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Antioxidant, Anti-hyperlipidemic and Cardioprotective Potentials of Diet Supplemented with *Parkia biglobosa* Roasted Seeds on Isoproterenol-Induced Cardiotoxicity in Wistar rats

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ABSTRACT

Metabolic diseases including overweight and cardiovascular diseases have increased to epidemic proportions with cardiovascular diseases as the most common non-communicable diseases globally. In this work, the major aim is to investigate the biological potentials of roasted *P. biglobosa* seed in the prevention of cardiovascular diseases. Dry *P. biglobosa* seeds were pulverized and 100 g were extracted in 85% ethanol for 72 h and filtered. Antioxidant potential was evaluated via DPPH scavenging and ferric reducing antioxidant power (FRAP). Thereafter, 24 male Wistar rats (100-150 g) were randomised into four groups and treated as follow: Group 1 (normal saline only), Group 2 (normal saline + ISO 85 mg/kg), Group 3 (1.8 mg/kg propanolol pretreatment + ISO 85 mg/kg); and Group 4 (fed with supplemented *P. biglobosa* roated seeds (20%) + ISO 85 mg/kg). Twenty-four hours after induction the rats were anaesthesised using diethylether and sacrificed. Blood samples were collected into EDTA bottles by cardiac puncture. Also, based on the earlier reports of the GC-MS profiles, five constituents were selected, optimized and docked against angiotensin converting enzyme (PDB ID: 1O8A) using *insilico* method. Results showed presence of saponins, phenols and there was a significant decrease in triglycerides and cholesterol in the extract-treated rats when compared with the cardiotoxicity control group. Meanwhile there was no significant difference in the concentration of HDL-c across the group. The study concluded that diet supplemented with *P. biglobosa* roasted seeds possessed cardioprotective activity which was mediated via lipid lowering and antioxidant mechanisms in isoproterenol challenged rats.

***Keywords*:** Antioxidants, Hyperlipidemic, Cardio-protective, Cardiotoxicity, *Parkia biglobosa*

Introduction

Cardiovascular Diseases (CVDs) are the number one cause of death globally.[1] CVDs kill more people annually than any other disease. In 2016, about 17.9 million people died as a result of CVDs (in which 85% are due to heart attack and stroke). This figure represents 31% of all global deaths.[1] Over three quarters of CVD deaths take place in low- and middle-income countries. Out of the 17 million death due to non-communicable diseases in 2015, 16.9 million were under the age of 70, and 82% happened in low- and middle-income countries, and 37% are caused by CVDs.[1]

CVD is a general term for conditions affecting the heart or blood vessels and include Coronary Heart Disease (CHD) and Coronary Artery Disease (CAD), Peripheral Arterial Disease and Acute Coronary Syndrome (ACS) among several other conditions.[2]

The health professionals frequently use the terms Cardiovascular Diseases (CAD) or Acute Coronary Syndrome (ACS) or Coronary Heart Disease (CHD) interchangeably, they are generally referred to diseases associated with heart dysfunctions. It is usually associated with a build-up of fatty deposits inside the arteries and an increased risk of blood clots. It can also be associated with pathologic process affecting the coronary arteries such as atherosclerosis.[3] CHD is a major cause of death and disability in developed countries.[4] Among patients suffering from acute myocardial infarction, 70% of fatal events are due to occlusion from atherosclerotic plaques. As atherosclerosis is the predominant cause of acute myocardial infarction, risk-factors for atherosclerotic disease are often mitigated in the prevention of disease. Modifiable risk factors which include cigarette smoking, exercise, hypertension, obesity, cholesterol, LDL, and triglyceride levels account for 90% (men) and 94% (female) of myocardial infarctions. In contrast, age, sex, and family history are non-modifiable risk factors for atherosclerosis.[5,6] Although the mortality for this condition has gradually declined over the last decades in western countries, it still causes about one-third of all deaths in people older than 35 years.[7,8,9] Coronary artery disease is very common in both developed and developing world. In one study, it was estimated that CAD represented 2.2% of the overall global burden of disease and 32.7% of cardiovascular diseases.[10] It costs over 200 billion dollars annually to the health care system in the United States.

