EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 7
315-324
Year: 2024

ANTIOXIDANT AND HYPOLIPIDEMIC EFFECTS OF METHANOL FRACTION OF *MANGIFERA HADEN* SEEDS ON ALBINO RATS INDUCED HYPERLIPIDEMIC RATS

Maryann Nonye Nwafor[1*], Onyinyechi Cynthia Onyekwere[1], Chinedu Shedrack Ogunwa[2], Michael Obinna Eji[2], Chinemelum Adaora Onodugo[3], Ikimi Charles German[4], Abba Babandi[5] and Whyte Jim Alalibo[6]

[1]Department of Chemistry & Biochemistry, Madonna University, Elele, Rivers State, Nigeria. P.M.B. 05.
[2]Department of Biochemistry, Alex Ekwueme Federal University Ndufu-Alike Ikwo, Abakaliki, Ebonyi State, Nigeria. P.M.B. 1010.
[3]Department of Biochemistry, Federal University, Oye-Ekiti, Ekiti State, Nigeria, P.M.B. 053.
[4]Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria. P.M.B. 126.
[5]Department of Medical Biochemistry, Federal University Dutse, Jigawa State, P.M.B. 7156.
[6]Department of Chemical Pathology, University of Abuja Teaching Hospital, Gwagwalada, Abuja, Nigeria, P. M. B. 228.

***Corresponding Author: Maryann Nonye Nwafor**
Department of Chemistry & Biochemistry, Madonna University, Elele, Rivers State, Nigeria. P.M.B. 05.

Article Received on 27/05/2024 **Article Revised on 17/06/2024** **Article Accepted on 07/07/2024**

ABSTRACT
Antioxidants are substances which inhibit oxidative damage by trapping free radicals to a target molecule. They are quite necessary to overcome the chronic metabolic disorder characterized by elevated circulating levels of lipids. The present study investigated the antioxidant and hypolipidemic potentials of *Mangifera haden* seeds in carbon tetrachloride induced hyperlipidemic rats. The animals were acclimatized to the laboratory conditions for a period of seven days. At the end of the acclimatization period, each rat was weighed and randomly divided into six (6) groups of five animals each, comprising of test animals and control groups. Proximate analysis, antioxidant activity, toxicity studies and lipid profile parameters were determined in the study using standard methods. Proximate composition of whole sample of *Mangifera haden* seeds revealed that the (%) values of protein, lipid, crude fibre, ash, moisture, and carbohydrate are (4.82, 17.18, 4.29, 2.77, 8.39, 62.55) respectively. The fraction of *Mangifera haden* seeds were shown to have high antioxidant activity. All carbon tetrachloride induced animals displayed hyperlipidemia as shown by their elevated levels of serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c) and reduction in high density lipoprotein (HDL-c). Methanol fraction of *Mangifera haden* seeds were administered at different doses (200 mg, 400 mg, 600 mg) of the fraction per kilogram body weight of the animals in carbon tetrachloride hyperlipidemic rats. Simvastatin was used as reference standards. Simvastatin was found to be an effective drug in lowering lipid profiles. The statistical analyses were carried out using one way ANOVA followed by Dunnett's Post Hoc Multiple Comparisons test. *Mangifera haden* seeds show significant decrease ($P < 0.05$) in the levels of serum Total Cholesterol, Triacylglycerols, LDL-C, VLDL-C, Atherogenic index (A.I) and significant increase ($P < 0.05$) of serum HDL-C against carbon tetrachloride-induced hyperlipidemic rats. The result also suggest that at 200 mg/kg *Mangifera haden* body weight concentrations are an excellent lipid lowering agent. *Mangifera haden seeds* exhibited quite hypolipidemic potential when compared with the reference drug, simvastatin. This indicates that the organic extracts could be explored as an alternative therapeutic agent in the treatment of hyperlipidemia, obesity and abdominal fats.

KEYWORDS: Antioxidant, *Mangifera haden*, hyperlipidemia, atherogenic index, and simvastatin.

1.0 INTRODUCTION
Antioxidants are substances which inhibit oxidative damage by trapping free radicals to a target molecule. It is the first line of defense against free radical damage, and are critical for maintaining optimum health and well-being (Atanu *et al.*[1] Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (Krishan *et al.*[2] In

normal cells, oxygen derivatives are neutralized or eliminated owing to the presence of a natural defense mechanism that involves enzymatic antioxidants (glutathione peroxidase, superoxide dismutase, catalase) and water or fat-soluble non-enzymatic antioxidants (vitamins C and E, glutathione, selenium). The human body has several mechanisms to counteract oxidative stress by producing antioxidants which are either naturally produced in the body or externally supplied through foods and/or supplements (Susinjan.[3] Endogenous and exogenous antioxidants are effective as free radical scavengers by donating their own electrons to ROS and thereby neutralize adverse effects of the latter. The antioxidant systems are classified into two major groups, protective or enzymatic antioxidants and non-enzymatic antioxidants (Atanu *et al.*[1] A recent study carried out by Thambi *et al.*[4] evaluated antioxidant effects of mango peel powder and proved that the acetone extract of the *Mangifera indica* peel exerts strong radical scavenging effects. A research carried out by Abbasi *et al.*[5] with nine mango varieties (Royal mango, Thai mango, Egg mango, Luzon, Narcissus, Big Tainong, Keitt, Australian mango, and Small Tainong) found in China found that the peel of small Tainong (Xiao Tainong) variety exerts the highest antioxidant potential among the tested varieties. Another study conducted by Sultana *et al.*[6] measured the antioxidant potential of water-methanol extracts of the peels of two mango varieties (Langra and Chaunsa) grown in Pakistan. Among these two peel extracts, water-methanol extract of Chaunsa exhibited strong antioxidant effects than Langra. Kim *et al.*[7] who studied antioxidant effects of ethanolic extracts of mango peel and flesh showed potent antioxidant effects of mango peel extracts compared to flesh extracts. Antioxidant effects of methanolic extract of *Mangifera indica* leaves have been reported by Mohan *et al.*[8]

