ANTI-HYPERGLYCEMIC AND ANTIOXIDANT POTENTIALS OF SOME BIOACTIVE COMPOUNDS IDENTIFIED FROM THE METHANOLIC KERNEL EXTRACT OF *Anacadium ocidentale in vitro* and *in silico* INVESTIGATIONS

S. A. Ahmed[1*], A. T. Ajiboye[1], Y. A. Ibrahim[1,2], A. T. Olagunju[1,3], S. Salau[1] & O. Adetunji[1,4]

[1]Department of Chemistry and Industrial Chemistry, Kwara State University, Malete, Nigeria
[2]School of Chemical Sciences, University of Chinese Academy of Sciences, Beijing 100049, P.R. China
[3] State Key Laboratory of Bio-Organic Chemistry, Chinese Academy of Sciences, 345 Ling Ling Road, Shanghai 200032, P.R. China.
[4]Missouri University of Science and Technology, 400 W. St., Rolla, MO 65409-0010 Schrenk Hall, United State of America.
*Corresponding author: akinyeye.ahmed@kwasu.edu.ng

ABSTRACT

The phytochemical components of roasted kernel of cashew nut (Anacadium ocidentale), its antioxidant and anti-hyperglycemic potentials were investigated via in vitro and in silico. The roasted kernel of cashew nut was extracted in 95 % methanol. Using boron trifloride (10 % in 1-butanol), the extract was derivatized and the derivatized sample was analyzed using a Gas Chromatographic–Mass Spectrometry (GC-MS) method to establish its chemical constituents. Antioxidant potential was evaluated through 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2'-azino-bis-(3-ethyl benzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) radical scavenging assays. The anti-hyperglycemic potential of the crude methanol extract was investigated through α-glucosidase assay. The results of phytochemical screening revealed the presence of phytochemicals such as phenols, glycosides, alkaloids, steroids, carbohydrate, flavonoids and terpenoids. Total numbers of 20 phytochemicals belonging to different classes of natural products were identified by GC-MS analysis. Carbonic acid, dodecyl vinyl ester (45.9 %) was found to be the major compound. Other compounds like N- serylserine, D- fructose, 3-0-methyl, β- Amyrone, β- Amyrin, Lupeol and 9- octadecenoic acid were also identified. The methanol extract of roasted cashew kernel showed highest DPPH scavenging activity (IC_{50} 10 ± 0.001 µg/mL) which is statistically similar to positive controls, vitamin C and rutin (IC_{50} = 4 ± 0.001 and 1 ± 0.0001 µg/mL) and also exhibited moderate ABTS scavenging activity (IC_{50} = 14 ± 0.001 µg/mL) which is statistically significant as compared to positive controls, vitamin C (IC_{50} = 13 ± 0.01 µg/mL) and rutin (IC_{50} = 16 ± 0.001 µg/mL) respectively. The result of enzyme inhibition assay showed that the extract possesses highest anti-hyperglycemic potential against α-glucosidase with IC_{50} value (0.00024 ± 0.00002 µg/mL) compared to standard drug acarbose (IC_{50} = 0.417 ±0.021 µg/mL). The in silico techniques comprising the molecular docking, bioactivity and toxicity studies provides insight into the inhibitory properties, structure-activity-relationship predictions and drug-likeliness activities of the identified compounds. The research reveals the bioactive components present in the roasted kernel of cashew nut that can be utilized for its antioxidant and anti-hyperglycemic properties. Hence consumption of roasted cashew kernel could be beneficial for the treatment of diabetes mellitus and prevention of oxidative stress-related degenerative disorders.

Key words: Phytochemicals, cashew kernel, antioxidant, anti-hyperglycemic, GC-MS and In silico

1. INTRODUCTION

Anacadium ocidentale commonly known as cashew tree is an important tropical and perennial crop belonging to Anacardiaceae family[1, 2]. It is a multipurpose tree with great economic importance, grown in Benin Republic, Brazil, Cote d'Ivore, Guinea Bissau, Ghana, India, Mozambique, Nigeria, Philippines, Sri Lanka, Tanzania and Vietnam[3]. The nut/kernel of cashew is considered as the most valuable product due to its phytochemical, phytonutrient contents and health benefits. Thus the roasted kernel of cashew is widely consumed as a snack-food, desert nut or as an ingredient for food, confectioneries and bakery products[4, 5]. Various parts of cashew tree are ethno-medicinally useful for the treatment of malarial, microbial infections, hypertension and obesity, coronary heart disease and diabetes[6, 7, 8, 9].

Phytochemical investigation of different parts of *A. occidentale* had resulted in identification of various phytochemicals. Thus, the presence of bioactive compounds such as saturated and unsaturated fatty acids, tocopherols, squalenes, phytosterols, β-carotene, lutein, zeaxanthin, α-tocopherol, γ-tocopherol, thiamin, stearic acid, oleic acid, and linoleic acid have been identified in cashew nuts[10, 11]. In addition, it is a rich source of substantial amounts of essential amino acids, vitamins and minerals which are seldom found in daily diets[12, 4]. Currently, studies on the antioxidant and anti-hyperglycemic potentials of roasted cashew kernel are scarce in scientific literature, although studies on nutrient contents of raw or roasted cashew nut have been reported[13, 14]. Therefore, the aim of the present study is to determine the phytochemical constituents of roasted cashew kernel, study its antioxidant and anti-hyperglycemic potentials using in vitro and in silico methods.

2. MATERIALS AND METHODS

2.1. Plant Collection and Extraction: The seed of *Anarcandium occidentale* was purchased from Baboko market, Oja-Tuntun in Ilorin, Kwara State, Nigeria. The cashew nuts were roasted at 125 ^{o}C for 30 minutes in an open pan. The nuts were mechanically removed from the shell and the edible kernels were easily obtained without being damaging. The edible kernels were pulverized and stored for further uses. A standard extraction procedure described by [4,5] was adopted in this study. The pulverized kernels (100 g) were macerated in 1 litre of 95 % methanol for 72 hours at room temperature with constant shaking. The supernatant obtained was filtered using Whatman filter paper (0.45 μm). This process was repeated three times under the same conditions. The extract was pooled and concentrated over a reduced pressure using rotary evaporator at 40^{0}C. The dry weight yield was calculated to be 7.8 %. The extract was then refrigerated at 4°C in dark sample bottle for subsequent experiments.

2.2 GC-MS Analysis of the Extract: The derivatized extract was diluted in methanol (1:10 *v/v*) and was analyzed using the HP5MS (GC-9890A system and Mass spectrometry -5975C). The capillary column (0.25mm x 0.320mm) was used for the separation of the components. Helium, at a flow rate of 1.6 mL/min (constant flow mode), was used as carrier gas. Initial oven

temperature was 80 ^{0}C which hold for 2min at 10 ^{0}C per min to 240 ^{0}C and wait for 6min. A volume of 2 µL of sample extract was injected in split less mode and the injection temperature was set at 250 ^{0}C. The mass spectrometer was operated in electron ionization (EI) mode within the mass range of 40–400 amu while scan speed was 769 amu/s start time and end time were 3.0 min and 27 min. respectively[15].

2.3. Identification of Phytochemical Constituents: The phytochemical constituents were identified by comparing the fragmentation patterns of the spectra obtained for all constituents with the data stored in the instrument database using the NIST. The components were identified upon comparison with the structures available in the computer library[15].