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It is estimated that 7.6% of men and 5.0% of women in the US lived with coronary artery disease from 2009 to 2012 based on the national health survey done by the American Heart Association (AHA). This amount to 15.5 million Americans afflicted with the disease during this time.[11]According to the World Health Organization (WHO), more than 80% of the world population uses traditional medicine to cope with health problems.[2] It is a well-established fact that fruits, herbs and spices-rich diets are associated with low risks of many ailments. Therefore, research is currently directed towards discovering useful bioactive compounds in medicinal plants that could be used as new strategies for treatment and management of cardiovascular diseases.[12,13] Recently, attention has been focused on phytochemicals and polyphenols such as the flavonoids, alkaloids, and xanthones derived from different plant species as potential therapeutic agents in the prevention and management of cardiovascular diseases due to their antioxidant nature and the implication of oxidative stress in the etiology of cardiovascular diseases. This suggests that antioxidant therapy represents a promising avenue for treatment.[14]

Parkia biglobosa, also called the African Locust Bean is a multipurpose tree indigenous to the tropical regions of West Africa belonging to the family Mimosaceae.[15,16] The analysis of the phytochemicals present in *P. biglobosa* revealed the presence of alkaloid, flavonoids, tannins, saponins, cardiac glycosides, sterols, resins and terpenes.[17-19] For a long time, different parts of *P. biglobosa* have been in use among native populations of West Africa to meet their nutritional and basic health care needs.[20] *Parkia biglobosa* bark extracts have been identified to have significant anti-inflammatory, analgesic, antibacterial and anti-helminthic properties. Recently, the cardioprotective potential, antioxidant, anti-carcinogenic and anti-trypanosomic activities of the bark and root of the plant have been reported.[21,22,23] Therefore, the aim of this study focused on investigating the biological potentials of roasted *P. biglobosa* seed in the prevention of cardiovascular diseases.

Materials and Methods

Materials

The seeds of *P. biglobosa* were purchased from Oja Jagun Ogbomoso, Oyo state, Nigeria in January, 2021. The seeds were identified by Dr. (Mrs) J.M Adelowo in the Department of Biology, Adeleke University, Ede with voucher number: AUE22-01 and were further sun dried and pulverised. Exactly 100 g of powdered seeds were extracted in 3 L of 85% ethanol by maceration method for 72 h and filtered. The filtrate was concentrated *in vacuo* on a rotary evaporator at 40 ºC to produce the crude extract.

Qualitative Phytochemical screening of P. biglobosa seed extract

The extract was screened for presence of saponins, phenols, alkaloids, steroids, terpenoids, glycosides, tannins and flavonoids using standard methods [24-25].

Quantitative Phytochemical screening

The total phenolics content in the extract was determined spectrophotometrically as described by Singleton *et al* [26] using tannic acid (10 µg/ml) standard. Different volumes of tannic acid solution were pipetted into clean test tubes. Then, 1.5 ml of Folin-Ciocalteu's phenol reagent (1:10 v/v) was added. This was followed by the additon of 7.5% sodium carbonate (1.5 ml) which was incubated at room temperature for 90 min.

Antioxidant Assays

FRAP Assay

The capacity of *P. biglobosa* to reduce Fe^{3+} to Fe^{2+} was carried out according to the modified method of Benzie and Strain[27]. Sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ 40 mM HCl solution and 20 mM $FeCl_3.6H_2O$ solution were used in the ratio of 10:1:1 to prepare the FRAP reagent and stored in dark cupboard. The reaction mixture contained 0.3 ml *P. biglobosa* seed extract (1 mg/ml) or standard solution in test tubes containing 2.7 ml of FRAP reagent respectively and were incubated for 5 min at 37 ^oC. The absorbance was measured at 539 nm against blank.

DPPH Assay

The DPPH radical scavenging activity of *P. biglobosa* seed extract was assayed according to the procedure of Blois[28] as reported by Padmanabhan and Jungle[29]. The stock solution of 0.3 mM DPPH contained 0.024 g of DPPH dissolved in 200 ml methanol. The reacting solution contained 0.3 ml *P. biglobosa* seed extract in test tubes containing 1.7 ml of DPPH reagent in triplicates and incubated in a dark chamber for 10 min. The absorbance was measured at 517 nm against the reagent blank.

Experimental Animal

Healthy male Wistar rats (100-150 g) were purchased from animal breeding house in Ede, Osun State and transported to the Animal Facility Unit at the Department of Biochemistry, Adeleke University Ede. The animals were allowed to acclimatise for 2 weeks with access to food and water *ad libitum*.

Ethical Clearance

The ethical clearance was obtained from the Ethical Committee of the Adeleke University Ede, Osun State with reference number: AUERC/FOS/IND/02.