The mango (*Mangifera indica)* is a tropical stone fruit that is luscious, sweet, fragrant and packed with nutrients like vitamins A, C and D. It is a member of the drupe family. It is a type of plant food with a fleshy outer section that surrounds a pit (shell). This pit contains a seed. Different types of mangoes vary in colour, shape, flavour, and seed size. Although mango skin can be green, red, yellow, or orange, its inner flesh is mostly golden yellow. *Mangifera indica* is a member of the cashew family, anarcardiaceae and one of the most important widely cultivated fruits of the tropical world. Mango is native to India and southern Asia. People have cultivated it for over 4,000 years. Hundreds of types of mango exist, each with its own characteristics taste, shape, size and colour. In Nigeria, there are only three common varieties in Nigeria. They are *Mangifera haden*, *Mangifera pirie*, and *Mangifera turpentine*.

Mangifera haden is the yellow or red mango whose fruits are ready for harvesting and consumption when they are matured and ripened (Shah *et al.*[9] When the fruit is still green in colour, they are sour in taste but turns sweet with characteristics aroma when riped. Mango seed is a single flat oblong seed that can be fibrous or hairy on the surface depending on the cultivar (leanpolchareanchai *et al.*[10] Mango seed consists of a tenacious coat enclosing the kernel. During the processing of mango, by-products such as peel and kernel are generated. Kernels take up about 17-22% of the fruit. The major components of mango seed are starch, fat, and protein. The oil of mango seed kernel consist of about 44-48% saturated fatty acids (mainly stearic acid) and 52-56% unsaturated (Shah *et al.*[9] Mango seed kernels have a low content of protein but they contain the most of the essential amino acids, with highest values of leucine, valine and lysine (Kumar *et al.*[11] Mango seed kernels were shown to be a good source of polyphenols, phytosterols such as campesterol, sitosterol and tocopherols. In addition, mango seed kernel could be used as a potential source for functional food ingredients, antimicrobial compounds and cosmetic due to its high quality of fat and protein as well as high levels of natural antioxidants (Kumar *et al.*[11]

Hyperlipidemia is a disorder of lipid metabolism that results in an elevated level of circulating cholesterol or triacylglycerols (TAG). It is a state of disease associated with different lipid disorders like hypercholesterolemia, hypertriglyceridemia. This disease could be inherited or acquired. Dyslipidemia is a prerequisite to the development of atherosclerosis. It is also closely associated with diabetes, insulin resistance and obesity.

Atherogenic index (A.I) is a new comprehensive lipid index composed of triacylglycerols and high density lipoproteins (HDL-C). It is a strong marker for predicting the risk of blood lipids levels, cardiovascular diseases (CAD) and an optimal indicator of dyslipidaemia. The decrease in A.I is an indication of lower risk of cardiovascular disease and stroke.

Statins are drugs that lowers the level of Low-Density Lipoprotein (LDL-c) in the blood. They are referred to as 3-hydroxyl-3 –methylglutaryl-CoA (HMG-CoA) reductase inhibitors. These drugs block the action of liver enzyme (HMG-CoA reductase inhibitors) that helps produce cholesterol. Statins also raise the levels of High –Density Lipoproteins (HDL-c). Low-Density Lipoprotein (LDL-c) is often known as "Bad Cholesterol". Statins reduce the production of cholesterol in the liver. By so doing, it prevents the hardening and narrowing of the arteries (atherosclerosis) and cardiovascular diseases. These drugs include fluvastatin, simvastatin, atorvastatin, pravastatin, rosuvastatin, lovastatin and pitastatin.

There is a great number of people living with cardiovascular diseases, obesity, high blood pressure and stroke in our society due to lifestyles changes, diet consumption, increase in overweight and sedentary life. Hyperlipidemia can be treated by the use of synthetic drugs such as statin and fibrates. These drugs are costly and exhibit high undesirable side effects. Therefore,

natural products from medicinal plants need to be investigated for antioxidant properties and hypolipidemic potentials.

2.0 MATERIAL AND METHODS

2.1 Plant Material

The plant materials are the seeds of *Mangifera haden*.

2.2 Methods

2.2.1 Collection, Preparation and Extraction of Mango Seeds

The plant materials, *Mangifera haden* seeds were collected from Mile One market, Port-Harcourt, Rivers State, Nigeria. The seed samples were identified and authenticated by Mr. Alfred Ozioko of the Bioresources Development and Conversation Programme (BDCP), Nsukka. The specimen *Mangifera haden* voucher number is InterCEDD/908. They were washed and air-dried. The kernel and kernel sheathes were removed manually from the seeds. Fresh kernel seeds and kernel sheathes were chopped and blended with distilled water at a ratio of sample/water of 1:3 (W/V). After filtration, the filtrate was lyophilized with a freeze –dryer. The extract was then stored in an airtight container in a refrigerator until use.

2.2.1.1 Fractionation of the Mangifera haden seeds extract

The crude extract of *Mangifera haden seeds* were subjected to solvent – solvent partitioning using protocol designed by Kupchan and Tsou,[12] with slight modifications. The crude extract of *Mangifera haden* seeds was dissolved in 10% aqueous methanol (Methanol:Water; 9:1 V/V) to make the solution which was successfully partitioned by solvents; methanol and ethyl acetate in the order of increasing polarity by using a separating funnel. 350 g of the sample was dissolved in 1400 ml of the solvent (methanol) in the ratio sample:solvent (1:4:). This was thoroughly mixed and poured into the separating funnel. The sample was allowed to settle into layers; water settled on the bottom, methanol lie above the water, and ethyl acetate settled at the middle. Resulting fractions were dried by evaporating respective solvent using rotary evaporator under high vacuum and kept in dessicator for further studies.

