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Original Research Article

Cardio-Protective Effect of *Hunteria umbellata* Seed: Experimental and *In-silico* Approaches

Adetoun E. Morakinyo[1]*, Femi E. Omoniyi[1], Samuel C. Nzekwe[1], Abel K. Oyebamiji[2], Joy M. Adelowo[2], Sulaiman A. Lawal [1], Angel A. Olumade [1], Elijah O. Olopade[1], Temitope A. Oyedepo[1]*

[1]*Department of Biochemistry, Adeleke University, Ede, Osun state, Nigeria*
[2]*Department of Basic Sciences, Adeleke University, Ede, Osun state, Nigeria*

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ABSTRACT

Cardiotixicity is one of the main causes related to sudden death in the world. The study investigated the ameliorative potential of *Hunteria umbellata* against isoproterenol (ISO)-induced cardiotoxicity using experimental and *in-silico* approaches. The ethanol extract of *H. umbellata* was prepared, phytochemically screened and quantified. *In vitro* antioxidant potential of the extract was also carried out. Male Wistar rats (30) were randomly and equally grouped into five and used for this study. After administration of the extract, the rats were sacrificed. Thereafter, the cardiac toxicity biomarkers (CK-MB, LDH, AST) and lipid profiles [Triglycerides (TG), Total cholesterol (TC) and High-density cholesterol (HDL-c)] were estimated from the blood plasma. Five molecular compounds from *H. umbellata* seed were selected based on their percentage yield and they were subjected to optimization using Spartan 14. The optimized compounds were docked against β_1-adrenergic receptor (PDB ID: 2ycw). Data obtained was analyzed using standard descriptive and inferential statistics. Pre-treatment with the extract caused a significant decrease in the levels LDH, AST, TC and TG with the most apparent effect at 100 mg/kg bwt. There was no significant difference in the activity of CK-MB. It was observed that compound (A), (C) and (E) inhibited β_1-adrenergic receptor than the referenced drug. In conclusion, *H. umbellata* exhibited ameliorative effects against ISO-induced myocardial damage, suggesting preliminary anti-dyslipidemia abilities possibly due to high phenolic and flavonoid contents.

Keywords: Cardio-protective, *Hunteria umbellata*, Propanalol, Phytochemicals.

Introduction

Cardiovascular diseases (CVDs) are disease conditions of the heart and blood vessels. They are often accompanied by life threatening complications resulting into heart attack, heart failure, stroke and other conditions. Global statistics have revealed cardiovascular diseases (CVDs) as the leading cause of death globally. WHO estimated 17.9 million people died from CVDs in 2019, representing 32% of all global deaths.[1] Although the prevalence of cardiovascular disease (CVD) is slowly declining in high-income countries, it is, however, on the rise in low-income and middle-income countries with over three quarters of CVD global deaths taking place in low and middle-income countries. [1,2] In Nigeria, WHO revealed in 2016 that an estimated 29% of total deaths in the country were as a result of non-communicable diseases, with 11% attributed to CVDs.[3] Factors responsible for cardiovascular diseases have been classified into modifiable risk factors and non-modifiable risk factors. The most important of the modifiable risk factors are the behavioural which include sedentary lifestyle, unhealthy diet, smoking, excessive use of alcohol and air pollution.[4] Other modifiable risk factors include high blood pressure, high LDL cholesterol and high blood sugar.[5]

*Corresponding author. E mail: adetounadejumobi05@gmail.com; topeoyedepo@adelekeuniversity.edu.ng
Tel: +234-7033744184; +234-8035838269

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The non-modifiable risk factor predisposes a subset to a higher risk of suffering CVDs irrespective of how healthy a lifestyle they live. These factors include age, gender, family history and ethnicity.[6]
The economic constraint of low and middle-income countries such as Nigeria has made a great number of her indigents unable to bear the cost of modern diagnosis and improved treatment options in cardiovascular health. This reality has remained a pivotal reason why people from these regions seek non-conventional interventions such as phytotherapy in the prevention and management of their diseased conditions. Furthermore, these medicinal plants are often believed to be safe and efficacious based on their long-standing use in various cultures.[7] They contain potent pharmacologically active constituents such as flavonoids, alkaloids, terpenoids, tannins, saponins, steroids amongst others.[8]
Hunteria umbellata (Apocynaceae) is an important medicinal plant predominant in the rainforest zone of West Africa and commonly called Abeere in South-west Nigeria.[9,10] The leaf, seed, root and bark of *H. umbellata* have been reportedly useful for the treatment or/and management of different human conditions: oxytocic effect,[11] anti-oxidative effect, erectile stimulating effect and anti-diabetic effect .[10,12,13] Since *H. umbellata* has been reported to exert anti-obesity function ,[14] this research was therefore aimed at investigating the cardio-protective potential of *H. umbellata* against cardiotoxicity in rats model.

