Effects of ethyl acetate fraction of *Mangifera haden*seeds on carbon tetrachloride induced hepatotoxicity in albino rats

Authors' Contributions

This work was carried out in collaboration among all authors. Authors MNN, CGI and CSO designed the study, wrote the protocol and supervised the work. Author AJW performed the statistical analysis. Author AB managed the analyses of the study. Authors MNN and OCOwrote the first draft of the manuscript, managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

ABSTRACT

The present study investigated the effects of ethyl acetate fraction of *Mangifera haden*seeds on carbon tetrachloride-induced liver damage in wistar albino rats. The phytochemical compositions and acute toxicity studies of crude extract and *Mangifera haden*seed fraction were determined using standard methods. Albino rats (60) randomly grouped into twelve of five rats each were used for this study. Determination of total bilirubin and protein, lipid peroxidation, liver enzymes marker, antioxidant enzymes, glutathione, lipid profile analysis and histological examination were carried out using standard methods. Phytochemical constituents of crude extract and ethyl acetate fraction of *Mangifera haden* seed showed that they contain relative amount of flavonoids, phenolics, saponins, tannins, terpenoids, triterpenes, alkaloids and coumarins. Liver injury was induced with carbon tetrachloride (CCl_4) at a dose of 1.0 ml/kg body weight of the animals. All the CCl_4 intoxicated rats displayed hyperlipidemia as shown by their elevated levels of serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein (LDL), and reduction in high density lipoprotein (HDL) levels. The administration of ethyl acetate fraction of *Mangifera haden* seeds significantly ($P = 0.05$) lowered the levels of serum TC, TAG, LDL, and increased HDL levels. The level of serum malondialdehyde (MDA) significantly ($P = 0.05$)increased in the CCl_4 group as compared to the normal and treated groups. The elevated level of serum MDA decreased ($P = 0.05$) significantly at the treatment of rats with 200, 400 and 600 mg/kg of ethyl acetate fraction. The rats treated with CCl_4 produced significant ($P = 0.05$) increase in serum ALT, AST and ALP when compared to the normal group. Animals treated with varying doses of ethyl acetate fraction produced significant ($P = 0.05$) reduction of serum ALT, AST and ALP activities when compared to the CCl_4-untreated group. The activities of hepatic antioxidant enzymes (SOD, CAT and GPx) and glutathione concentration significantly ($P = 0.05$) decreased in CCl_4-untreated group compared to the normal group. The decreased activities of the antioxidant enzymes and glutathione concentration in CCl_4 intoxicated rats were ameliorated/modulated more effectively in rats treated with 200, 400 and 600 mg/kg ethyl acetate fraction of *Mangifera haden* seed. Total bilirubin (TB) concentrations significantly ($P = 0.05$) increased in CCl_4-untreated group when compared to the normal group. Oral treatment of rats with varying doses (200, 400, 600 mg/kg) of ethyl acetate fraction caused a significant ($P = 0.05$) decrease in the serum TB levels. Administration of CCl_4 showed a reduction in serum total proteins. Treatment of the animals with 200, 400 and 600 mg/kg of ethyl acetate fraction caused significant ($P = 0.05$) increase in serum total potein. Histological examination showed hepatoprotective properties of plant's fractions. The compounds found in the different fractions (ethyl acetate and n-hexane) of *Mangifera haden* seeds are 9-Octadecenoic acid (Z)-, methyl ester, (Z) –Oleic acid and so on. Therefore the medicinal importance of the plant is been established.

KEYWORDS: *Mangifera haden;* Carbon tetrachloride; Malondialdehyde; Ethyl acetate fraction; Hepatotoxicity.

1.0 INTRODUCTION

Carbon tetrachloride is a potent hepatotoxin used to induce toxicity in experimental models (Zarezade*et al.,* [1].“It is metabolized by cytochrome P_{450} to trichloromethyl and trichloromethyl peroxyl radicals” (Wang *et al.,*[2].“These trichloromethyl radicals bind to the tissue macromolecule to induce peroxidative degradation of endoplasmic reticulum membrane lipids, which are rich in polyunsaturated fatty acids. This development eventually leads to the formation of lipid peroxides, which in turn provide other products, such as malondialdehyde (MDA). These products lead to membrane injury and in extreme cases damages the liver” (Su *et al.,*[3]. “The liver is the largest internal organ and gland in the human body” (Adrian and Uruj, [4]. “It plays a pivotal role in regulating various physiological processes. An imbalance between aggressive and protective forces results in liver damage. It has also been observed that a complex mechanism is involved in the induction of hepatotoxicity by various environmental and chemical agents” (Krishna, [5]. “It is therefore necessary to design pharmacological strategies for their prevention and treatment. Mango (*Mangifera indica*) is a tropical fruit that is rich in a diverse range of bioactive phytochemicals” (Parvez, [6].“It belongs to the family Anarcardiaceae in the order of sapindales. It is called the king of all fruits because of its rich, luscious, aromatic flavour and delicious taste in which sweetness and acidity are delightly mixed” (Neguse *et al.,*[7]. It is grown in many parts of the world, especially in tropical countries. There are more than 1,000 varieties of mango available worldwide, of the available varieties, only a few are grown and traded on a commercial scale. The known varieties in Nigeria are *Mangifera haden*, *Mangifera pirie* and *Mangifera turpentine*. *Mangifera haden* is referred to as Indian large or purple/red/yellow mango (Parvez, [6]. “These are mangoes whose fruits are ready for harvesting when they are fully matured and ready for consumption when ripe. They are usually sour in taste when they are still green, but when ripe they are sweet with a characteristic aroma and taste. Ripe mangoes are a suitable choice for hypertensive patients as they are good sources of potassium and contain traces of sodium” (Parvez, [6]. “Mangoes are highly recommended for pregnant woman and individuals suffering from anaemia because of its iron content” (Yashoda and Prabha, [8]. “Mango products are good complementary food for weaning children as they contain necessary vitamins” (Maldonado – Celis *et al.,* [9]. “Mango has been found to improve the appetite and is an effective antidote for various body toxins” (Parvez, [6]. “The juice helps prevent mental weakness and improves concentration and memory” (Parvez, [6]. “Mango leaves have anti-inflammatory, diuretic and cardiotonic properties” (Kumar *et al.,* [10]. “Dried