2.3 Proximate Analysis of the whole Sample of *Mangifera haden* Seeds

The proximate analysis of whole sample of *Mangifera haden* seeds was carried out using the method of Association Official of Analytical Chemists.[13]

2.4 Toxicity Studies

2.4.1 Acute Toxicity Test

The mean lethal dose (LD_{50}) for methanol fraction of *Mangifera haden* seeds were determined following the method described by Lorke.[14]

2.5 *In Vitro* - Antioxidant Tests

2.5.1 Total Antioxidant Activity (TAC)

The total antioxidant capacity of the fraction was determined with phosphomolybdenum method using ascorbic acid as the standard. The assay was based on the reduction of Mo (vi) to Mo (v) by the fraction and the subsequent formation of a green phosphomolybdate (v) complex at acidic pH. 0.1 ml of the fraction was mixed with 3 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95^0C for 90 min. After the samples had cooled to room temperature, absorbance of the aqueous solution of each was read at 695 nm against blank in a spectrophotometer. The blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the sample. The antioxidant capacity was expressed as the equivalent of ascorbic acid. Total antioxidant capacity of the fraction was estimated using the phosphomolybdate method as reported by Jan *et al.*[15]

2.5.2 FRAP (Ferric Reducing Antioxidant Power)

Ferric ions reducing power was measured according to the method of Oyaizu,[16] with a slight modification. In this method, to measure the reducing power of the fraction, 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution was added to 1 ml of fraction with various concentrations. After mixing and incubating the mixture at 50^0C for 30 minute, the reaction was stopped by adding 2.5 ml of trichloroacetic acid (10%) solution to each sample. The samples were mixed and then centrifuged for 10 minute. The supernatant (2.5 ml) and 2.5 ml of $FeCl_3$ (0.1% W/V) were added to the mixture and was kept for 10 minute. The absorbance at 700 nm was measured in a spectrophotometer as the reducing power. The higher absorbance of the reaction mixture indicated the increased reducing power. Ascorbic acid was used as positive control. All tests were performed in triplicate.

2.5.3 DPPH Free Radical Scavenging Assay

DPPH free radical scavenging assay was carried out according to the method of Brand-Williams *et al.*[17] In the DPPH (2, 2- diphenyl – 1- picrylhydrazyl) assay based on the scavenging ability of stable radical DPPH, fresh methanolic solution of DPPH (1 ml) was added to 2ml of each of the methanol fraction solutions with a concentration range of 2.5 – 100 µg/ml. The final DPPH was 0.1 mM. The mixture was shaken and incubated for 30 minute at room temperature in the dark and then absorbance was measured at 517 nm using a UV-Spectrophotometer. Methanol was used as a blank and ascorbic acid (Sigma, USA) was used as a positive control. All tests were performed in triplicate.

The activity was calculated based on the following equation

$$\% \text{ Inhibition} = 100 \frac{x\ (A_O - A)}{A_O}$$

Where $\mathbf{A_O}$ was the absorbance of the control reaction (containing no test compound) and A was the absorbance of the test compound.

2.5.4 Determination of Anti Radical Power (ARP)

The Anti Radical Power of the fraction was determined by the method of Mishra *et al.*, 2012.

$$\text{Anti Radical Power (ARP)} = \frac{1 \quad x \quad 100}{IC_{50}}$$

(Mishra *et al.*,[18]

2.6 Experimental Design

A total of thirty (30) male adult wistar albino rats weighing between 150 g – 250 g were selected and used for the study. They were randomly divided into six (6) groups of five (5) wistar albino rats each. A suspension of the fraction was prepared in Tween - 80 and different doses of methanol fraction of *Mangifera haden* seeds (200, 400, 600 mg/kg body weight) were administered to the animals for 14 consecutive days.

Curative Model

Group 1: Normal control
Group 2: (Carbon tetrachloride, CCl_4 (1.0 ml/kg body weight)
Group 3: CCl_4 + 200 mg/kg body weight of fraction of *Mangifera haden* seeds
Group 4: CCl_4 + 400 mg/kg body weight of fraction of *Mangifera had*en seeds
Group 5: CCl_4 + 600 mg/kg body weight of fraction of *Mangifera haden seeds*
Group 6: CCl_4 + 10 mg/kg body weight of Simvastatin

2.6.1 Induction of Hyperlipidemia

Hyperlipidemia was induced in rats with a dose of 1.0 ml/kg body weight of CCl_4. Carbon tetrachloride (CCl_4) in its concentrated form was dissolved in olive oil in the ratio of 1:1 (v/v) prior to administration to all groups with the exception of normal control group. Carbon tetrachloride was injected as a double dose via intra – peritoneal route on days 1 and 2, the methanol fraction of *Mangifera haden* seeds were administered from days 3 to 14. On Day 14, the animals were sacrificed following an overnight fast and blood samples collected via occular puncture with sterile syringes directly into neatly labelled plain sample tubes.

2.7 Lipid Profile Tests

2.7.1 Determination of Total Cholesterol Concentration

The concentration of total cholesterol was determined using the method of Abell *et al.*[19]

Test procedure

Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample respectively. To the blank, was added 10 µl distilled H_2O, 10 ul standard specimen to the standard test tube and 10 µl sample (serum) to the sample test tube. To each of these test tubes was added 1000 µl of the cholesterol reagent. It was thoroughly mixed and incubated for 10 minute at room temperature (20-25^{0}C). The absorbance of the sample (A sample) against the blank was taken within 60 minute at 500 nm.

$$\text{Conc. of cholesterol in sample (mg/dl)} = \frac{\Delta A_{sample} x \text{ conc. of standard}}{\Delta A_{standard}}$$

Where Concentration of the standard = 202.65 mg/dl

2.7.2 Determination of Low-Density Lipoprotein Concentration

The concentration of low density lipoprotein (LDL) was determined using the method of Friedwald *et al.*[20]

Calculation

LDL-C (mg/dl) = Total Cholesterol –High Density Lipoprotein – Triglyceride/5.