Materials and Methods

Sample collection and preparation
Fresh *H. umbellata* seed were purchased from Oja Jagun Ogbomoso, Oyo state, Nigeria in January, 2021. The seeds were identified in the Department of Biology, Adeleke University, Ede. The seeds were thoroughly washed and air dried at room temperature to eliminate dirt.

When completely dried, the seeds were dislodged from their outer shell and pulverized to fine light brown powder using a domestic blender. The powdered seed (1000 g) was soaked in 3 L of 100% ethanol for 48 h with intermitent shaken and sieved with a double layered muslin cloth and cotton wool respectively. The filtrate was concentrated to dryness using rotary evaporator. The extract obtained was stored at -4ºC until further use. The percentage yield for the plant extract was calculated using the formula below;

$$\%\ Yield = \frac{\text{Weight of Extract}}{\text{Weight of powdered sample}}\ X\ 100$$

Chemical and reagents
Diagnostic kits for the assays of Alanine Transferase (ALT) and AST were products of Randox Laboratory Limited, CrumLin, U.K. The Lactose dehydrogenase (LDH) kit and Creatinine kinase- muscle/brain (CK-MB) were products of Agappe Diagnostics, Cham, Switzerland. Isopreterenol (ISO) was purchased from Sigma Aldrich (St. Louis, MO,USA). Other chemicals used were of analytical grade and water used was glass distilled.

Preliminary phytochemical screening and quantification
The plant extract was screened for the presence of alkaloid, saponins, phenols, tannins, glycosides, steroids, and terpenoids using standard procedures.[15,16]

Estimation of total phenolic content
Estimation of the total phenolic content of *H. umbellata* was determined using the Folin-Ciocalteu assay as described by Singleton *et al.*[17] using tannic acid (100 µg/mL) as standard. The standard curve was prepared by pipetting 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of tannic acid solution (100µg/mL of tannic acid) in triplicate into clean and dried test tubes. The volume was made up to 1 mL with distilled water. Folin-Ciocalteu's phenol reagent (1:10) dilution (1.5 mL) was added to each and followed by the addition of 1.5 mL of Sodium carbonate (7.5%). The reaction mixtures were incubated for $1^1/_2$ hours at room temperature. The absorbance was read against the blank at 725 nm. The standard calibration was prepared by plotting the absorbance against the concentrations of tannic acid.
A diluted solution of ethanol *H. umbellata* seed extract (0.2 mL and 0.5 mL of 1mg/mL) in triplicate was made up to 1 mL with ethanol. The reaction mixture was treated as described for standard, tannic acid and the total phenolics concentrations were extrapolated from the standard calibration curve and expressed as mg tannic acid equivalent per gram of the extract (mg TAE/g of extract).

Estimation of total flavonoid concentration
Estimation of the total flavonoid concentration of *H. umbellata* seed extract was determined according to the spectrophotometric method described by Sun *et al*. [18] using rutin (0.1 mg/mL) as standard. The standard curve was prepared by pipetting 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of rutin solution (0.1 mg/mL) in triplicate into clean and dried test tubes. The volume was made up to 2 mL with distilled water.This was followed by the addition of 0.3 mL of freshly prepared 5 % (w/v) $NaNO_2$, 0.3 mL of 10 % (w/v) $AlCl_3$ and mL of 4 % (w/v) NaOH. The reaction mixtures were incubated for 10 min at room temperature and absorbance was taken against the blank at 500 nm. The standard calibration curve was prepared by plotting the absorbance against rutin concentrations.
H. umbellata (0.2 mL and 0.5mL of 1 mg/mL) in triplicate was pipetted and made up to 2 mL with distilled water in clean test tubes followed by the addition of 0.3 mL (5%) $NaNO_2$, 0.3 mL (10%) $AlCl_3$ and 2 mL (4%)NaOH. The reaction mixture was treated as described for standard, rutin and the total flavonoid concentrations were extrapolated from the standard calibration curve and expressed as mg rutin equivalent per gram of the extract (mg RE/g of extract).

DPPH radical scavenging assay
The DPPH-radical scavenging activity of the *H. umbellata* seed extract was assayed as described by Brand-Williams *et al.*[19] with ascorbic acid as reference drug. Different volumes of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ mL working concentrations made from the extract and standard ascorbic acid (1 mg/ mL in methanol) was put in clean and dried test tubes in triplicates followed by the addition of DPPH (1 mL, 0.3 mM) dissolved in methanol. The mixture was properly mixed by inversion and then incubated in a dark chamber for 30 min. The absorbance was read at 517 nm against the reagent blank. The percentage free radical scavenging activities of the standard and extract was calculated as the percentage inhibition of DPPH using the expression:

$$\%\text{scavenging activity} = 100\ \text{x}\ \frac{\Delta\text{Abs}_{\text{control}} - \Delta\text{Abs}_{\text{sample}}}{\Delta\text{Abs}_{\text{control}}}$$