Preparation of P. biglobosa Seed-Supplemented Diet

The seeds were roasted and pulverised. Thereafter, 20 g of the powdered seed was mixed with the commercial feed (80 g) to form the diet. The animals were fed with this supplemented diet.

Induction of Cardiotoxicity

Cardiotoxicity was induced in rats using isoproterenol as described by Akinlusi *et al*.[30] Twenty four male Wistar rats (100-150 g) were randomised into four groups and treated as follow: Group 1 (normal saline only), Group 2 (normal saline + ISO 85 mg/kg), Group 3 (1.8 mg/kg propanolol pretreatment + ISO 85 mg/kg); and Group 4 (fed with supplemented *P. biglobosa* roated seeds – 20g/80g diet + ISO 85 mg/kg). The animals were allowed to acclimatise for 2 weeks with access to food and water *ad libitum* and twenty four hours after induction the rats were anaesthesised using diethylether and sacrificed. Blood samples were collected into EDTA bottles by cardiac puncture. in The blood samples were centrifuged on Bench centrifuge model 800D (Pathway Medicals England, U.K) at 3000 rpm for 10 min. The plasma (supernatant) was collected and used for biochemical analyses.

Lipid Profile Assays

The concentrations of total cholesterol, triglycerides and HDL were carried out using Randox diagnostic kits.[31]

Determination of Cholesterol concentration

The estimation of total cholesterol in the plasma was carried out according to the method Richmond as described by Akinlusi et al.[30] The plasma and standard samples (0.01 ml) were pipetted in duplicate followed by the addition of reagent 1 solution (1 ml). The reagent blank contained distilled water (0.01 ml) and 1 ml reagent solution containing 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^oC for 10 min and the absorbance was read at 546 nm within 1 hr against the reagent blank.

Determination of triglycerides concentration

The concentration of triglycerides was determined according to the method of Kostner *et al*.[32] The plasma and standard samples (0.01 ml) were pipetted and 1 ml of reagent solution was added. The constituents of Reagent 1 were 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^oC for 10 min and absorbance read at 546 nm within 1 hr against the reagent blank containing distilled water to sample.

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Determination of concentration of HDL-c

The concentration of HDL-c was determined according to the method of Tietz[33]. 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 centrifuged for 10 min at 4000 rpm. The supernatant was collected and used for estimation of HDL-c. 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 hr.

Aspartate Aminotransferase (AST)

The assay for aspartate aminotransferase (AST) was carried out according to the method of Reitman and Frankel[34] and Schmidt and Schmidt[35] using Randox diagnostic kit . Basically aspartate aminotransferase activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine. Sample (0.1 ml) was mixed with 0.5 ml of reagent containing phosphate buffer {100 mM, pH 7.4}, L-aspartate {100mM} and α-oxoglutarate {2 mM}. The reaction mixture was incubated for 30 min at 37^{o}C. Then 0.5 ml of reagent 2 contaning 2, 4-dinitrophenylhydrazine {2 mM}) was added and incubated at room temperature for 20 min. Thereafter 5.0 ml sodium hydroxide (0.4 M) were added to terminate the reaction process. The absorbance of the sample was read at 546 nm.

Lactate Dehydrogenase (LDH) Assay

The assay for the LDH activity was carried out according to the method described by Akinlusi *et al.*[30] using Agappe diagnostic kit. The plasma (0.01 ml) was mixed with 1.0 ml working reagent [containing 4 ml of Tris buffer [pH 7.4, 80mM], pyruvate [1.6 mM] and sodium chloride [200 mM]] and 1 ml of reagent containing NADH [240 mM]. The mixture was incubated at 37^{o}C for 1 min. The absorbance was read at 340 nm per minute within 3 minutes interval.