2.7.3 Determination of High-Density Lipoprotein Concentration

The concentration of high-density lipoprotein (HDL) was determined using the method of Toth *et al*.[21]

Procedure

The precipitant solution (0.1 ml) was added to 0.3 ml of the serum sample and mixed thoroughly and allowed to stand for 15 minute. This was centrifuged at 2,000 x g for 15 minute. The cholesterol concentration in the supernatant was determined.

Calculation

$$\text{Concentration of HDL cholesterol in sample} = \Delta A\text{sample} \times \frac{\text{concentration of Standard}}{\Delta \text{ Astandard}}$$

Where concentration of the standard = 52.5 mg/dl.

2.7.4 Determination of Triacylglycerol Concentration

The concentration of triacylglycerol (TAG) was determined using the method of Otvos.[22]

Method: A quantity of the sample (0.1 ml) was pipetted into a clean labelled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 minute. The supernatant was decanted and reserved for use. The assay procedure was carried out as shown below. Three test tubes were set up and labelled as blank, standard and sample. To the blank test tube was added distilled water (0.5 ml), TCA (0.5 ml) and reagent mixture (1 ml). To the standard tube was added, standard solution (0.5 ml), TCA (0.5 ml) and reagent mixture (1 ml) while the sample tube was filled

with 1 ml of the supernatant and 1 ml of the reagent mixture. The mixtures were allowed to stand for 20 minute at 25 ^{0}C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

Calculation: The concentration of triacylglycerol in serum was calculated as follows.

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{standard conc.}}{1} \text{ (mg/dl)}$$

Where concentration of the standard = 196 mg/dl

2.7.5 Determination of Atherogenic Index

Atherogenic index of methanol fraction was determined using the method of Chakraborty *et al.*[23]

$$\text{Atherogenic Index} = \frac{\text{TC - Total serum HDL-C}}{\text{Total serum HDL-C}}$$

Where TC = Total Cholesterol and HDL-C = High Density Lipoprotein Cholesterol

2.7.6 Determination of Percentage Protection of Methanol Fraction

The % protection of methanol fraction against hyperlipidemia and liver damage was determined using the method of Dhandapani.[24]

$$\% \text{ Protection} = \frac{\text{AI of control – AI of treated group}}{\text{AI of Control}} \times 100$$

Where AI of Control = Atherogenic Index of Control

And AI of the treated group = Atherogenic Index of the treated group.

2.8 Blood Collection and Serum Preparation

After 24 hr CCl_4 administration, all animals were euthanized by diethyl ether and blood samples were collected promptly from occular puncture. The collected blood samples were centrifuged at 2500 rpm at room temperature for 20 minute and serum was separated. The serum was carefully pipetted into another set of sterile plain tubes and stored in the refrigerator for further biochemical analysis.

2.9 Statistical Analysis

The biochemical data obtained from the study were analysed using IBM statistical product and service solutions (SPSS) version 20, and the results were expressed as Mean ± Standard deviation. Statistical difference between means were obtained using one-way analysis of variance (ANOVA), followed by Post Hoc Multiple Comparison Test (PHMCT). ($P < 0.05$) was considered statistically significant.

3.0 RESULTS

3.1 Ferric Reducing Antioxidant Power (FRAP) of methanol fraction of *Mangifera haden* seeds

The Ferric Reducing Antioxidant Power (FRAP) of methanol fraction of *Mangifera haden* seeds are shown in table 1 below. The higher the concentrations of the fraction, the higher the absorbance recorded.

Table 1: Ferric Reducing Antioxidant Power (FRAP) of methanol fraction of *Mangifera haden* seeds.

	FRAP Assay	
	Absorbance at 700nm	
Concentrations (µg/ml)	**Methanol**	**Standard**
31.25	0.39 ± 0.17^{a}	1.28 ± 0.02^{a}
62.50	1.09 ± 0.05^{b}	1.57 ± 0.02^{b}
125	$1.23 \pm 0.06^{b,c}$	1.61 ± 0.02^{b}
250	1.31 ± 0.01^{c}	1.66 ± 0.02^{b}
500	1.50 ± 0.04^{d}	1.64 ± 0.13^{b}

Standard (Ascorbic Acid); Results are expressed as Means ± SD (n = 3)

Mean values with different superscripts (a,b,c,d) down the column are considered significantly different at $P < 0.05$.

3.2 Total Antioxidant Capacity (TAC) of methanol fraction of *Mangifera haden* seeds

The Total Antioxidant Capacity (TAC) of methanol fraction of *Mangifera haden* seeds are shown in table 2 below. The higher the concentrations of the fraction, the higher the absorbance recorded. The IC_{50} of methanol fraction was 3.13 at a correlation of 0.7766 while the standard (ascorbic acid) is 1.87 at a correlation of 0.9805. Since, the lower the IC_{50}, the higher the antioxidant capacity, thus methanol fraction exhibited a high amount of total antioxidant capacity (TAC).

Table 2: Total Antioxidant Capacity (TAC) of methanol fractions of *Mangifera haden* seeds.

	TAC Assay	
	Absorbance at 695nm	
Concentrations (µg /ml)	**Methanol**	**Standard**
31.25	0.008 ± 0.001^{a}	0.24 ± 0.02^{a}
62.50	0.014 ± 0.004^{b}	0.47 ± 0.03^{b}
125	0.018 ± 0.002^{b}	0.56 ± 0.04^{c}
250	0.024 ± 0.0034^{c}	0.86 ± 0.03^{d}
500	0.059 ± 0.003^{d}	1.07 ± 0.03^{e}

IC_{50}	3.13	1.87
ARP	31.94	53.48

Standard (Ascorbic Acid); Anti Radical Power (ARP)
Results are expressed as Means ± SD (n = 3).
Mean values with different superscripts (a,b,c,d) down the column are considered significantly different at P < 0.05.