Where ΔAbs- Change in absorbance

Ferric reducing antioxidant power assay
The ferric reducing antioxidant power of *H. umbellata* seed extract was determined according to the spectrophotometric method described by Benzie and Strain[20] with Ascorbic acid as standard. The standard curve was prepared by pipetting 30 µL of different concentrations (0.1, 0.2, 0.3, 0.4, 1.0 and 2.0mM) prepared from stock solution of Ascorbic acid (1 mM) into clean and dry test tubes. The solutions were made up to 1mL with distilled water and 300 µL of FRAP reagent ({1.627g sodium acetate + 16 mL Glacial acetic acid make up to 1 L with distilled water}, 10mM TPTZ (2,4,6-tri[2-pyridyl]-s-triazine)[0.0093g in 3 mL of 40 mM HCl {1.46mL of conc 32% HCl in 1 L of d.H_2O}]and 20 mM Iron (III) Chloride hexahydrate ($FeCl_3.6H_20$) all mixed together) was added. The mixture in the test tubes was then incubated at 37ºC and the absorbance was read at 593 nm.
The *H. umbellata* seed extract (0.02 mL of 1 mg/mL) in triplicate was pipetted and made up to 2 mL with distilled water in clean test tubes followed by the addition of the FRAP reagent and the mixture was incubated at 37^0C. The absorbance was then read at 593 nm. The concentrations were extrapolated from the standard calibration curve.

Experimental animals
Thirty (30) healthy adult male Wistar rats (195 ± 2.60 g) were procured from the central animal house, Ladoke Akintola University of Technology Ogbomoso, Nigeria. The animals were housed in stainless steel cages and kept in a room where 12 h light/dark cycle was maintained throughout the period of experiment. The animals were acclimatized for 21 days and allowed to ad libitum access to water and standard commercial rat chow pellets (ACE Feeds Nigeria Ltd). The principle of laboratory animal care (NIH publication No 85-23) guidelines were followed in this study and the ethical approval for the study was obtained from the ethical committee, Adeleke University, Ede with the ethical reference number AUERC/FOS/IND/01.
The animals were later divided into five groups of six animals each (n = 6) as follows: Group 1: control, rats were fed with standard diet and normal saline; Group 2, negative control, rats were fed with standard diet and induced with Isoproterenol hydrochloride (85 mg/kg bwt); Group 3, positive control, rats were fed with standard diet and propanolol (1.8 mg/kg bwt), and induced with Isoproterenol hydrochloride (85 mg/kg bwt); Group 4, rats were fed with Standard diet and 100 mg/kg bwt of *H. umbellata* seed extract, and induced with Isoproterenol hydrochloride (85 mg/kg) and Group 5, rats were fed with standard diet and 200 mg/kg bwt of *H. umbellata* seed extract and induced with Isoproterenol hydrochloride (85 mg/kg bwt). Normal saline, propanolol and extract were administered orally to experimental rats once daily for 21 days before induction of cardiotoxicity.

Induction of cardiotoxicity
Isoproterenol hydrochloride (ISO) (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for 2 days (21^{st} and 22^{nd}day) to induce cardiotoxicity.

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Collection of Blood Samples for Biochemical Estimations

Approximately twenty-four hours after the last induction of cardiotoxicity with Isoproterenol hydrochloride, the experimental animals were sacrificed humanely by subjecting to mild anesthesia using diethyl ether. Blood samples collected were centrifuged at 3000 rpm for 10 min. Blood plasma was collected into labeled sterile anti-coagulant bottles and stored in a refrigerator at -20^{o}C for biochemical analyses.

Estimation of total cholesterol concentrations

The estimation of total cholesterol in the plasma was carried out according to the method of Richmond using Randox Diagnostic kit.[21] The plasma/homogenate and standard (0.01 mL) were pipetted in duplicate into separate test tubes and reagent 1 solution was gently added (1 mL). The reagent blank contained distilled water (0.01 mL) and 1 mL reagent 1 solution {PIPES buffer (80 mmol/L, pH 6.8), 4-aminoantipyrine (0.25 mmol/L), phenol (6 mmol/L), peroxidase (0.5 U/mL), cholesterol esterase (0.15 U/mL), and cholesterol oxidase (0.10 mmol/L)}. The reaction mixtures were incubated at 37^{0}C for 10 min. The absorbance was read at 546 nm within 1 hour gainst the reagent blank.

The concentration of total cholesterol was calculated using the expression:

$$\text{Conc. of plasma cholesterol (mg/dL)} = \frac{\text{A}_{\text{sample}}}{\text{A}_{\text{std}}} \times \text{Conc. of Std.}$$

Estimation of triglycerides concentrations

This was carried out according to the method of Tietz using Randox diagnostic kits.[22] The plasma/homogenate and standard (0.01 mL) were pipetted in duplicate into separate test tubes and reagent 1 solution was gently added (1 mL). The reagent blank contained distilled water (0.01 mL) and 1 mL reagent 1 solution {pipes buffer (40 mmol/L, pH 7.6), 4-chloro-phenol (5.5 mmol/L), magnesium ions (17.5 mmol/L), 4-aminophenazone 0.5 (mmol/L), ATP (1.0 mmol/L) lipases (150 U/mL), glycerol kinase (0.4 U/mL) glycerol-3-phosphate oxidase (1.5 U/mL), peroxidase (0.5 U/mL)}. The reaction mixtures were incubated at 37^{0}C for 10 min. The absorbance was read at 546 nm within 1 h against the reagent blank.