2.4 Antioxidant Assay: The anti-oxidant activity of the extract was investigated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS$^{\bullet+}$) radical scavenging assays[15, 16, 17].

2.4.1 DPPH Assay

The determination of the free radical scavenging activity of the extract was carried using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay based on the procedure described by Cervato *et al.*, 2000[16] and Ajiboye *et al.*, 2017[15] with slight modifications. Briefly, DPPH solution was prepared by dissolving 0.0197 g DPPH in 1L methanol. The mixture was kept in dark brown bottle and incubated for 16 hours. The prepared DPPH solution (60 µL) was mixed with 100 μl of extract at various concentrations (20-100 μg/mL). The mixture was left in the dark for 30 min before the absorbance (Abs) values were measured at 517nm with a microplate reader ((BioTek Instruments, Synergy 4, Winooski, VT). The decrease in absorbance of DPPH· on addition of test samples relative to the control (Vitamin C and Rutin) was used to calculate the percentage inhibition (% Inhibition) according to equation (1): The IC_{50} value (μg/mL), which represents the concentration of a sample required to scavenge 50 % of the DPPH radicals, expressed as mean standard deviation was calculated.

$$\%\ \text{inhibition of DPPH Radical} = \frac{\text{Absorption of control} - \text{Absorption of test sample}}{\text{Absorption of control}}\ x\ 100\%$$

2.4.2 ABTS Assay. The assay utilizes ABTS radical generated by the oxidation of $ABTS^{2-}$ with potassium persulfate. The ABTS radical scavenging assay was performed using the method described by Re *et al*. [17] with slight modification. The ABTS radical cation was prepared by reacting equal portions of 7mM ABTS solution at pH 7.4 (5mM NaH_2PO_4. 5mM Na_2HPO_4, 154mM NaCl) and 2.45mM potassium persulfate solution. The mixture was incubated at room temperature for 16 hours in the dark at 25 ^{0}C and then diluted with 96% ethanol until its absorbance of 734 nm reached 0.7 ± 0.02. The prepared $ABTS^{\bullet+}$ solution (100 μL) was mixed with 100 μL of extract at various concentrations (500 – 31.25 μg/mL). Each concentration was analyzed in triplicate (n = 3) and the percentage decrease

of absorbance at 734 nm with a microplate reader (BioTek Instruments, Synergy 4, Winooski, VT) was calculated for each point and the antioxidant capacity of the tested extract was expressed as percent inhibition (%). The radical scavenging activity of the extract was measured according to the equation (1) used in the DPPH assay and IC_{50} value (μg/mL), which represents the concentration of a sample required to scavenge 50% of the ABTS radicals, expressed as mean standard deviation was calculated. Vitamin C and Rutin were used as standard.

$$\% \text{ inhibition} = \frac{\text{Absorption of control} - \text{Absorption of test sample}}{\text{Absorption of control}} \, x \, 100\% \qquad (1)$$

2.5 in vitro α-Glucosidase Inhibitory Assay: α-glucosidase inhibitory activity of the extract was carried out followed the standard method described by Pistia-Brueggeman and Hollingsworth [18] with minor modification. Briefly, 1 mg of extract was dissolved in 10 % of DMSO as the stock solution, while, for the positive control, 1 mg of acarbose (Sigma-Aldrich, USA) was dissolved in 1mL of DMSO. The assay was performed by the addition of α-glucosidase enzyme (0.1U/mL) to the extract starting at a concentration of 0.025mg/ml and making further serial dilutions. The reaction was initiated by the addition of substrate (p-nitrophenyl α-glucoside; 2.5 mM) and incubating the reaction mixture at 37 ^{0}C for 45 min. The reaction was terminated by the addition of 100 µM of sodium carbonate. The absorbance of the resultant product was measured at 405 nm. Acarbose was used as a reference standard. The % of inhibition was calculated using equation (2) and IC_{50} value (μg/mL), representing the concentration of sample needed to inhibit 50 % α-glucosidase, was determined using linear regression analysis.

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \, x \, 100\% \qquad (2)$$

2.6 Computational methodology

Ligand preparation

Thirteen compounds (ligands) alongside the two standards (i.e. metformin and ascorbic acid) were selected for *in-silico* studies. Table 4 shows the names of the selected compounds, and standards with their assigned code names. All ligands were modeled using the Chemdraw12 software[19]. Using molecular mechanics force field method (MMFF), and conformation search methods, a reasonable input geometry with minimized energy of stable conformation was generated using the Spartan 14 version 1.1.4 software[20]. The resulting conformer were optimized using the equilibrium geometry Density Functional Theory method (DFT) at B3LYP and 6-31+G* basis set**.** The ligand file was converted and saved as (*.pdbqt)* AutoDock file using AutoDock 4 software[21].

Receptor preparation

The fact that *in vitro* analysis defines the plant extract as possessing anti-diabetic and antioxidant activities, the Human Dipeptidyl peptidase IV (DPP4) and Human Peroxiredoxin-5-Mitochondria (HP5M) were selected as target receptors for the study. From Protein Data Bank, two crystal structures (2RGU and 3MNG) were obtained from the RCSB database website (http://www.rcsb.org/pdb)[22]. The resolution for both 2RGU and 3MNG are 2.60Å and 1.45Å respectively, which falls within the recommended value (2Å) for a good receptor[23]. Water molecules were removed from both crystal structures to avoid inaccurate interaction at the binding sites. Polar hydrogens were added and all non-polar hydrogens merged. Kolman charges were computed and resulting structures also saved as *(.pdbqt)* AutoDock file.

The binding site of each receptor were obtained for 2RGU from the associated literature defining residues SER630, HIS740, ASP708, GLU205, GLU206, TYR662 and ARG125 as the active sites (Eckhardt *et al.*, 2007)[24] while maximum box size was chosen for the docking mode of 3MNG due to its small structural dimensional size.

Molecular Docking Studies

AutoDock/vina tool[25] was used for the docking procedure on virtual screening software called PyRx[26]. The *.pdbqt* files earlier saved were generated using AutoDock4 tools[27]. The docking scores were graded according to their increase in energy or decrease in stability (poses). The most stable poses or lowest energy poses were chosen for visualization using the BIOVIA Discovery studio version 2021 software[28]

Toxicity studies

Toxicity studies performed on the selected compounds include; drug induced hERG toxicity, carcinogenicity, Human oral bio-availability, AMES mutagenesis, acute oral toxicity, water solubility and bio-degradation were investigated using the admetSAR webserver (http://lmmd.ecust.edu.cn/admetsar2/)[29]

Prediction of Activity Spectra Studies (PASS) and bio-activity studies

The Prediction of Activity Spectra studies (PASS) and bio-activity studies were conducted on the selected compounds and compared to that of the standards using the PASS online webserver[30].

3. RESULTS AND DISCUSSION

3.1 *GC-MS analysis*: The phytochemicals present in the extract obtained from the roasted kernel of cashew nut are presented in Table 1. The total ion chromatogram (TIC) of the extract presented in Figure 1a and 1b showed the retention time and signals that correspond to the phytochemicals present in the extract. The molecular formula, molecular weight, and percentage area of various components of the extract are given in Table 1.