3.3: DPPH Radical Scavenging Power of methanol fraction of *Mangifera haden* seeds

Table 3 shows the DPPH radical scavenging power of methanol fraction of *Mangifera haden* seeds. For DPPH radical scavenging activity, the higher the concentration of the fraction, the higher the % inhibition of DPPH radical. Thus, at the highest concentration 500 µg/ml, the best efficacy of the fraction was exhibited. The IC_{50} of methanol fraction was 1.08 at a correlation of 0.8214. Since the percentage inhibition has direct relationship with the concentrations of the fraction. The lower the $IC_{50,}$ the higher the antioxidant capacity and the higher the $IC_{50,}$ the lower the antioxidant potential. It was asserted that methanol fraction has high antioxidant potential.

The Anti-Radical Power (ARP) of methanol and standard (ascorbic acid) were 92.59 and 62.11 respectively. The higher the (ARP), the more potent the fraction. Methanol fraction has a very high Anti-Radical Power of 92.59.

Table 3: DPPH Radical Scavenging Power of Methanol Fraction.

	DPPH Assay	
	Absorbance at 517nm	
Concentrations (µg/ml)	% Inhibition (Methanol)	% Inhibition (Standard)
31.25	82.86 ± 2.02^{b}	64.19 ± 1.34^{a}
62.50	89.02 ± 2.91^{c}	63.01 ± 0.78^{a}
125	$91.30 \pm 1.41^{c,d}$	63.30 ± 0.68^{a}
250	77.51 ± 0.80^{a}	64.12 ± 0.72^{a}
500	95.85 ± 3.04^{e}	64.39 ± 0.50^{a}
IC_{50}	1.08	1.61
ARP	92.59	62.11

(Results are expressed as Means ± SD; n = 3);
Standard (Ascorbic Acid); ARP (Anti-Radical Power);
Mean values with different superscripts (a,b,c,d,e) down the column are considered significantly different at P < 0.05.

3.4: Acute Toxicity of methanol fraction of *Mangifera haden* seeds

The acute toxicity studies of methanol fraction of *Mangifera haden seeds* were shown in Table 4. Acute toxicity test showed no death or adverse reaction up to 5000 mg/kg body weight.

Table 4: Phase I and II of the acute toxicity test

Phase/Groups	Dosage of fraction (mg/kg b.w)	Mortality rate
Phase 1		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

n = 3

3.5 Effect of methanol fraction of *Mangifera haden* seeds on the lipid profile of CCl_4-Intoxicated Rats

Table 5 shows the effect of methanol fraction of *Mangifera haden* seeds on lipid profile of CCl_4-intoxicated rats. At the administration of $CCl_{4,}$ there was a significant ($p<0.05$) increase in the concentration of Total cholesterol (TC), Triacylglycerol (TAC), Low Density Lipoprotein (LDL-C) and significant ($p<0.05$) decrease in HDL-C as seen in (CCl_4 administered group) when compared to that of the normal control group. Groups that were treated with fractions and drugs (group 3 - 6) showed a significant ($p<0.05$) reduction in TC, TAG, LDL and significant ($p<0.05$) increase in HDL-C when compared to that of the untreated group (group 2).

Table 5: Effect of methanol fraction of *Mangifera haden* seeds on lipid profile of CCl_4-Intoxicated Rats.

Treatment Group	TC (mg/dl)	TAG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Group 1	5.86 ± 0.04^{d}	$1.02 \pm 0.06^{a,b}$	3.29 ± 0.12^{c}	2.36 ± 0.12^{c}
Group 2	7.94 ± 0.04^{e}	1.41 ± 0.07^{c}	6.34 ± 0.07^{e}	1.32 ± 0.05^{a}
Group 3	5.07 ± 0.03^{b}	0.85 ± 0.04^{a}	2.63 ± 0.10^{b}	2.27 ± 0.09^{c}
Group 4	5.68 ± 0.05^{c}	0.94 ± 0.03^{b}	3.50 ± 0.12^{d}	1.99 ± 0.12^{b}
Group 5	5.65 ± 0.04^{c}	1.0 ± 0.07^{b}	$3.38 \pm 0.09^{c,d}$	2.06 ± 0.08^{b}
Group 6	4.92 ± 0.05^{a}	0.84 ± 0.07^{a}	2.42 ± 0.12^{a}	2.33 ± 0.10^{c}

(Results are expressed as Means ± SD; n=5); Mean values with different superscripts (a,b,c,d,e) down the column are significantly different from each other at P<0.05.

3.6: Atherogenic Index and percentage protection of methanol fraction of *Mangifera haden* seeds

Table 6 shows the atherogenic index and percentage protection of methanol fraction of *Mangifera haden* seeds. Atherogenic index was significantly ($p<0.05$) higher in CCl_4 untreated group when compared to that of the normal control group. However, those of the treated groups (group 3-6) were significantly ($p<0.05$) decreased in atherogenic index levels compared to the CCl_4 untreated group. Group 6 (standard drug) exhibited the lowest atherogenic index of (1.11) and the highest percentage protection (77.84%) on the cell membrane of the animal.

Table 6: Atherogenic Index and % Protection of methanol fraction.

Treatment Groups	Atherogenic Index	Percentage Protection
Group 1	1.49 ± 0.13^{c}	--------
Group 2	5.01 ± 0.22^{f}	---------
Group 3	1.24 ± 0.09^{b}	75.25
Group 4	1.86 ± 0.17^{e}	62.87
Group 5	1.74 ± 0.09^{d}	65.27
Group 6	1.11 ± 0.10^{a}	77.84

(Results are expressed as Means ± SD; n=5);
Mean values with different superscripts (a,b,c,d,e) down the column are considered significantly different at $P < 0.05$.