The concentration of triglycerides was calculated using the expression:

$$\text{Plasma Triglyceride Concentration (mg/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{Standard Conc. (mg/dL)}$$

Isolation and Estimation of Plasma High-Density Lipoprotein Cholesterol (HDL-c)

The isolation of plasma HDL-c was carried out according to the method of Gidez *et al*. using Randox Diagnostic Kit.[23] The sample (plasma or standard (0.2 mL) was precipitated with 0.5 mL (4:1) of precipitating reagent (Phosphotungstic acid, 0.55 mM and Manganese Chloride, 25 mM). The suspension was mixed, allowed to settle and followed by centrifugation for 10 min. at 4000 rpm. The supernatant was carefully collected and used for estimation of HDL-c.

The estimation of HDL-c concentration was carried out, using Randox Diagnostic Kit. The reaction mixture contained the 0.1 mL supernatant/standard in duplicate and 1 mL Reagent 1. The reagent blank contained distilled water (0.1 mL) and 1 mL Reagent 1 {pipes buffer (80 mmol/L, pH 6.8), 4-aminoantipyrine (0.25 mmol/L), phenol (6 mmol/L), peroxidase (0.5 U/mL), cholesterol esterase (0.15 U/mL), and cholesterol oxidase (0.10 mmol/L). The reaction mixtures were incubated for 10 min. at 37ºC. The absorbance was read against the reagent blank at 500 nm within 1 h. The concentration of HDL-c was calculated from the expression:

$$\text{HDL} - \text{c concentration} \left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{standard}}} \text{X Standard concentration (mg/dL)}$$

Estimation of aspartate transaminase (AST) activity

The assay for AST activity was carried out according to the method of Reitman and Frankel and Schmidt and Schmidt using Randox Diagnostic Kit.[24,25] The sample (0.1 mL) was mixed with 0.5mL of Reagent 1 (Phosphate buffer {100mMol/L, pH 7.4}, L-aspartate {100 mMol/L} and α-oxoglutarate {2 mMol/L}) and incubated for exactly 30 min at 37^{0}C after which 0.5 mL of Reagent 2 (2, 4-dinitrophenylhydrazine {2 mMol/L}) was then added. The mixture was allowed to stand for exactly 20minutes at 20 to 25^{0}C after which 5.0mL of Sodium hydroxide (0.4 mol/L) was added. The absorbance of the sample was then read at 546nm against reagent blank containing distilled water instead of the sample after 5 minutes. The AST activity was extrapolated from a standard curve where one unit of aspartate aminotransferase activity was defined as the amount of protein that liberated one µmole oxaloacetate/ mL per minute under experimental conditions.

Estimation of lactate dehydrogenase (LDH) activity

The assay for the LDH-P activity was carried out according to the method reported by Akinlusi *et al*. using Agappe Diagnostic Kit.[26] The plasma (0.01 mL) was mixed with 1mL of working reagent in a test tube. The working reagent was prepared by mixing 4 volumes of Reagent R1 (Tris buffer [pH 7.4, 80 mMol/L], pyruvate [1.6 mMol], sodium chloride [200 mMol/L]) with 1 volume of ReagentR2 (NADH [240 mMol/L]) after which the mixture was incubated at 37^{0}Cfor 1 minute and the change in absorbance was measured per minute during 3 minutes.

The LDH-P activity was calculated as shown below:

LDH-P activity (U/L) = (ΔOD/min) x 16030

ΔOD/min: change in Absorbance per minute

Estimation of creatinine kinase activity

The assay for the CK-MB activity was carried out according to the method described by Akinlusi *et al*. using Agappe Diagnostic Kit.[26] The plasma (0.04mL) was mixed with 1mL of working reagent in a test tube. The working reagent was prepared by mixing 4 volumes of Reagent R1 (Imidazole [pH 6. 7, 125mMol/L], D-Glucose [25mMol/L], N-Acetyl-L-Cysteine [25mMol/L], Magnesium acetate [12.5mMol/L], NADP [2.52mMol/L], EDTA [2.02mMol/L], Hexokinase >6800 U/L, Antihuman polyclonal CK-M antibody (sheep) sufficient to inhibit up to 2000 U/L of CK-MM) with 1 volume of Reagent R2 (Creatinine phosphate [250mmol/L], ADP [15.2 mmol/L], AMP [25mmol/L], Diadenosine pentaphosphate [103mmol/L], -6-PDH >8000 U/L) after which the mixture was incubated at 37^{0}c for 100seconds and the change in absorbance was measured per minute during 5 minutes.