Table 1: GC-MS Analysis of the Methanol Extract of Roasted Cashew Kernel

S/N	Compound name	Molecular formular	Molecular weight	Retention time	% Area

1.	N- serylserine	$C_6H_{12}N_2O_5$	192	14.210	1.462
2.	D- fructose,3-0-methyl	$C_7H_{14}O_6$	194	14.394	1.041
3.	Trimethyl silyl 23-acetoxy-3, 6, 9, 12, 15, 18, 21- heptaoxatricosan-1-oate	$C_{21}H_{39}SO_{11}$	486	14.418	0.181
4.	Pentadecanoic acid, 13-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	14.527	0.888
5.	n- hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.553	0.114
6.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	14.727	0.627
7.	9, 12- octadecadienoic acid (z,z)	$C_{18}H_{32}O_2$	280	14.843	0.831
8.	9- octadecenoic acid (z)- methyl ester	$C_{19}H_{36}O_2$	296	14.959	0.649
9.	Cyclohexanol, 5- methyl-2-(1-methylethyl)-,(1α,2 α,3α)	$C_{10}H_{21}O$	156	15.946	1.299
10.	Methyl stearate	$C_{19}H_{38}O_2$	298	16.557	4.627
11.	9- octadecenoic acid	$C_{18}H_{34}O_2$	282	16.814	1.906
12.	(E)-9-octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	18.162	1.089
13.	(z)-3-(pentadec-8-en-1-yl)phenol	$C_{21}H_{34}O$	302	18.244	4.705
14.	Bis(2-ethyl hexyl) phthalate	$C_{24}H_{38}O_4$	390	18.623	1.263
15.	Carbonic acid, dodecyl vinyl ester	$C_{15}H_{28}O_3$	256	19.111	45.99
16.	Heptadecane, 8-methyl	$C_{18}H_{38}$	254	19.219	10.466
17.	β- Amyrone	$C_{30}H_{48}O$	424	23.865	1.722
18.	β- Amyrin	$C_{30}H_{51}O$	426	24.836	0.949
19.	Lupeol	$C_{30}H_{50}O$	426	29.269	0.761
20.	Hydroquinone,2TMS derivative	$C_{12}H_{22}O_2Si_2$	254	30.02	0.679

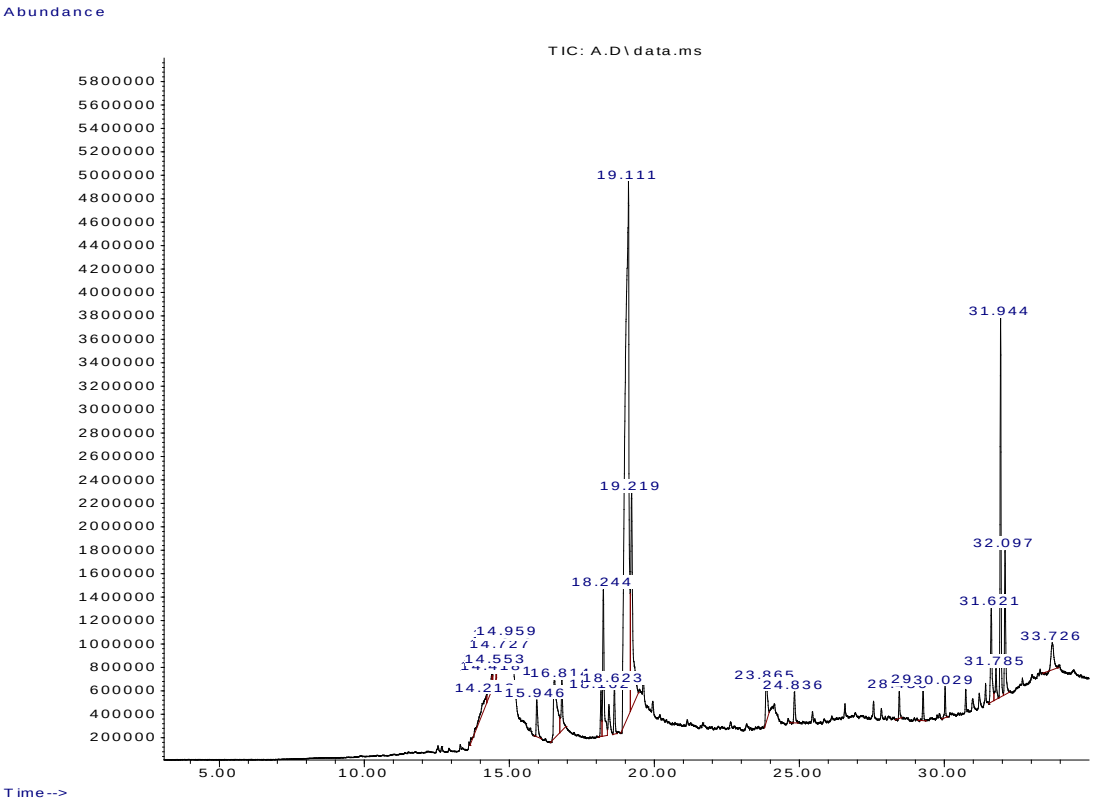


Figure 1a: Total ion GC-MS Chromatogram of the methanol extract of roasted cashew kernel.