DISCUSSION

Substances with high antioxidant capacities have the tendency to release its electrons and mop up reactive oxygen species and free radicals. This eliminate a lot of diseases from the body and so individuals can live a healthy life. Total Antioxidant Capacity (TAC) assay is also known as Phosphomolybdenum assay (PM). TAC assay is a quantitative method used to investigate the reduction reaction rate among antioxidants, oxidants and molybdenum ligand which gives a direct estimation of reducing capacity of antioxidant. In this study, for Total Antioxidant Capacity (TAC), the higher the concentrations of fraction of *Mangifera haden* seeds, the higher the absorbance. Antioxidant activity depends on the presence of its bio-active compounds mainly phenolics, flavonoids, triterpenes, alkaloids and coumarins. This suggests that the concentration of the bioactive compounds present in the fraction is important to showing antioxidant activity. Thus, higher concentration of the fraction shows higher antioxidant activity. The fraction showed a good total antioxidant activity that increased with increasing concentration. The methanol fraction radical scavenging activity was shown to be at IC_{50} value of (3.13 µg/ml) in comparison to the IC_{50} value of ascorbic acid the standard (1.87µg/ml).

The Anti-Radical Power (ARP) is defined as the reciprocal of IC_{50} multiply by 100. ARP is used to show the capacity of an antioxidant. The higher the ARP, the more potent the sample. The ARP of methanol fraction was remarkably increased and this indicates high antioxidant capacity of the fraction. The results of Abdurahman *et al.*,[25] is quite similar to this study, whose research on the total antioxidant capacity of the aqueous, butanol and petroleum ether extracts of *Irvingia gabonensis* at 100, 125 and 250 µg/ml were significantly ($p<0.001$) reduced compared to ascorbic acid. The work of Matsinkousoh *et al.*[26] is in consonant with this study who reviewed the antioxidant activity of the kernel peel of African mango (*Irvingia wombolu*) and the lower the IC_{50} value, the higher the radical scavenging activity.

The Ferric Reducing Antioxidant Power (FRAP) assay is a novel method used to assess the reduced concentration of ferric ion. It is based on the redox antioxidant reaction that measures the reducing potency of fraction and standard antioxidant (Phatak and Hendre.[27] Higher absorbance indicates higher reducing potency. In this study, increased concentrations showed increased absorbance of free radicals. Thus, at 500 µg/ml concentration, the highest reducing potency in the fraction were exhibited. In this study, the fraction showed a good concentration-dependent reducing power, which was consistent with the findings of Ewere *et al.*[28] that documented that the reducing power of *Irvingia gabonensis* (O' Rorke) baill ethanolic leaf extract increased in a concentration dependent manner. The results of Abdurahman *et al.*[25] is quite similar to this

study, who studied the ferric ion reducing antioxidant activity of the aqueous, butanol and petroleum ether extracts of *Irvingia gabonensis* at 100, 125 and 250 µg/ml were significantly ($p<0.001$) reduced compared to ascorbic acid.

DPPH method is a spectrophotometric procedure and quick method to analyze the free radical activity of natural compounds (Shahzor *et al.*[29] The antioxidant activity of a substance can be expressed as its ability to scavenge the DPPH free radical. It is a rapid, simple, inexpensive and widely used method to measure the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods, extract and fraction. For DPPH radical scavenging activity, the higher the concentrations, the higher the % inhibition of the fraction. Thus, at a higher concentration, the higher efficacy of the fraction were exhibited. This is due to the fact that the fraction have more tendency to donate electrons to mop up the free radicals. However, impurities could likely be the cause of decrease percentage inhibition in some fraction. This is due to the fact that impurities are bad absorber and bad emitter (radiator) of electrons. According to Iwu *et al.*[30], phenolic compounds act as electron donors and are readily oxidized to form phenolate ions, this gives rise to protonated phenol which is used as a cleaning agent. IC_{50} is the minimum concentration of the fraction(s) that can inhibit 50% of the free radicals. IC_{50} values are typically expressed as molar concentration. The IC_{50} of methanol fraction was 1.08 at a correlation of 0.8214. Since the inhibition of the DPPH free radicals has direct relationship with the concentrations of the fractions. The higher the concentrations, the higher the % inhibition of the DPPH free radicals. The results of Abdurahman *et al.*[25] is comparable to this study. This is because the DPPH radical scavenging activity of the aqueous, butanol and petroleum ether extracts of *Irvingia gabonensis* at 100, 125 and 250 µg/ml was significantly ($p<0.001$) reduced compared to ascorbic acid. More so, this study is in line with the findings of Ewere *et al.*[28] that established that *Irvingia gabonensis* (O' Rorke) baill ethanolic leaf extract scavenged DPPH radical in a concentration dependent manner and the percentage inhibition of the extract was significantly ($p<0.05$) higher than that of the ascorbic acid (standard) used at the respective concentrations.

This study revealed that all CCl_4 – intoxicated rats displayed hyperlipidemia as shown by their elevated levels of serum Total Cholesterol (TC), Triacylglycerols (TAG), Low Density Lipoproteins (LDL) and reduction in High Density Lipoproteins (HDL) level. The elevation of serum total cholesterol and low density lipoprotein (LDL) cholesterol has been implicated as a primary risk factor for cardiovascular disease. The large increase in serum cholesterol and triacylglycerols is mainly due to an increase in VLDL secretion by the liver. CCl_4 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues. This results to increased blood lipid concentration, increased hepatic synthesis of cholesterol and induction of hyperlipidemia.