The CK-MB activity was calculated as shown below:

CK-MB activity (U/L) = (ΔOD/min) x 8254

U/L: Enzyme unit

ΔOD/min: change in absorbance per minute

Molecular docking study

In this work, five selected molecular compounds (Figure 1) from active constituents of *H. umbellata* seed were optimized for further study and the descriptors obtained were observed and reported.[27] The optimized compounds and β_1-adrenergic receptor (PDB ID: 2ycw) were subjected to docking study.[28] β_1-adrenergic receptor was subjected to EduPyMOL-v1.7.4.4-Win32 in order to remove any material which were not amino acid and the active site in the treated receptor was located (center (X = 50.51, Y = -1.532, Z = 33.296) and size (X = 64, Y = 70, Z = 78) via auto dock tool 1.5.6 and then converted to .pdbqt format in preparation for docking calculation using auto dock vina 1.1.2. The name of the studied compounds were 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (A), (1S,3R,4R,5R)-3-(((E)-3-(3,4-dihydroxyphenyl)acryloyl)oxy)-1,4,5-trihydroxycyclohexanecarboxylic acid (B), 2,3,7,8-tetrahydroxychromeno[5,4,3-cde]chromene-5,10-dione (C), 3,4,5-trihydroxybenzoic acid (D) and 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (E).

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Statistical Analysis

Data are expressed as mean ± SEM. Differences between the mean values of the control and treated groups were determined by One-way Analysis of Variance with a Dunnett post hoc test using the Graph Pad Prism 5. Significant difference was considered if $p < 0.05$.

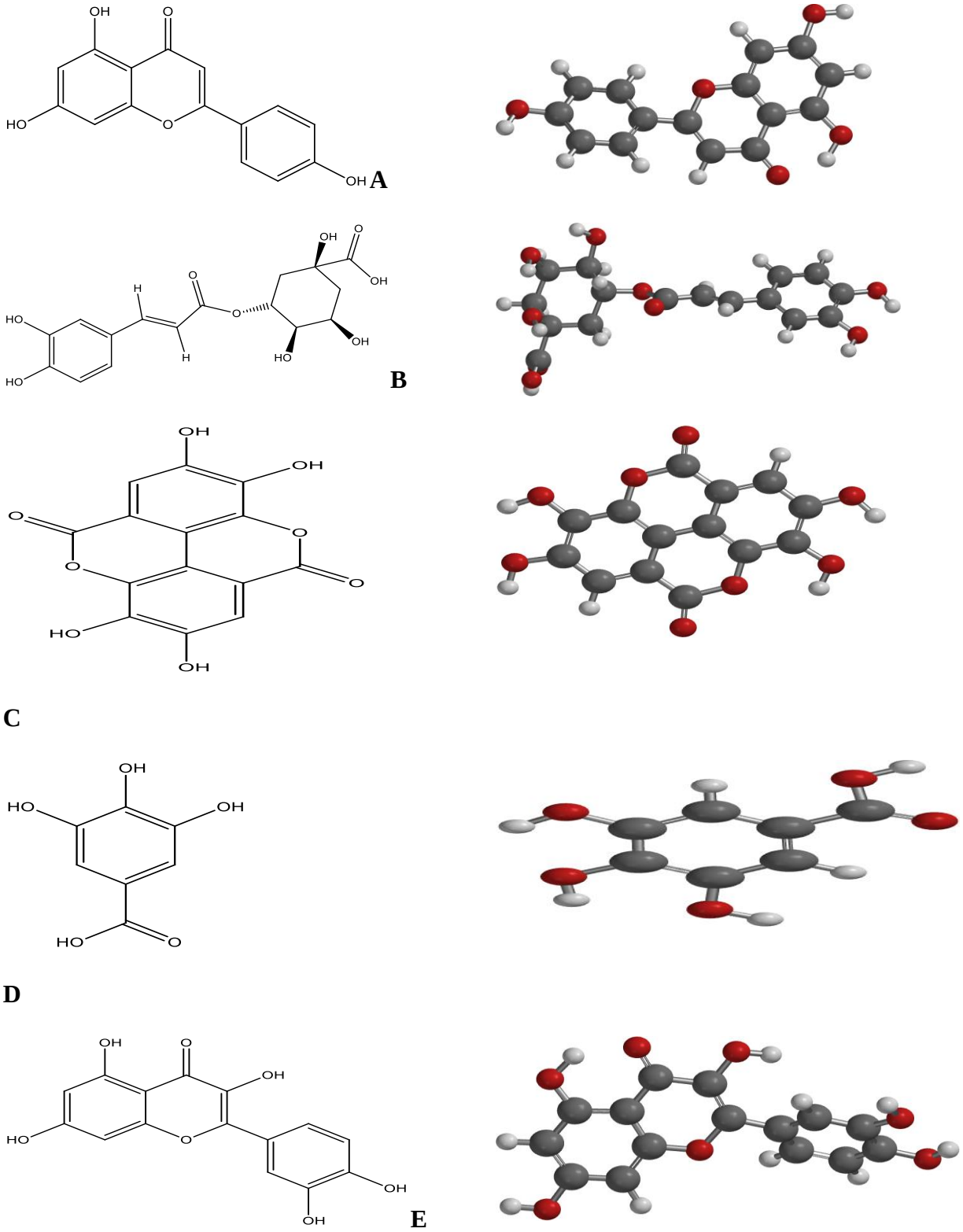

Figure 1: 2D and 3D structure of the selected compounds from *H. umbellata* seed

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Results and Discussion

Extractive value and phytochemical constituents of ethanol seed extract of H. umbellata