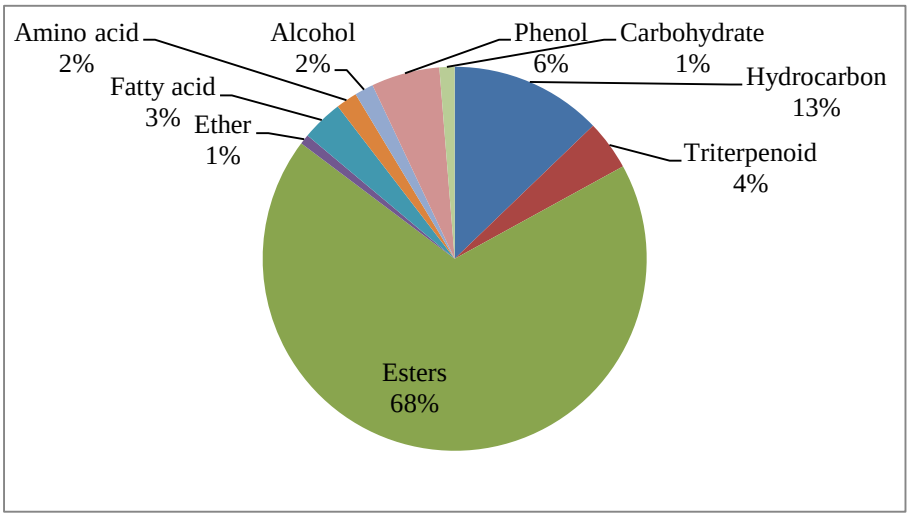
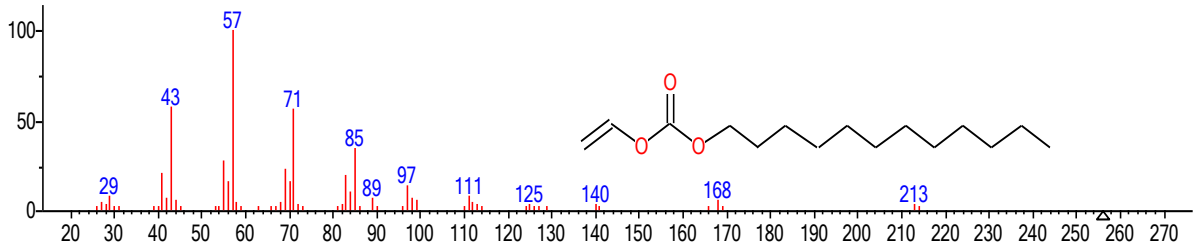
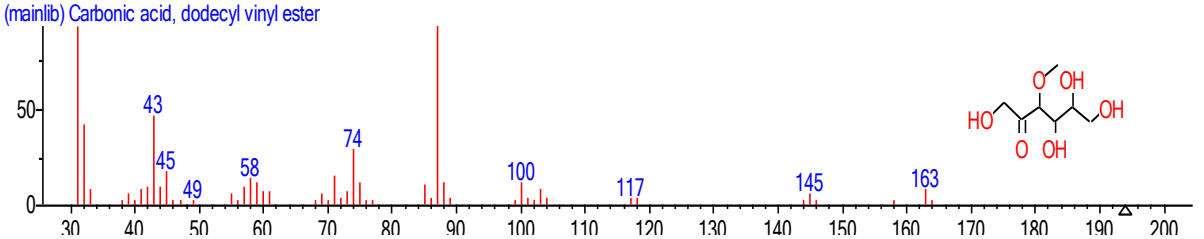
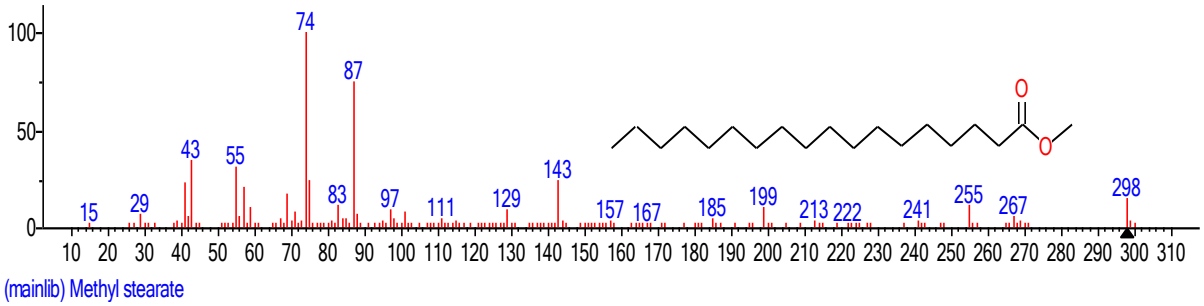
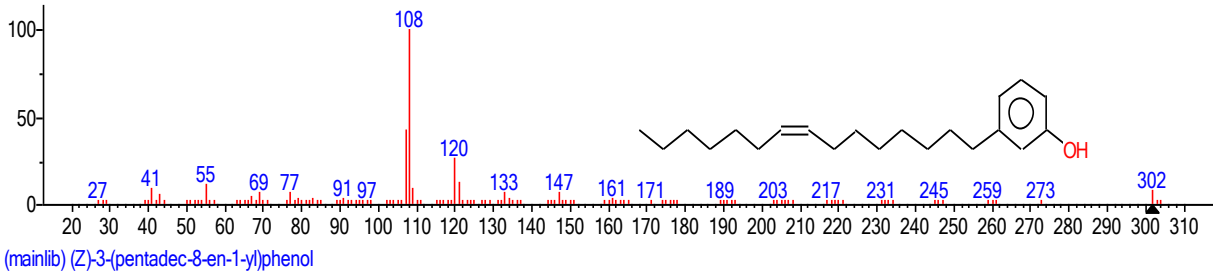


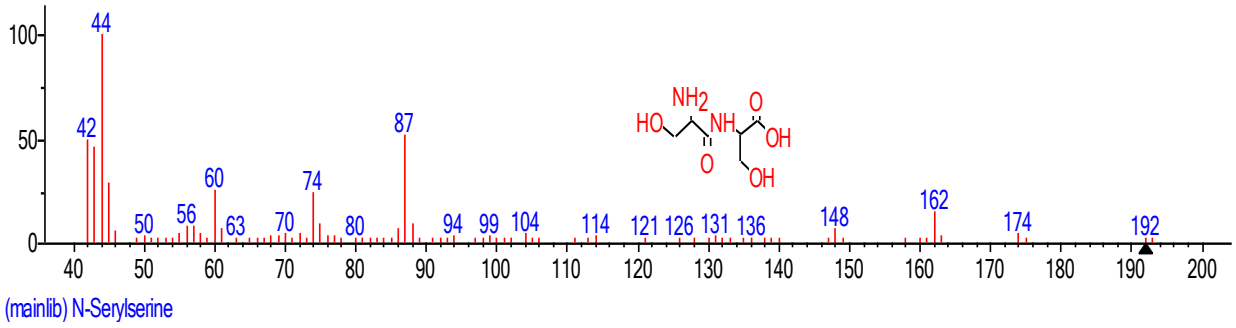
Figure 1b: Percentage of various phytochemicals identified in the methanol extract of roasted cashew kernel.