The administration of methanol fraction of *Mangifera haden* seeds markedly lowered the levels of serum TC, TAG, LDL-C and increased HDL-C level. The significant reduction in total cholesterol levels observed in the study could be attributed to the enhanced excretion of cholesterol and its catabolism to bile salts due to the presence of unsaturated fatty acids found abundantly in oils from *Mangifera haden* seeds. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids or lipolysis. The hypolipidemic potentials of these fractions may be due to their antioxidant and antihyperlipidemic effects due to the phytochemicals present (Kuyooro *et al.*[31] The present study is in agreement with the work of Osorio – Esquivel *et al.*[32] whose studies on hypercholesterolemic mice with diets supplemented with microwave dehydrated mango powder showed a significant ($p<0.05$) decrease in total serum cholesterol, LDL-c, triacylglyerols and significant increase in HDL-c compared to the hypercholesterolemic mice and the conventional – dehydrated mango powder group. More so, Gururaja *et al.*[33] findings is in tandem with this study who studied on "The methanol extract of *Mangifera indica* showed a significant cholesterol – lowering activity at 90 mg/kg and significant decrease in plasma triacylglycerols". The findings of Kuyooro *et al.*[31], are not consistent with this study, in his work, there was a significant ($p<0.05$) decrease in total cholesterol and LDL cholesterol but no significant change in HDL cholesterol and triacylglycerols. The findings of Arogba and Omada[34] of "Effects of diet substitution with defatted kernels of mango (*Mangifera indica*) and wild mango varieties (*Irvingia gabonensis* and *Irvingia wombolu*) was quite similar with this study since the plasma lipid profile of the animals after 21 days of feeding showed that total cholesterol (TC) and LDL-c decreased with increasing defatted test sample substitution while HDL-c increased.

Atherogenic index is a novel index composed of triacylglycerols and high Density Lipoproteins (HDL-C). It is the strongest marker used to quantify blood lipid levels and an optimal indicator of dyslipidaemia and cardiovascular diseases. Atherogenic index was considerably decreased in plant fraction treated groups when compared to hyperlipidemic group (CCl_4 administered group). At 200 mg/kg of methanol fraction of *Mangifera haden* seeds confers the most protective effect of the plant fraction (75.25%) against hyperlipidemia. However, atherogenic index (A.I) was remarkably decreased in standard drug (simvastanin, 10 mg/kg) when compared to untreated control (CCl_4 administered group). Arogba and Omada[34] findings are also consistent with this study, this is because as the TC, LDL-c and atherogenic index significantly ($p<0.05$) decreased, the HDL-c also significantly ($p<0.05$) increased offering cardio protection to the cell of the

animals. Furthermore, Dzeufiet *et al.*[35] supported this study who demonstrated that the seeds and extracts of *Irvingia gabonensis* reduced total cholesterol, triacylglycerols, LDL-c, atherogenic index and improved HDL-c in diabetic rats.

CONCLUSION

Mangifera haden seeds has shown to possess high antioxidant potency and hypolipidemic activities. Therefore, it is suggested that *Mangifera haden* seeds fraction should be incorporated in foodstuffs because of its rich antioxidant capacities and hypolipidemic potential. Consuming a diet made with this seed kernel will not only nourish the body system but also boost the immune system against infectious diseases.

ACKNOWLEDGEMENT

This study was not supported financially by any body, organization nor grant. Dr. Emeka Osagwa is acknowledged for the technical assitance that guided this research work in Central Research and diagnostic Laboratory, Ilorin, Kwara State, Nigeria.

Competing Interests: Authors have declared that no competing interests exist.

Ethical approval: The Department of Biochemistry, University of Nigeria, Nsukka approved the use of animals for this research study. All the experiments has been examined and approved by the approprioate ethics committee.

REFERENCES

1. Atanu OF, Ikeojukwu A, Owolabi PA, Avwioroko O. Evaluation of chemical composition, *in vitro* antioxidant and antidiabetic activities of solvent extracts of *Irvingia gabonensis* leaves. *Heliyon*, 2022; 8: 1 -10.
2. Krishan M. Patterns of necrosis in liver disease: Clinical liver disease. *A Multimedia Review Journal, AASLD-American Association for liver Disease*, 2017; 10(2): 53-56.
3. Krishna M. Microscopic anatomy of the liver. *Clinical Liver Disease, A Multimedia Review Journal*, 2022; 2(1): 4-7.
4. Susinjan B. Reactive oxygen species and cellular defense system. V. Rani and U.C.S. Yadav (Eds.), *Free Radicals in Human Health and Disease*, 2015; DOI 10.1007/978-81-322-2035-0_2.
5. Thambi PA, John S, Lydia E, Iyer P, Sarah J, Monica SJ. Antimicrobial efficacy of mango peel powder and formulation of recipes using mango peel powder (*Mangifera indica* L.). *International Journal of Home Science*, 2016; (2): 155–161.
6. Abbasi AM, Liu F, Guo X, Fu X, Li T, Liu RH. Phytochemical composition, cellular antioxidant capacity and antiproliferative activity in mango (*Mangifera indica* L.) pulp and peel. *International Journal of Food Science & Technology*, 2017; 52(3): 817–826.
7. Sultana B, Hussain Z, Asif M, Munir A. "Investigation on the antioxidant activity of leaves, peels, stems, bark, and kernel of Mango (*Mangifera indica* L.)," *Journal of Food Science*, 2012; 77(8): 849–852.
8. Kim H, Moon JY, Kim H. Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. *Food Chemistry*, 2010; 121(2): 429–436.
9. Mohan CG, Deepak M. Viswanatha GL. Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*. *Asian Pacific Journal of Tropical Medicine*, 2013; 6(4): 311-314.
10. Shah KA, Patel, MB, Patel RJ, Parmar PK. *Mangifera indica* (Mango). *Pharmacognosy Review*, 2010; 4**(**7): 42-48.
11. Leanpolchareanchai I, Padois K, Falson F, Bavovado R, Pithayanukul P. Microemulsion system for topical delivery of thai mango seed extract: Development, Physicochemical characterization and ex vivo skin permeationstudies. *Molecules*, 2014; 19(11): 17107-17129.
12. Kumar M, Saurabh V, Tomar M, Hasan M, Changan S, Sasi M, Maheshwari C, Prajapati U, Mekhemar M. Mango (*Mangifera indica*) Leaves: Nutritional composition, phytochemical profile and health-promoting bioactivities. Antioxidants, 2021; 10(2): 299-309.
13. Kupchan S, and Tsou G. A novel cytotoxic cucurbitacin 20-acetate from *Datisca glomerata. Journal of Organic Chemistry*, 1973; 38(7): 1420-1421.
14. A.O.A.C Association of Official Analytical Chemists, Official Methods of Analysis of the A.O.A.C., International, 18th ed. Association of Official Analytical Chemists, Gathersburg, MD, 2005.
15. Lorke D. A new approach to practical acute toxicity testings. Archives of Toxicology, 1983; 55: 275-287.
16. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia fruit*, Osong. *Public Health and Research Perspective*, 2013; 4: 246-254.
17. Oyaizu M. Studies on products of browning reaction prepared from glucosamine. Jpn J. Nutr, 1986; 44: 307.
18. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technology, 1995; 28: 25-30.
19. Mishra K, Ojha H, Chaudhury, N. Estimation of antiradical properties of antioxidant using DPPH assay: Critical review and results. *Food Chemistry*, 2012; 130(2): 1036-1043.
20. Abell LL, Levy BB, Brodie BB, Kendall EF. A simplified method for the estimation of total cholesterol in serum and demonstration of its