The extractive value was used to obtain the quantity in percentage of the extract from powdered seed of *H. umbellata.* The extraction procedure yielded 50.78 g from 1000g of starting material which equals 5.078%. The preliminary phytochemical screening of the ethanolic *H. umbellata* seed extract revealed the presence of alkaloid, saponins, phenols, tannins, glycosides, steroids, and terpenoids as shown in Table 1.

Total flavonoid and phenolic content of H. umbellata seed extract

Phenolic compounds as well as flavonoids are known to possess anti-oxidative properties and exert other important bioactive functions beneficial for human health in curing and preventing many diseases. [29] Intake of plants rich in polyphenols may offer cardio-protective effects through various mechanisms including attenuating cardiac oxidative stress and inflammatory signaling, and reduced mitochondrial dysfunction. [30] The total phenolic content and total flavonoid of ethanol *H. umbellata* seed extract is presented in Table 2. The results showed a total phenolic content of 231.00 ± 26.00 mg/g(TAE) and total flavonoid content of 435.00 ± 50.00 mg/g(RE).

FRAP Assay and DPPH free radical scavenging activity

The antioxidant potential of *H. umbellata* seed extract was assessed through ferric reducing antioxidant power and DPPH radical scavenging activities (expressed as % $DPPH^{\cdot}$ scavenged). This study revealed that *H. umbellata* exhibited FRAP activity of 248.00 ± 26.00 µg/g (AAE) and scavenged DPPH radical at various concentrations as shown in Table 3 and Figure 2 respectively. The anti-oxidative effect exerted may be due to the presence of polyphenols which have been proven to scavenge radicals, and possess the ability of reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in polyphenol rich samples.[31] This implied that *H. umbellata* seed extract can act as electron donors and may reduce the oxidized intermediates of lipid peroxidation processes.

Lipid profile assay

Lipid profile analysis employs chemical/enzymatic methods of measuring total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C). These lipid measures are used universally to guide treatment and management of cardiovascular diseases.[32] This study revealed that isoproterenol induced a statistically significant increase ($p < 0.05$) in the plasma total cholesterol, thereby elevating total cholesterol activity. In animal groups treated with ethanol seed extract of *H. umbellata*, a dose-dependent reduced activity of total cholesterol was observed. Isoproterenol also gave rise to an increase in the triglyceride level. This increased level was mitigated in rats pre-treated with ethanol seed extract of *H. umbellata*. The HDL-cholesterol level of normal control rats was significantly different ($p < 0.05$) when compared to other groups as shown in Table 4.

Cardiac biomarkers assay

The effects of *H. umbellata* seed extract on cardiac biomarkers as presented in Table 5, showed no significant difference in the activity of creatinine kinase – myocardial band (CK-MB) when compared to the treated and untreated groups. However, the group that received 100 mg/mL *H. umbellata* showed reduced CK-MB level and an elevated activity was observed in the group that received 200 mg/mL. A significant decrease was observed in the activity of lactate dehydrogenase (LDH) in the treated groups when compared with the control groups. There was a significant increase in the AST activity of normal control rats when compared with the negative control.

As observed in this study, intraperitoneal injection of ISO resulted in hypertriglyceridemia and hypercholesterolemia which is consistent with reports from previous studies.[33,34] The results of the lipid profile analysis showed anti-dyslipidemia effects of *H. umbellata* against ISO induced myocardial damage, by attenuating the ISO induced altered lipid profiles and ameliorating to near positive control values. The ability of *H. umbellata* to attenuate the observed hypertriglyceridemia and hypercholesterolemia might be due to the presence of dyslipidemia-inhibiting phytochemicals and/or the ability of these phytochemicals to interact synergistically with other bioactive compounds to exhibit dyslipidemia inhibitory activity. The anti-dyslipidemia activities observed in this study corroborates earlier studies of Morakinyo *et al.*[35] and Adeneye *et al.*[36]

Calculated descriptors

In this work, series of descriptors were obtained and five descriptors were selected to determine the drug-likeness of the studied compounds. The selected descriptors were molecular weight, Log P, hydrogen bond donor, hydrogen bond acceptor and number of rotatable bonds.