(mainlib) Carbonic acid, dodecyl vinyl ester




(mainlib) Methyl stearate


(mainlib) (Z)-3-(pentadec-8-en-1-yl)phenol


(mainlib) N-Serylserine

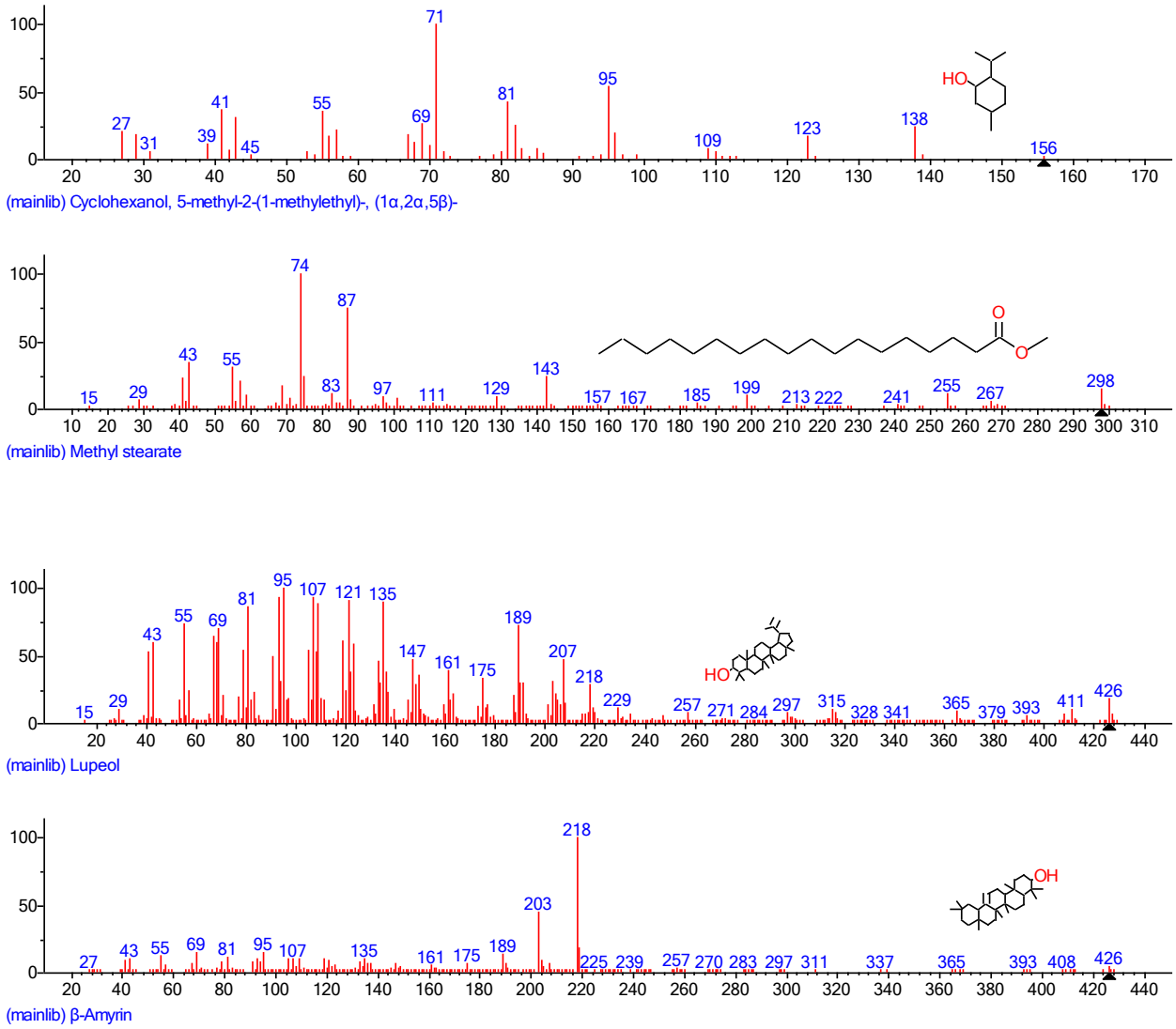


Figure 2: Some bioactive components detected in roasted cashew kernel nut.

3.2 Antioxidant activity of the extract: *In vitro* antioxidant activity of the extract was measured using DPPH and ABTS assays.

3.2.1 DPPH Radical Scavenging Assay: DPPH radical scavenging activity is a widely used method to evaluate the free radical scavenging ability of various samples. This method is based on the reduction of DPPH in the presence of a radical scavenger or hydrogen donors due to the formation of non-radical form of DPPH-H [31]. Result in Table 2 revealed that methanol extract of roasted cashew kernel expressed the lowest value of IC_{50} (10±0.001 μg/mL), significantly when compared to the positive controls, vitamin C (13±0.01 μg/mL) and rutin (16±0.001μg/mL) respectively. The impressive antioxidant potential showed by cashew kernel extract suggests that the extract is a rich source of antioxidant

compounds that may be helpful in sustaining good health.

3.2.2 ABTS Radical Scavenging Assay: The ABTS assay in this study was based on the ability of the extract to scavenge $ABTS^{2-}$ radical. Using this method, the obtained result is presented in Table 2. The cashew kernel extract showed moderate ABTS radical scavenging activity. The IC_{50} value of the extract (14±0.001 µg/mL) is higher compare to those of standard drugs, Vitamin C (4±0.001 µg/mL) and rutin (1±0.001 µg/mL).

Table 2: IC_{50} values of the extract for DPPH and ABTS Inhibition Assays

Sample	IC_{50} (µg/mL)	
	DPPH	ABTS
Extract	10±0.001	14±0.001
Vitamin C	13±0.01	4±0.001
Rutin	16±0.001	1±0.001

Values are expressed as Mean ± SEM obtained from at least three independent experiments

3.3 *α-Glucosidase Inhibitory Activity:* The *α*-glucosidase assay was used to investigate the inhibitory activity of the crude extract of cashew kernel. The inhibitory activity of the extract was measured against *α*-glucosidase using *p*-nitrophenyl-*α*-D-glucopyranoside (p-NPG) as a substrate and this was compared with acarbose (Table 3). The crude extract showed significantly high inhibitory activity against *α*-glucosidase enzyme, with IC_{50} value of (0.24 ± 0.002 µg/mL). This result demonstrated that the kernel of cashew nut possessed impressive anti-hyperglycemic activity and could play a preventive role in the treatment of diabetes mellitus.

Table 3: IC_{50} value of the extract for α-Glucosidase Inhibitory Assay

Samples	α-Glucosidase Inhibitory IC_{50} (µg/mL)
Extract	0.24 ± 0.002
Acarbose	417 ±0.021

Values are expressed as Mean ± SEM obtained from at least three independent experiments

3.4 Molecular Docking analysis

The results obtained from the docking of 13 identified compounds against both DPP4 and HP5M were shown in Table 4. Binding affinity is a function of the inhibitory properties of the plant extracts against DPP4 and HP5M. The table shows compound names, code name, binding affinities and the inhibition constant of the identified compounds and standards against 2RGU and 3MNG crystal structure.

Table 4: Compound name, code name, binding affinities and the inhibition constant of pre-selected compounds and standards against 2RGU and 3MNG crystal structure

			2RGU		3MNG	
S/N	Compound names	Code name	ΔG (kcal/mol)	Inhibition constant (K_i), μM	ΔG (kcal/mol)	Inhibition constant (K_i), μM
1	(E)-9-octadecenoic acid ethyl ester	L1	-5.9	46.96	-4.5	499.77
2	9- octadecenoic acid (z)- methyl ester	L2	-6.0	39.66	-4.6	422.01
3	9Z, 12Z-octadecadienoic acid	L3	6.4	20.18	-4.6	422.01
4	β- Amyrin	L4	-8.6	0.49	-7.8	1.9
5	β- Amyrone	L5	-9.5	0.11	-6.8	10.27
6	Bis(2-ethyl hexyl) phthalate	L6	-6.8	10.27	-5.2	153.2
7	Carbonic acid, dodecyl vinyl ester	L7	-5.5	92.29	-4.2	829.58
8	Hexadecanoic acid, ethyl ester	L8	-5.6	77.95	-4.1	982.23
9	Lupeol	L9	-9.3	0.15	-7.0	7.32
10	Methyl stearate	0	-5.4	109.28	-4.5	499.78
11	n- hexadecanoic acid	L11	-5.8	55.6	-4.5	499.78
12	N- serylserine	L12	-5.0	214.77	-5.0	214.77
13	Pentadecanoic acid, 13-methyl-, methyl ester	L13	-5.6	77.95	-4.2	829.58

14	Metformin	ST-1	-5.5	92.29	**	**
15	Ascorbic acid	ST-2	**	**	-5.7	65.83

** Not conducted

L4 = β-amyrine, L5 = β-amyrone, L9 = Lupeol, ST-1 = Metformin, ST-2 = Ascorbic acid

3.5 Toxicity Studies

In silico toxicity studies includes the drug induced human Ether-á-go-go-Related Gene (hERG) toxicity, carcinogenicity, human oral bio-availability, AMES mutagenesis, acute oral toxicity, water solubility, bio-degradation, etc. shown in Table 5.

Table 5 Toxicity prediction profile of selected compounds with the standards

Toxicity	L4	L5	L9	ST-1	ST-2
hERG toxicity	0.4256	0.6513	0.3607	0.7503	0.7840
	NO	NO	NO	NO	NO
Carcinogenicity	0.9571	0.9571	0.9714	0.5600	0.8589
	NO	NO	NO	NO	NO
Human oral bio availability	0.6286	0.5143	0.5571	0.8286	0.5857
	YES	YES	YES	YES	YES
AMES mutagenesis	0.8700	0.8900	0.7300	0.6600	0.9400
	NO	NO	NO	NO	NO
Acute oral toxicity rating	III	III	III	III	IV
Bio-degradation	0.8250	0.8747	0.7750	0.9000	0.5250
	NO	NO	NO	NO	NO

L4 = β-amyrine, L5 = β-amyrone, L9 = Lupeol, ST-1 = Metformin, ST-2 = Ascorbic acid

3.6 Prediction of Activity Spectra (PAS) and bioactivity studies of the selected compounds

In order to ascertain that the selected compound or hits are potent enough as anti-diabetes and anti-oxidant drugs, their respective Prediction of Activity Spectra Studies (PASS) and bio-activity studies were conducted and compared to that of the standards. Table 6 shows the prediction (structure-activity relationship) of each of the selected compounds and the bio-activity properties of the selected compounds together with the standards are shown in Table 7.