specificity, Journal of Biological Chemistry, 1952; 195(1): 357-366.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density Lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, 1972; 18: 499-502.
22. Toth LS, Cicchetti D. A developmental psychopathology perspective on child maltreatment. Social and emotional development (Vol 3, 7th eds.) New York, NY: John Wiley, 2013; 35.
23. Otvos J. Supplement: Triglycerides as a risk factor in cardiac heart disease. Clinical cardiology, 1999; 22(2): 11-27.
24. Chakraborty M, Patel JS, Kamath J. Antihyperlipidemic activity of *Divya methipachak* against Triton-induced hyperlipidemia in rats. *International Research Journal of Pharmacy*, 2012; 3(8): 226-229.
25. Dhandapani R. Hypolipaedemic activity of *Eclipta prostrate* (L) leaf extract in atherogenic diet induced hyperlipidemic rats. *Indian Journal of Experimental Biology*, 2007; 45: 617-619.
26. Abdurahman ME, Abubakar A, Onyemelukwe GC. *In-Vitro* antioxidant activities of different stem bark extracts of *Irvingia gabonensis* (Irvingiaceae). *Tropical Journal of Natural Product Research*, 2020; 4(6): 223-227.
27. Matsinkousoh R, Azantsa BKG, Oben J, Dakam W, Ngondi JL. Measurement of antioxidant activity of African mango (*Irvingia wombolu*) kernel peels. *World Journal of Pharmaceutical Research*, 2018; 6(4): 148-162.
28. Phatak RS, Hendre AS. Total Antioxidant Capacity (TAC) of fresh leaves of *kalanchoe pinnata*. *Journal of Pharmacognosy and Phytochemistry*, 2014; 2(5): 32-35.
29. Ewere GE, Uka E, Usunobun U. Phytochemical composition, *in vitro* antioxidant activity and acute toxicity of Irvingia gabonensis (O'Rorke) baill ethanolic leaf extract. *International Journal of Biological Research*, 2016; 4(1): 36-41.
30. Shahzor MD, Gnanaraj C, Haque AT, Iqbal M. Antioxidant and chemopreventive effects of *Nephrolepis biserrata* against carbon tetrachloride-induced oxidative stress and hepatic dysfunction in rats. *Pharmaceutical Biology*, 2015; 53(1): 31-39.
31. Iwu IC, Chijioke-Okere M, Onu UL, Ukaoma A, Onwumere F. Minerals and phytochemical constituents of *Mangifera indica* seed kernel obtained from Eastern Nigeria. *African Journal of Agriculture and food Science*, 2018; 1(2):1-11.
32. Kuyooro ES, Abam OE, Agbede BE. Hypolipidemic effects of *Irvingia gabonensis*-supplemented diets in male albino rats. Biochemistry and Analytical Biochemistry, 2017; 6(2): 316-321.
33. Osorio-Esquivel O, Cortes-Viguri V, Garduno-Siciliano L, Ortiz-Moreno A, Sanchez-Pardo ME. Hypolipidemic activity of micro-wave-dehydrated mango (*Mangifera indica*) powder in mice fed a hypercholesterolemic Diet. Journal of Biomedical Science and Engineering, 2014; 7: 809-817.
34. Gururaja GM, Mundkinajeddu D, Kumar AS, Dethe SM, Agarwal A. Evaluation of cholesterol-lowering activity of standardized extract of *Mangifera indica* in albino wistar rats. Pharmacognosy Research, 2017; 9(1): 21-26.
35. Arogba SS, Omada AA. Effects of diet substitution with defatted kernels of mango (*Mangifera indica*) and wild mango varieties (*Irvingia gabonensis* and *Irvingia wombolu*) on weight and plasma lipid profile of wistar rats. *African Journal of food Science*, 2020; 14(1): 10-15.
36. Dzeufiet D, Ngeutse DF, Dimo T, Tedong L, Nguegim TF, Tchamadeu M, Kamtchouing P. Hypoglycemic and hypolipidemic effects of *Irvingia gabonensis* (*Irvingiaceae*) in diabetic rats. *Pharmacology online*, 2009; 2: 957–962.