Table 1: Preliminary Qualitative Phytochemical Screening of *Hunteria umbellata* Seed Extract

Phytochemical Constituents	Result
Saponin	+
Phenols	+
Alkaloids	+
Steroids	+
Terpenoids	+
Glycosides	+
Tanins	+

**(+): present, (-): absent

Table 2: Total Phenol content and total flavonoid Content of Ethanol *H. umbellata* Seed Extract

Phytochemical Content	Concentration mg/g
Total phenolics	231.00 ± 26.00 mg/(TAE)
Total flavonoids	435.00 ± 50.00 mg/g(RE)

Each value is expressed as mean ± SEM, n=2. TAE; Tannic acid equivalent, RE; Rutin equivalent

Table 3: Ferric reducing antioxidant potential of the ethanol *H. umbellata* seed extract

Extract	Concentration (µg)
***H. umbellata* seed**	248.00 ± 26.00 (AAE/g)

****Each value is expressed as mean ± SEM, n=2. AAE; Ascorbic acid equivalent

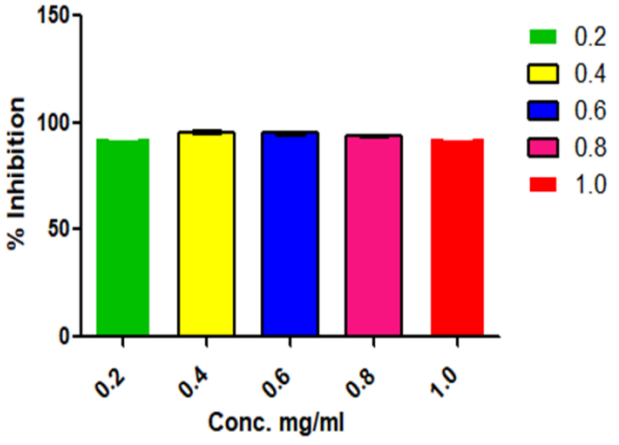

Figure 2: DPPH-Radical Scavenging Activity of the *H. umbellata* ethanolic seed extract
* Each value represented mean ± SEM of n = 3 readings

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Table 4: Effect of Ethanolic Seed Extract of *H. umbellata* on the Lipid Profile of Tested Rats

	TRIG. Conc. (mg/dL)	Total Cholesterol Conc. (mg/dL)	HDL-c (mg/dL)
Group 1 Normal Control	85.05 ± 3.911	78.700 ± 1.443	0.166 ± 0.001
Group 2 Negative Control	155.700 ± 2.813[a]	113.800 ± 9.135[a]	1.105 ± 0.009[a]
Group 3 (Positive control)	114.400 ± 2.918[ab]	100.800 ± 5.531	1.215 ± 0.003[ab]
Group 4 (*H. umbellata* 100 mg/kg bwt)	111.900 ± 2.627[ab]	110.300 ± 4.544[a]	0.869 ± 0.006[abc]
Group 5 (*H. umbellata* 200 mg/kg bwt)	152.400 ± 5.131[ac]	98.35 ± 4.691	0.091 ± 0.049[ac]

**Each value is represented as Mean ± SEM, n = 6 readings. Value of $p < 0.05$ was considered significant. The values across column with superscript (a) implied significant difference from normal control, (b) implied significant difference from negative control, and (c) implied significant difference from positive control groups.

Table 5: Effects of *H. umbellata* Seed Extract on Cardiac Biomarkers

	CK-MB	LDH	AST
Group 1 Normal Control	4.337 ± 0.613	16.40 ± 7.521	131.20 ± 0.924
Group 2 Negative Control	4.083 ± 0.783	16. 64 ± 1.459	183.8 ± 8.737[c]
Group 3 (Positive control)	4.400 ± 0.550	15.93 ± 2.009	83.77 ± 16.28[b]
Group 4 (*H. umbellata* 100 mg/kg bwt)	2.200 ± 0.550	4.207 ± 0.357[abc]	137.7 ± 10.020
Group 5 (*H. umbellata* 200 mg/kg bwt)	4.953 ± 0.407	4.867 ± 0.053[abc]	194.5 ± 36.84[c]

**CK-MB: Creatinine kinase – myocardial band; (LDH): lactate dehydrogenase; (AST): Aspartate amino transaminase. Each value represented Mean ± SEM (n=5). Each mean value in a row was compared with the Cardiac control with superscript 'a' and different alphabet in superscript indicates a significant difference ($p < 0.05$) between the cardiac control and a considered value.

Table 6: Obtained descriptors from optimized compounds from *H. Umbellate*

	Molecular Weight (≤500)	Log P (≤5)	HBD (≤5)	HBA (≤10)	Number of Rotatable Bonds (≤10)
1	270.24	2.58	3.00	5.00	1.00
2	354.31	-0.65	6.00	8.00	4.00
3	302.19	1.31	4.00	8.00	0.00
4	170.12	0.50	4.00	4.00	1.00
5	302.24	1.99	5.00	7.00	1.00