Table 6: Prediction of activity spectra for selected compounds and standards

2MNG	L4	L5	L9	ST-1	ST-2
Pa	0.410	0.337	0.309	0.414	0.268
Pi	0.041	0.314	0.196	0.030	0.102

Biological activity	Anti-diabetic	Diabetic neuropathy treatment	Diabetic neuropathy treatment	Diabetic neuropathy treatment	Anti-diabetic
3MNG					
Pa	0.405	0.281	0.280	**	0.928
Pi	0.012	0.027	0.027	**	0.003
Biological activity	Antioxidant	Antioxidant	Antioxidant	**	Antioxidant

L4 = β-amyrine, L5 = β-amyrone, L9 = Lupeol, ST-1 = Metformin, ST-2 = Ascorbic acid

** Not available

Table 7: Bio-activity analysis of the selected compounds and standards

	2RGU				3MNG			
Bio-activity	L4	L5	L9	ST-1	L4	L5	L9	ST-2
ΔG (kcal/mol)	-8.60	-9.50	-9.30	-5.50	-7.80	-6.80	-7.00	-5.70
K*i* (μM)	0.49	0.11	0.15	92.29	1.90	10.27	7.32	65.83
miLogP	4.76	4.32	2.91	0.34	4.76	4.32	2.91	-0.31
LE (*kcal*/mol/heavy atoms)	0.31	0.32	0.58	0.61	0.30	0.21	0.44	0.48
FQ	0.83	0.92	1.18	0.96	0.75	0.32	0.84	0.82
LELP	17.16	14.55	5.01	0.56	18.92	0.66	6.65	-0.65

L4 = β-amyrine, L5 = β-amyrone, L9 = Lupeol, ST-1 = Metformin, ST-2 = Ascorbic acid

3.8: Binding interaction of selected compounds and standards

The binding interactions of the selected compound and standard with the Human Dipeptidyl peptidase IV (DPP4) and Human Peroxiredoxin-5-Mitochondria (HP5M) crystal structures were as shown in Table 8 and 9.

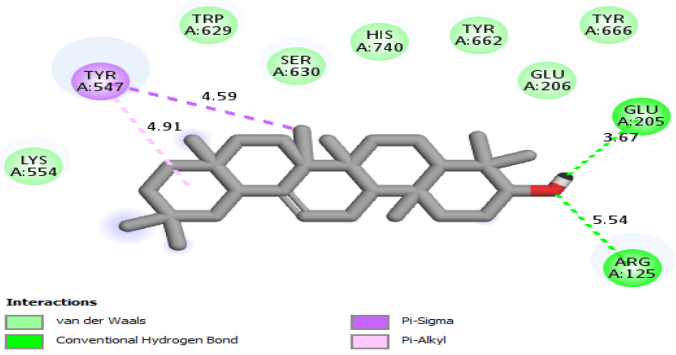
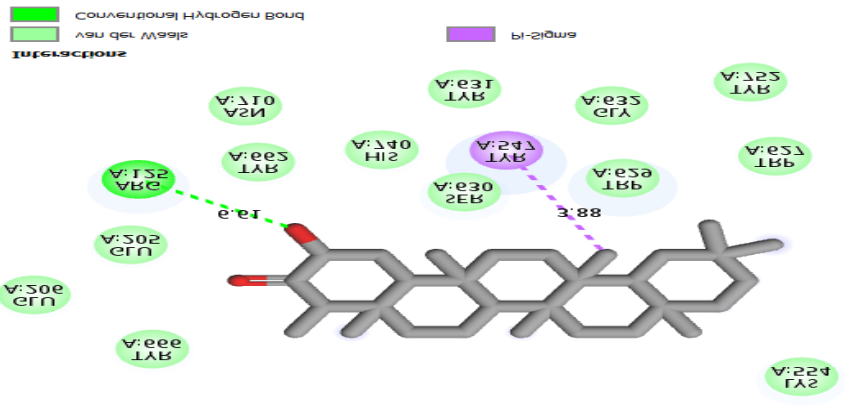
Table 8: Docking scores, binding sites and inhibition constants of selected compounds against DPP4 and HP5M crystal structure with H-bond length.