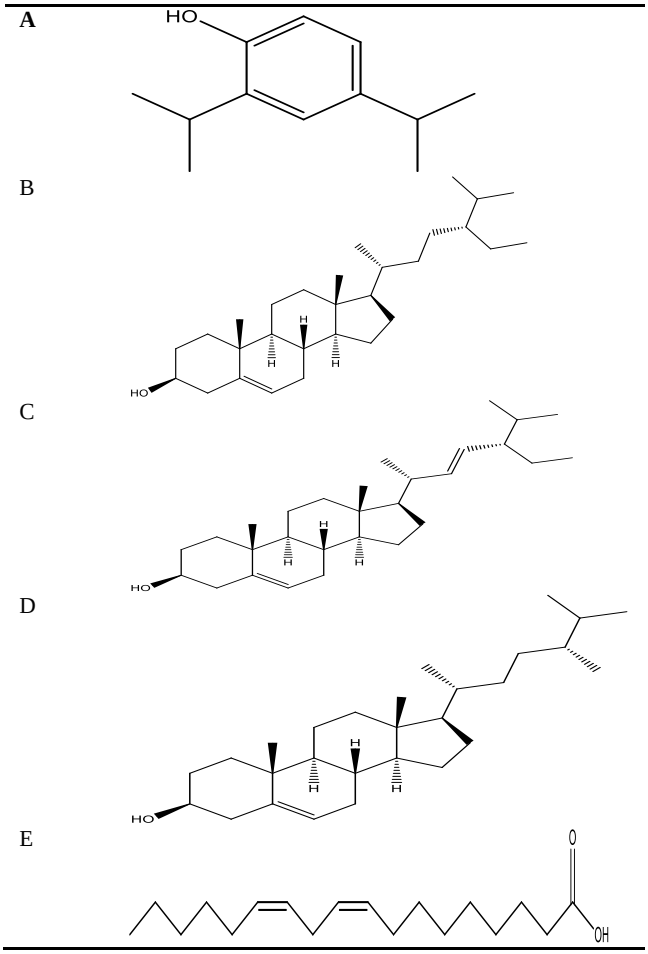
Creatinine kinase-myocardial band (CK-MB) Assay

The assay for the CK-MB activity was carried out according to the method of DGKC[36] as modified by Witt and Trendelendurg[37] using Agappe diagnostic kit. The measurement of CK-MB was carried out to evaluate the ability of antibody in sample to inhibit the activity of CK-MB when mixed with reagent containing Imidazole [pH 6. 7, 125 mM], D-Glucose [25 mM], N- Acetyle-L-Cysteine [25 mM], Magnesium acetate [12.5 mM], NADP [2.52 mM], EDTA [2.02 mM], Hexokinase >6800 U/L and 1 ml of another reagent containing creatinine phosphate [250 mM], ADP [15.2 mM], AMP [25 mM], diadenosine pentaphosphate [103 mM], -6-PDH >8000 U/L. This was incubated at 37 ^{o}c for 2 min and absorbance was measured at 340 nm per min within 5 min interval.

Computational Details

Five molecular compounds selected from *Parkia biglobosa* seeds were optimized using density functional theory method via Spartan 14[38]. The optimized compounds were 2,4-di(propan-2-yl)phenol (**A**), (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol(**B**), (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(*E*,2*R*,5*S*)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol (**C**), (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol (**D**), (9*Z*,12*Z*)-octadeca-9,12-dienoic acid (**E**)[39] (Table 1). The optimized compounds were docked against angiotensin converting enzyme (PDB ID: 1O8A)[40] using molecular docking method. The software involved in the studied docking method were EduPyMOL-v1.7.4.4-Win32, Autodock tool 1.5.6, autodock vina 1.1.2 and discovery studio.

Table 1: 2D structure of the selected compounds from *Parkia biglobosa* seeds.



The downloaded receptor was subjected to Pymol software in order to remove water molecules and ligand that were downloaded with the receptor from protein data bank. The binding site in the clean receptor was located using Autodock Tool software and it was saved in .pdbqt so as to be accepted by autodock vina which will perform the docking calculation. The observed grid box for this docking study was as follows: center (X = 40.66, Y = 37.366, Z = 43.463) and size (X = 74, Y = 70, Z = 78) as well as the spacing was set to be 1.00Å.

Statistical Analysis

Data were expressed as Mean ± SEM. Differences between the mean values of the control and treated groups were determined by One-way analysis of variance with Dunnett post-hoc test using Graph pad prism 5. Significant difference was considered if $p < 0.05$.

Results and Discussion

The phytochemical constituents of *P. biglobosa* seed extract showed the presence of phenols, saponins, steroids, terpenoids, tannins and flavonoids (Table 2). The total phenols and flavonoids present in the seed extract were 328.5 ± 2.50 TAE/g and 175.4 ± 1.50 QE/g respectively (Table 3). The FRAP value (0.205 ± 0.004 mg/ml) and percentage inhibition of DPPH-radical were shown in Table 4 and Figure 1 respectively.

Anti-hyperlipidemic Effect

The result of the anti-hyperlipidemic effect of *P. biglobosa* seed on diet-fed rats was presented in Table 5. The negative control group showed a significantly higher level of triglyceride (TRIG) (155.7 ± 2.813 mg/dl) and total cholesterol (CHOL) (113.8 ± 1.135 mg/dl) than the normal control (85.05 ± 3.911 mg/dl and 78.70 ± 1.443 mg/dl).

