flavonoid compounds in the stem bark of *A. carambola* (Silva et al., 2021; Rhaman et al., 2022). A review of some indigenous medicinal plants from southern Nigeria showed that their phenolic and flavonoid content was lower than that of *A. carambola* (Awah et al., 2012; Mbaebie et al., 2012; Nwozo et al., 2023), suggesting that *A. carambola* has relatively high amounts of these compounds. Flavonoids, saponins, alkaloids, and tannins have demonstrated antidiabetic, anti-inflammatory, and antioxidant properties. These compounds have enhanced the function of pancreatic tissues by regulating glucose absorption and insulin secretion (Aba & Azusu, 2018). Saponins have reduced oxidative stress, increased serum insulin levels, inhibited disaccharide activity, and prevented the progression of alpha-amylase and alpha-glucosidase inhibitors (Perez Gutierrez, 2016; Wang et al., 2019). Alkaloids raise plasma insulin levels, lower serum lipids and lipid peroxidation, improve liver glucose content, increase insulin sensitivity, promote glucose uptake, and reduce excessive reactive oxygen species (Singh et al., 2022; Kumar et al., 2019). Flavonoids enhance the recovery of hepatic insulin and leptin sensitivity, glucose transport, and cell proliferation, while also inhibiting pancreatic α-amylase and α-glucosidase and improving insulin tolerance (Babu et al., 2013; Alkhalidy et al., 2018). The analysis of the DPPH radical scavenging results showed that all fractions exhibited radical scavenging activity against DPPH radicals, with varying IC_{50} values range. The ethyl acetate fraction demonstrated the highest antioxidant activity among all the extracts (IC_{50} values <100 µg/mL). The ferric reducing antioxidant power was determined by plotting the average absorbance values against the extract concentrations (µg/mL), and the percentage inhibition was ranked as follows: ethyl acetate > aqueous > butanol > dichloromethane > hexane fraction. The extract's ability to form stable iron (II) chelates is

linked to its capacity to reduce free ferrous ions, thereby influencing the Fenton reaction associated with various human diseases (Halliwell and Gutteridge, 1984). The strong antioxidant activity observed, especially in the ethyl acetate fraction, is likely due to its high phenolic and flavonoid content, indicating a correlation between antioxidant activity and polyphenols. *Averrhoa carambola* stem bark extract showed inhibitory effects on α-amylase and α-glucosidase in rats, as noted in this study. All groups treated with the extract had their blood glucose levels reduced to normal at either the middle or high dose throughout the research period. After the oral administration of a starch load, blood glucose levels increased but returned to normal after 120 minutes, with only the middle dose group showing a percentage increase. The digestion of dietary polysaccharides like starch is facilitated by the combined action of α-amylase and α-glucosidase enzymes. α-Amylase breaks down α-linkages in polysaccharides into disaccharides like maltose, and α-glucosidase enzymes on the cell membrane further convert disaccharides into monosaccharides (Kalra, 2014; de Melo et al., 2006). Inhibiting these enzymes slows the digestion of carbohydrates, leading to a smaller increase in blood glucose levels after a carbohydrate meal, thus delaying glucose absorption in the intestines and potentially preventing postprandial glucose spikes, as observed in this study. When combined with maltose and sucrose, *Averrhoa carambola* stem bark extract significantly inhibited blood glucose increases, a similar effect was noted for Acarbose, the standard drug. Studies suggest that managing postprandial hyperglycemia by inhibiting α-glucosidase and α-amylase can be an effective strategy for controlling infant-stage of diabetes. This slow glucose absorption and reduce blood sugar spikes after meals (Park and Han, 2015; Hiyoshi et al., 2017; Morais et al., 2020). Common antidiabetic medications, such as acarbose, voglibose, and miglitol, are used for this purpose, although side effects like flatulence, stomach discomfort, and allergic reactions are reported (Shehadeh et al., 2021). Plant extracts, rich in bioactive compounds, have been found to inhibit the activities of α-amylase and α-glucosidase (Proença et al., 2017; Yang et al., 2019; Bnouham et al., 2006; Unuofin and Lebelo, 2020). Studies have shown that plant-based bioactive compounds exert antidiabetic effects by targeting various human enzymes and proteins (Egbuna et al., 2021; Bogle and Mendes, 2015; Salehi et al., 2019). Polyphenols, in particular, have been identified as strong inhibitors of α-amylase and α-glucosidase, playing a key role in diabetes management by influencing glucose transport and intestinal glycosidase activity (Dembinska-Kiec, 2008; He et al., 2019). According to de Sousa et al. (2004), phenolics and flavonoids reduce oxidative stress and regulate enzyme activity under hyperglycemic conditions. Other research has shown that these compounds protect against lipid peroxidation, lower blood glucose levels, and reduce oxidative markers in the kidneys (Pandey and Rizvi, 2010; Aguirre et al., 2011; Lee et al., 2009). Furthermore, the analysis of the bioactive compounds in the ethyl acetate fraction using gas chromatography-mass spectrometry identified compounds with documented bioactivity. For instance, phenol, 2,6-dimethoxy-4-(2-propenyl)- has demonstrated antioxidant, antiseptic, and anaesthetic properties (Gusmailina & Komarayati, 2015). Squalene has shown antioxidant, antimicrobial, anticancer, and cardioprotective effects (Lozano-Grande et al., 2018). Thiazolo(3,2-a)benzimidazol-3(2H)-one-2-(2-fluorobenzylideno)-7,8-dimethyl is known to possess anti-inflammatory, antimicrobial, antiulcer, antiviral, anthelmintic, and anticancer activities (Al-Rashood & Abdel-Aziz, 2010), while phenol, 4-(2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl)-2-methoxy- has been reported to exhibit antioxidant effects in a DDPH radical scavenging assay (Asika et al., 2016). The observed antioxidant properties of the extract and fractions, along with the antidiabetic potential of the crude extract from *A. carambola*, emphasize the promise of these natural products as complementary treatments for existing hyperglycemic medications. With the rising prevalence of diabetes and oxidative stress-related disorders, natural products present a valuable avenue for developing new drugs. Future studies should aim to explore this plant for additional therapeutic potentials, as well as isolate and characterize the phytochemicals accountable for these activities, understanding their pharmacokinetics, and exploring the synergistic effects of these compounds when combined with other antidiabetic drugs.

Conclusion

The findings of this study showed that the stem bark of *Averrhoa carambola* is a valuable source of phytocompounds, displaying strong DPPH radical scavenging, ferric reducing, and metal chelating activities, with the ethyl acetate fraction being the most potent. Furthermore, the extract exhibited significant antidiabetic effects across all models tested. GC-MS analysis identified numerous therapeutically relevant compounds in the extract, suggesting that these phytoconstituents may contribute to the observed antioxidant and antidiabetic properties.

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Author contributions

Conceptualisation and writing of draft manuscript (GNE), critical reviewing of the manuscript and editing (BNI), acquisition of data (UNU, UOJ and OA), acquisition of funding (BSA), software and data analysis (JEO & PST).

AI usage declaration

We did not use artificial intelligence in writing this research in any way.

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Conflict of interest

The authors declare no conflict of interest. The manuscript has not been submitted for publication in other journal.

Ethics approval

Approval for the animal studies was granted by the College of Health Sciences Animal Ethics Committee at the University of Uyo (UU/CS/AE/14/63). All ethical guidelines for the care and use of lab. animals, which regulate experiments involving living animals, were meticulously followed.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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