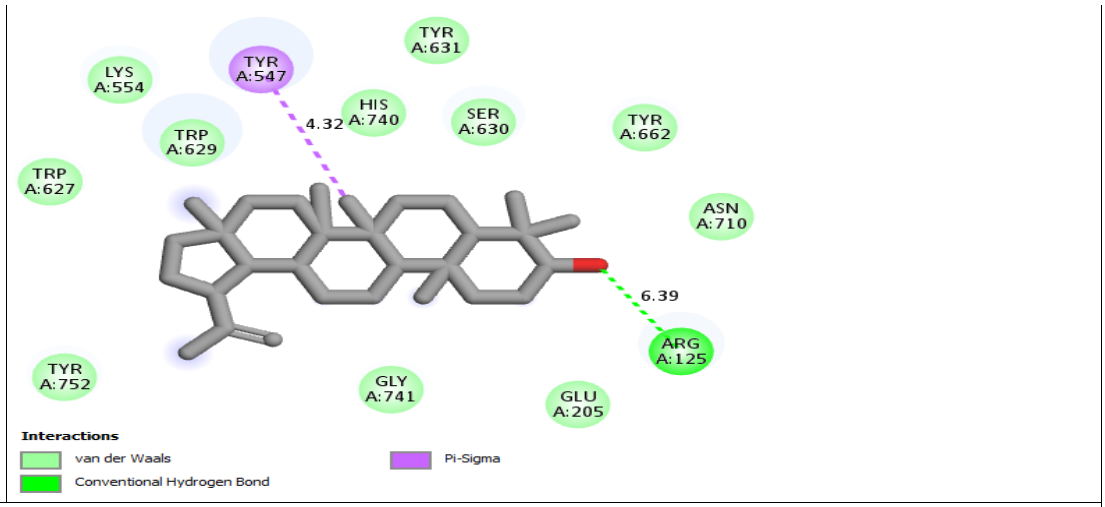
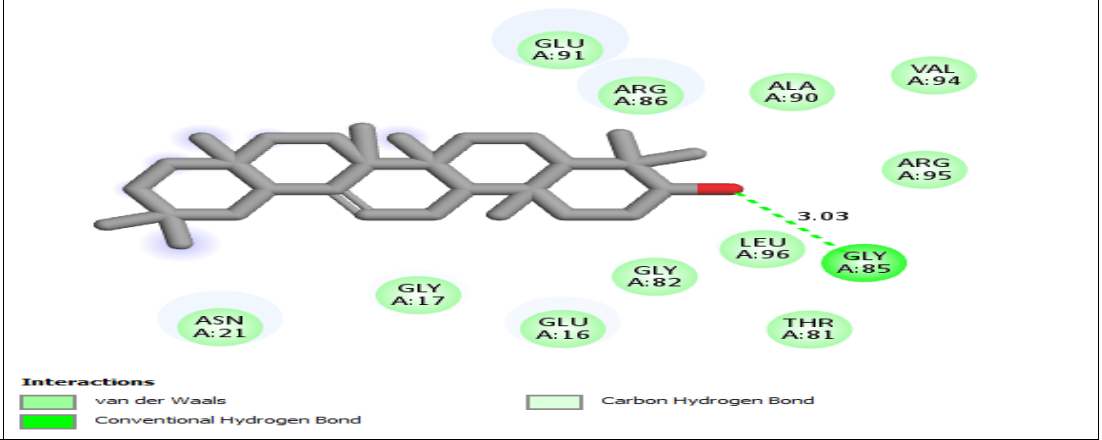
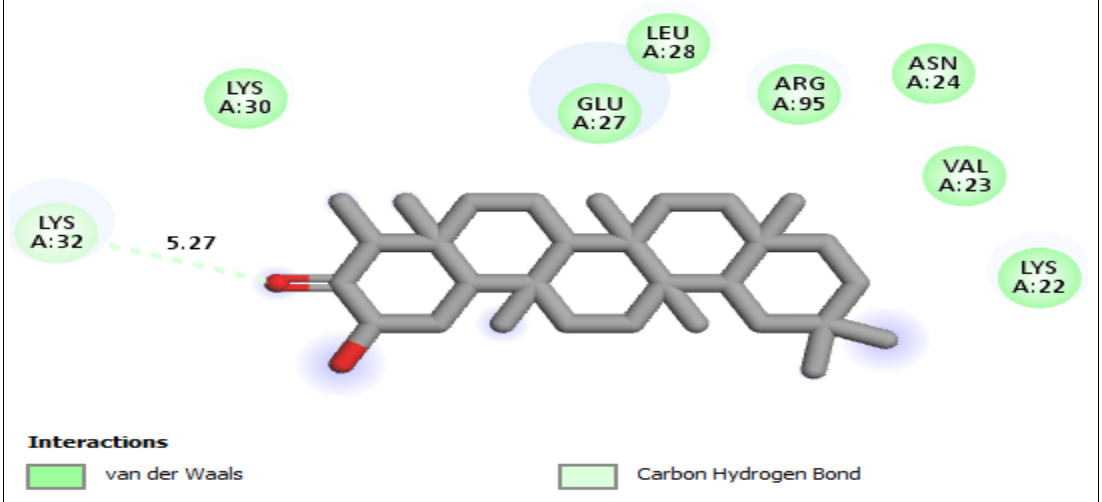
	Ligands	ΔG (kcal/mol)	Ligand-Receptor interaction with H-bond (bond length)	Ligand-Receptor interaction with electrostatic/hydrophobic interactions	Inhibition constant, K_i (μM)
2RGU	β-amyrin (L4)	-8.6	GLU205 (3.67) ARG125 (5.54)	TYR547	0.49
	β-amyrone (L5)	-9.5	ARG125 (6.61)	TYR547	0.11
	Lupeol (L9)	-9.3	ARG125 (6.39)	TYR547	0.15

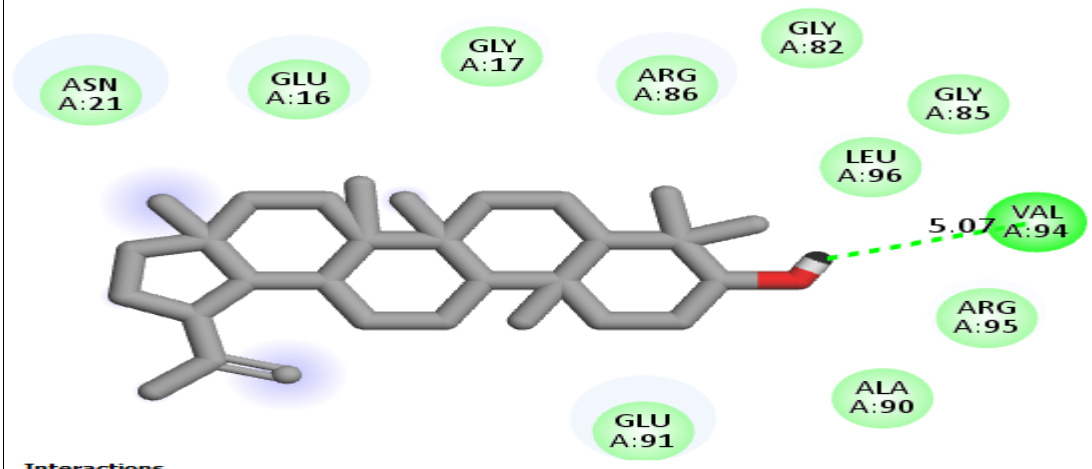
3MNG	β-amyrin (L4)	-7.8	GLY85 (3.03)		1.9
	β-amyrone (L5)	-6.8	**	LYS32	10.27
	Lupeol (L9)	-7.0	VAL94 (5.07)	**	7.32

**Not available

Table 9: 3-D Molecular interaction of selected compounds against 2RGU and 3MNG crystal structure respectively

	Ligands	Interactions
2RGU	L4	
	L5	
	L9	

3MNG		 TYR A:631 LYS A:554 TYR A:547 HIS A:740 4.32 SER A:630 TYR A:662 TRP A:629 TRP A:627 ASN A:710 6.39 ARG A:125 TYR A:752 GLY A:741 GLU A:205 Interactions van der Waals Pi-Sigma Conventional Hydrogen Bond
	L4	 GLU A:91 ARG A:86 ALA A:90 VAL A:94 ARG A:95 3.03 LEU A:96 GLY A:85 GLY A:82 GLY A:17 ASN A:21 GLU A:16 THR A:81 Interactions van der Waals Carbon Hydrogen Bond Conventional Hydrogen Bond
	L5	 LEU A:28 LYS A:30 GLU A:27 ARG A:95 ASN A:24 VAL A:23 LYS A:32 5.27 LYS A:22 Interactions van der Waals Carbon Hydrogen Bond
	L9	



Discussion

3.9. *In vivo* analysis

The result of GC-MS analysis showed that, total number of twenty phyteochemicals were identified in the extract of cashew kernel and this is amounting to 81.249 %. There were eight esters present in maximum percentage (68 %) along with one hydrocarbon (13 %), one phenolic derivative (6 %), three pentacyclic triterpene (4 %), three fatty acids (3 %), one amino acid (2 %), one alcohol (2 %), one carbohydrate (monosaccharide) (1 %) and one ether (1 %) as shown in figure 2. The predominant constituents detected in the extract are Carbonic acid, dodecyl vinyl ester (45.99 %), heptadecane, 8-methyl (10.46 %), (z)-3-(pentadec-8-en-1-yl) phenol (4.71 %) and Methyl stearate (4.63 %). Other phytochemical constitutes present include N-serylserine (1.46 %), D- fructose, 3-*O*-methyl (1.04 %), trimethyl silyl 23-acetoxy-3, 6, 9, 12, 15, 18, 21- heptaoxatricosan-1-oate (0.181 %), (E)-9-octadecenoic acid ethyl ester (1.089 %), bis(2-ethyl hexyl) phthalate (1.26 %), pentadecanoic acid, 13-methyl-, methyl ester (0.88 %), 9- octadecenoic acid (z)- ethyl ester (1.08 %), hexadecanoic acid, ethyl ester (0.62 %), n- hexadecanoic acid, (0.11%), 9, 12- octadecadienoic acid (z,z), (0.83 %), 9- octadecenoic acid, (1.91 %), cyclohexanol, 5- methyl-2-(1-methylethyl)-,(1α,2 α,3α) (1.29 %), hydroquinone,2TMS derivative (0.67 %), β- amyrone (1.72 %), β-amyrin (0.95 %) and lupeol (0.761 %). The extract exhibited good antioxidant capacity with both high inhibitory activity against ABTS·+ radical cations and strong scavenging activity against DPPH radicals. The IC_{50} values of less than 100 mg/mL indicated the potential sample as chemoprevention agent (Meiyanto *et al*., 2008) [32]. The result shows that cashew kernel extract play a significant role in its antioxidant activity. This extract presents a reservoir of important bioactive compounds that may be helpful in sustaining good health.