Table 7: Calculated binding affinity and residues involved in the interaction

	Binding Affinity (kcal/mol)	Residues involved in the interactions	Types of Non-bonding interaction involved
A	-9.5	Asn829, Phe201, Phe306, Val125, Thr126, Phe307, Val122	conventional Hydrogen Bond, unfavourable hydrogen bond, Pi-Sigma, Pi-Pi T shaped, Pi-Alkyl
B	-8.3	Val202, Thr203, Cys199, Asn329, Asp200	conventional Hydrogen Bond, unfavourable hydrogen bond, Pi-Alkyl
C	10.0	Phe201, Phe306, Asp121, Asn329, Val122, Phe307	conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Pi T-Shaped, Pi-Sigma, Pi-Alkyl
D	-6.2	Asn310, Ser211, Val122, Phe307	Conventional Hydrogen Bond, Pi-Pi T-Shaped, Pi-Alkyl
E	-9.4	Ser215, Ser212, Ser211, Phe307, Asp121, Phe201, Val122	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Pi-Anion, Pi-Sigma, Pi-Pi Stacked, Pi-Pi T-Shaped, Pi-Alkyl
Propranolol	-8.6	-	-

It was observed from Table 6 that the selected compounds obeyed the Lipinski rule of five [37] and this revealed that the studied compounds have the ability to act as drug which therefore showed the potency of *H. umbellata* seed as anti-cardiovascular diseases.

Molecular docking analysis

The selected studied compounds were subjected to docking study so as to observe the biochemical interactions, binding affinity and residues involve in the interactions between the selected compounds from cardiovascular diseases and β_1-adrenergic receptor (PDB ID: 2ycw). The calculated binding affinity for compounds **1** to **5** were -9.5, -8.3, -10.0, -6.2 and -9.4 kcal/mol respectively (Table 7).

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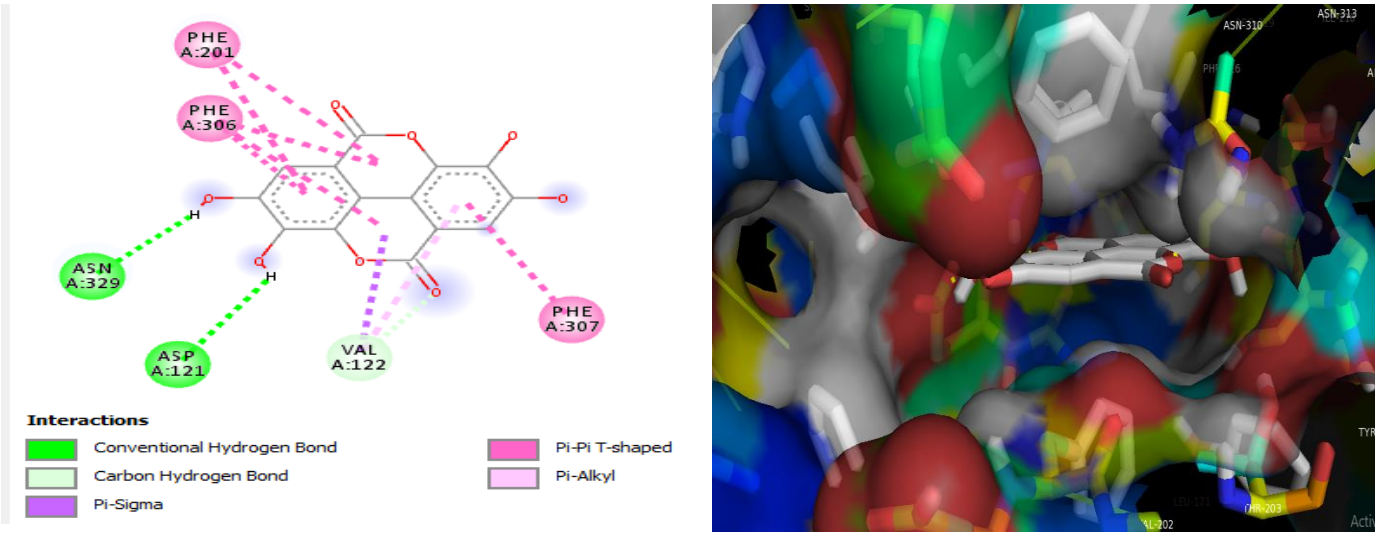

Figure 3: 2D and 3D structure of interactions between compound **C** and β_1-adrenergic receptor (PDB ID: 2ycw)

According to report by Akintelu *et al*.[38] drug-like compounds with lower binding affinity possess the ability to inhibit well [38]; thus, it was observed that 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**A**) with -9.5 kcal/mol, 2,3,7,8-tetrahydroxychromeno[5,4,3-cde]chromene-5,10-dione (**C**) with -10.0 kcal/mol and 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (**E**) with -9.4 kcal/mol as one of the vital phenolic compounds present in *H. umbellata* seed have the ability to inhibit well than Propranolol (reference drug) thereby revealed the efficacy of the *H. umbellata* seed as anti-cardiovascular diseases (Figure 3).

Conclusion

In this work, *H. umbellata* showed ameliorative effects against ISO-induced myocardial damage, suggesting preliminary anti-dyslipidemia abilities possibly due to high phenolic and flavonoid contents. Validation of it effects as anti-dyslipidemia via *in silico* approach were confirmed via efficient activity of compound (A), (C) and (E) against β_1-adrenergic receptor than the referenced drug.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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