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Table 2: Phytochemical Constituents of *P. biglobosa* Seed Extract

Phytochemical	Result
Saponins	+
Phenols	+
Alkaloids	-
Steroids	+
Terpenoids	+
Glycosides	-
Tannins	+
Flavonoids	+

(+): present (-): absent

Table 3: Antioxidant Properties of *P.biglobossa* Seeds

Antioxidants	
Flavonoid (QE/g)	175.5 ± 1.50
Total Phenolics (TAE/g)	328.5 ± 2.50

Each value represented Mean ± SEM (n=3).

Table 4: Ferric reducing antioxidant power of ethanol extract of *P. biglobosa* seed extract.

Concentation (mg/mL)	0.205 ± 0.004

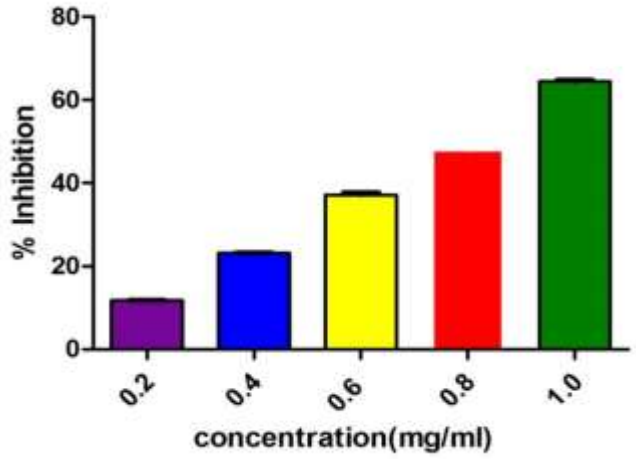

Figure 1: DPPH Scavenging activity of *P. biglobosa* seed

Table 5: Effects of Diet Supplemented with *P. biglobosa* (PB) Seed on Lipid Biomarkers

Group	TRIG (mg/dl)	CHOL (mg/dl)	HDL (mg/dl)
Control	85.05 ± 3.91	78.70±1.44	0.16 ± 0.0
Cardiac control	155.7±2.81[a]	113.8±1.13[a]	1.22±0.0[a]
ISO + Propanalol	114.40±2.91[ab]	100.80±5.5	1.11±0.01[ab]
ISO + PB	129.9±8.16[ab]	84.86 ± 2.53[c]	0.20±0.02[ab]

Each value represented Mean ± SEM (n=6). Value of $p < 0.05$ was considered significant. The values across column with superscript (a) implied significant difference from control while (b) implied significant difference from cardiac control.

However, treatment with *P. biglobosa* reduced the concentrations of TRIG (129.9 ± 8.16 mg/dl) and CHOL (84.86 ± 2.53) respectively. Conversely, there was a significant reduction in the levels of HDL-c of the normal control (0.166 ± 0.001 mg/dl) when compared with the negative control group (1.215 ± 0.003 mg/dl). Pre-treatment with *P. biglobosa* had no significant effect on the HDL-c level when compared with the negative control group.

Effect on Cardiac Biomarkers

The effects of the roasted seeds-supplemented diet on cardiac biomarkers were presented in Table 6. There was no significant difference in the activity of creatinine kinase-myocardial band (CK-MB) in both the treated and untreated groups. There was a non-significant decrease in the activity of lactate dehydrogenase (LDH) of the *P. biglobosa* fed group (14.81 ± 0.053 U/ml) when compared with the negative control group (16.40 ± 7.521 U/ml). The negative control group showed a significant increase in AST level (183.8 ±8.737 U/ml) when compared with the normal control group (131.20 ± 0.924 U/ml); suggesting a cardiac injury. However, treatment with *P. biglobosa* caused a significant decrease in AST level to 108.30 ±9.726 U/ml. The effect might be due to the presence of the phytoconstituents in the seed extract of *P. biglobosa*.