3.10

3.10.1: Molecular docking analysis

Table 4 reveals the inhibitory properties or binding affinity of the 13 pre-selected compounds. From the result, L4; L5 and L9 has better binding affinity to the 2RGU crystal complex while L4; L9 and L5 has better affinity to the binding site of 3MNG crystal structure. Most importantly, all selected compounds except for L12 and L10, has better binding affinity than Metformin (ST-1), the current control drug for Diabetes Type II. Likewise, L4; L5 and L9 have better binding affinity compared to ascorbic acid (ST-2), a popular anti-oxidant control drug. It's therefore imperative to subject the compounds to further studies in order to validate the inhibitory properties of the compounds over the standards. Hence, compounds L4; L5; and L9 were selected for further studies, to be compared alongside with the standard (ST-1), against 2RGU, also, compounds L4; L9; and L5 were selected to be compared with the standard (ST-2), against 3MNG crystal complex

3.10.2: Toxicity Studies

In contrast to conventional methods of toxicity discoveries, modern and computer-aided drug discovery has advantages of speed, cost effectiveness and safety over conventional methods. Toxicity studies are needed to determine the possible adverse effects of a drug in humans, animals, plants and the environment. Results on table 5 show that all selected compounds (i.e. L4, L5 and L9) were recorded as non-blockers and non-inhibitors of the human Ether-á-go-go-Related Gene (hERG), likewise the standards which is in accordance with the toxicity prediction. The inhibition of (hERG) potassium ion channels is toxic for the heart and may produce chronic cardiac arrhythmia. (Sanguinetti and Tristani-frouzi 2006) [33]. It was also found that all the three selected compounds are non-carcinogenic, bio-available and non-biodegradable. The ability of a drug to cause mutation to the DNA is revealed by its AMES toxicity value which could be a reasonable reason to exclude a potential drug in the discovery process. Table 5 shows that all three selected compounds are non-genotoxic. Similarly, all selected compounds show a type III rating for acute oral toxicity which translates, (slightly toxic), although, the rating can still be improved to IV (non-toxic) during further optimization.

3.10.3: Prediction of Activity Spectra Studies (PASS)

From Table 6, *Pa* is the probability that the drug is active while *Pi* is the probability that the drug is inactive. The probability of a drug to be validated experimentally must follow the order *Pa*> *Pi* [30]. It's noticeable from Table 6 that β-amirin (L4) shows a wide *Pa:Pi* difference and its activity in the treatment of diabetes can be likened to the activity of the standard drug (Metformin®). Unlike Metformin®, β-amirin (L4) has the ability to serve as a stand-alone drug in the treatment of both type I and type II stages of diabetes. β-amyrone (L5) and Lupeol (L9) can also be likened to the activity of Metformin®. It's also obvious from the result that ascorbic acid (Vitamin C) shows the brightest activity as an antioxidant drug, as it is commonly known for, but flashes of brilliance were also noticed in all selected

compounds, especially in β-amyrin (L4). Prediction of Activity Spectra Studies (PASS) and bioactivity analysis of all selected compounds reveal that they all possess excellent anti-diabetic and at the same time, antioxidant activities and could be further explored in the design of a more potent antioxidant and anti-diabetes alternatives to the current administered drugs.

3.10.4: Bio-activity analysis

Table 7 shows the bioactivity properties such as binding energy ΔG), inhibition constant (K*i*), Ligand Efficiency (LE), Ligand Efficiency- Lipophilicity (LELP) and Fit Quality (FQ), etc. Inhibition constants for the (3) selected compounds falls in the range of 0.107µM and 0.491µM against 2RGU and within 1.89µM and 10.267µM against 3MNG receptor. These values are in agreement with one of the criteria that must be satisfied by a potential drug (i.e. the inhibition constant of a potential drug is expected to be in the range of 0.1µM to 100µM) according to [34].

All the three selected compounds has ligand efficiency within the recommended value range of (≥0.3) against the 2RGU receptor, while only L4 and L9 has LE values within the recommended range against the 3MNG receptor. Meanwhile, only L9 falls between the recommended value-range for LELP (-10 ≥ LELP ≤10). Furthermore, against the 2RGU receptor, all selected compounds had values for FQ within the recommended range (≥0.8), which is higher than the rating for the standard, ST-1. Meanwhile, only L9 falls within the reported range against 3MNG receptor [35].

3.10.5: Binding interaction of selected compounds and standards

In order to ease the improvement of ligand affinity to the binding pocket at the drug optimization stage of drug discovery, the binding interactions of selected compounds has to be visualized. The binding interactions of the selected compound and standard with the Human Dipeptidyl peptidase IV (DPP4) and Human Peroxiredoxin-5-Mitochondria (HP5M) crystal structures were as shown in Tables 8 and 9. It's deducible from the Tables that all selected compounds targeted at 2RGU formed hydrogen bond interactions with the receptor across the active sites with similar electrostatic interaction which is easily understood due to their structural similarities. β-amyrin was found to bind to the 2RGU receptor best, forming strong H-bond interactions. Likewise, β-amyrin also formed strongest interactions with the 3MNG receptor among all the three selected compounds.

CONCLUSION

This present study identified twenty different bioactive compounds from the roasted kernel of *A. occidentale* extract using GC-MS technique. The *In vitro* free radical scavenging and α-glucosidase enzyme inhibitory assays suggested that the extract

possessed potent antioxidant and antihyperglycemic activities. The molecular docking study on 13 pre-selected identified compounds revealed that 11 out of these compounds significantly demonstrated antidiabetics and antioxidant activities by showing impressive binding affinity than Metformin (ST-1) and ascorbic acid (ST-2) standard drugs respectively. However, three out of these compounds (L4, L5 and L9) significantly demonstrated better binding modes with 3MNG and 2RGU crystal structure. Therefore, they were subsequently subjected for further studies in order to validate their inhibitory properties by comparing alongside with the standard (ST-1), against 2RGU, standard (ST-2), against 3MNG crystal complex. The result showed that the three compounds possess excellent anti-diabetic and antioxidant activities. More so, they are non-genotoxic. Therefore, these three compounds β-amyrin (L4); β-amyrone (L5) and lupeol (L9) could be responsible for the antioxidant and antidiabetic properties of kernel of *A. occidentale*. Hence, these compounds could be further explored in the design of a more potent antioxidant and anti-diabetes alternatives to the current administered drugs.

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