Molecular Docking

The optimized compounds (Figure 2) from *Parkia biglobosa* seeds were docked against angiotensin converting enzyme (ACE) (PDB ID: 1O8A) and the calculated binding affinity were reported in Table 7. Usually, the lower the binding energy value, the higher the bioactivity of the compound. In this study however, compound **C**: (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(*E*,2*R*,5*S*)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol**;** compound **D**: (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol and compound B which showed lower binding energies of 9.5, 9.5 and 9.0 kcal/mol are potent inhibitors angiotensin converting enzyme (ACE) than the reference drug (propranolol -6.8 kcal/mol). The presence of these compounds **B**, **C** and **D** in *P. biglobosa* seeds could be responsible for the observed cardio-protective effect.

Table 6: Effect of Diet Supplemented with *P. biglobosa* (PB) Roasted Seeds on Cardiac Biomarkers

Group	CK-MB (U/L)	LDH (U/L)	AST (U/L)
Control	4.33 ± 0.61	16.40± 7.52	131.20 ± 0.92
Cardiac control	4.08 ± 0.78	16.64±1.45	183.8 ± 8.73[a]
ISO+ Propanalol	4.40 ± 0.55	15.93± 2.00	83.77 ± 16.28[ab]
ISO + PB	5.50 ± 0.55	14.81± 0.05	108.30 ±9.72[ab]

Each value represented Mean ± SEM (n = 6). Value of $p < 0.05$ was considered significant. The values across column with superscript (a) implied significant difference from control while (b) implied significant difference from cardiac control.

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

	Binding Affinity (kcal/mol)
A	-6.5
B	-9.0
C	-9.5
D	-9.5
E	-5.8
Propranolol	-6.8

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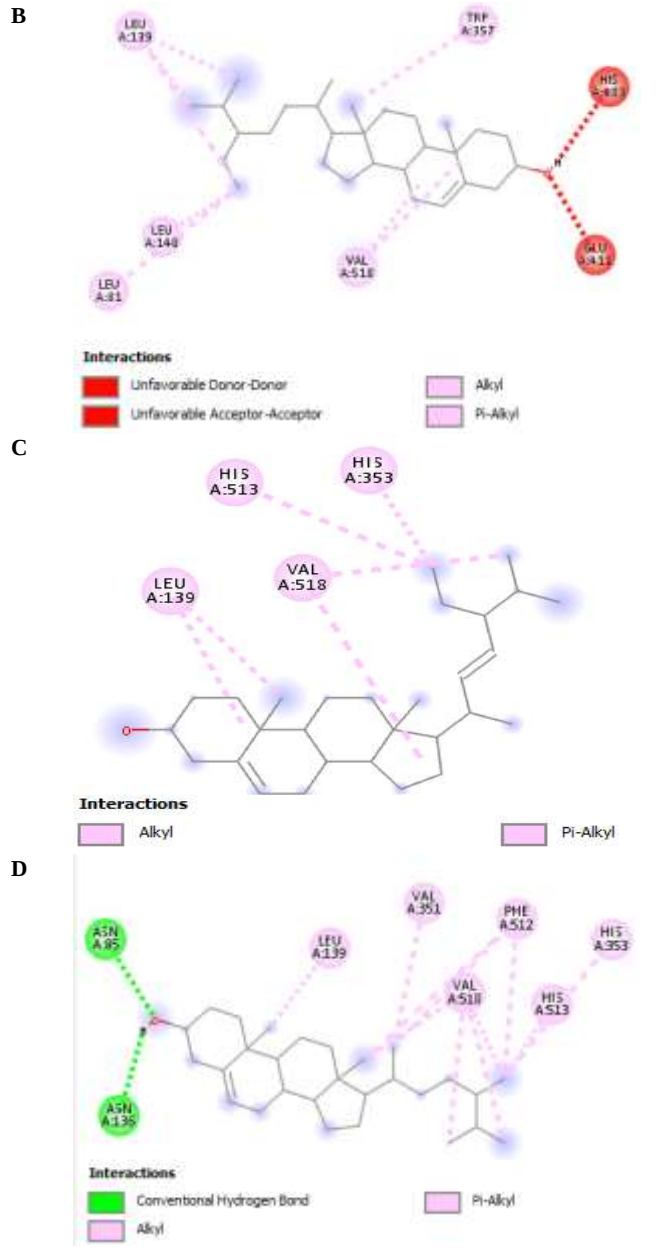

Figure 2: 2D structure of interactions between compound **B**, **C** and **D** and angiotensin converting enzyme (PDB ID: 1O8A)

Conclusion

In conclusion, the study showed that *P. biglobosa* roasted seeds possessed cardioprotective activity which was mediated via lipid lowering and antioxidant mechanisms. This activity might have been mediated through the inhibition of angiotensin converting enzyme by the seed extract constituents.

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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