and powdered mango leaves are used to expel kidney stones and improve hair growth"(Kumar *et al.,* [10]. "Mango bark is also effective in treating hemotysia, hemorrhaging, nasal catarrh, ulcers, rheumatism and diphtheria" (Parvez, [6]. "The major components of mango seed are starch, fat and protein" (Maldonado – Celis *et al.,* [9]. "Seed kernels are rich in polyphenols with strong antioxidant activity, but ironically, the seeds are discarded as waste during processing and consumption" (Torres-Leon *et al.,* [11]."The mango fruit is classified as a deliquescent drupe; it contains a single seed surrounded by a fleshy mesocarp covered by a fibrous husk. The mango seed consists of a tough shell surrounding the core. The kernels occupy about 17-22% of the fruit. Mango seed oil consists of about 44–48% saturated fatty acids (mainly stearic acid) and 52–56% unsaturated fatty acids" (Torres-Leon *et al.,* [11]. "Mango seed kernels are low in protein but contain a high concentration of essential amino acids, with the highest values of leucine, valine and lysine" (Kumar *et al.,* [10]. "Mango seed kernels have been shown to be a good source of polyphenols and phytoisoterols, especially camesterol, sitosterol and tocopherols" (Torres-Leon *et al.,* [11]. "In addition, mango seed kernel could be used as a potential source of functional food ingredients, antimicrobial compounds and cosmetics due to its high quality of fat and protein as well as high levels of natural antioxidants" (Kumar *et al.,* [10]. "Mango stone obtained after decortication of mango seeds can be used as an adsorbent" (Torres-Leon *et al.,* [11]. "Studies conducted with *Mangifera indica* leaf, bark, and flower extracts have been found to protect hepatocytes, lymphocytes, neutrophils, and macrophages from oxidative stress" (Siaka, [12]. Liver diseases are a major global health problem, with a high prevalence in developing countries. Despite the advances of modern medicine, there is no effective drug available to regenerate liver cells. There are also reports that currently available hepatoprotective drugs are associated with a number of adverse effects. To overcome the adverse effects of these drugs, there is an urgent need to develop new hepatoprotective drugs from natural sources. Plant extracts are relatively non-toxic, safe with little or no side effects. The seeds of *Mangifera haden* are native to Nigeria and are widely used in traditional medicine to treat many ailments, including liver disorders. Despite the folkloric use of *Mangifera haden* seeds, there is little scientific evidence based on a thorough literature search and thus forms the basis of this research.

2.0 Material and Methods

2.1 Plant Material

The plant material are the seeds of *Mangiferahaden.*

2.2 Methods

2.2.1 Collection, Preparation and Extraction of Mango Seeds

The plant material, *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. (InterCEDD Means International Centre for Ethnomedicine and Drug Development). 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.

2.2.2 Partitioning/Fractionation of the extract

The crude extracts of *Mangifera haden seeds* were subjected to solvent – solvent partitioning using protocol designed by Kupchan and Tsou, [13] 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; ethyl acetate and methanol in the order of increasing polarity by using a separating funnel. 350 g of the sample was dissolved in 1400 ml of the solvent (ethyl acetate) 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.

2.2.3 Animal Preparation

Sixty (60) adult male albino wistar rats were used for the study. The animals were acclimatized to the laboratory conditions for a period of seven (7) days. The animals were grouped and housed in aluminium cages and maintained at an ambient temperature (25 $\pm 2^0$C) and relative humidity (40-60%), with 12/12 hours of light and dark cycle in a well –ventilated animal house. Bedding material (saw dust) was removed and replaced as often as necessary to keep the animals clean and dry. The animals were fed with commercial grower's mash feed and water provided *ad libitum*. At the end of the acclimatization period, they were weighed and randomly divided into twelve (12) groups of five animals each, comprising of test animals and control groups.

2.2.4 Qualitative Phytochemical Screening of Crude Extract and Fraction of *Mangifera haden*Seeds

Phytochemical analysis of the extract and fraction of *Mangifera haden*seeds were carried out using the method described by Odebiyi and Sofowora, [14] for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

Alkaloids: A known volume (500 µl) of *Mangifera haden* seed solution, was added 1 ml (1% HCl) in a test tube. The mixture was heated for 20minute, cooled and filtered. The filtrate was

used in the following tests: 2 drops of Wagner's reagent was added to 1 ml of the extracts. A reddish brown precipitate indicates the presence of alkaloids.

Tannins: A known volume (1000 µl) of *Mangifera haden* seed solution, has 1 ml of freshly prepared 10% KOH added. A dirty white precipitate indicates the presence of tannins.

Phenolics: A known volume (1000 µl) of *Mangifera haden* seed solution, was added 2 drops of 5% $FeCl_3$ in a test tube. A greenish precipitate indicates the presence of phenolics.

Glycosides: A known volume (500 µl) of *Mangifera haden* seed solution, has 10 ml of conc. H_2SO_4 added, the mixture was heated in boiling water for 15minute. 10 ml of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.

Saponins: Frothing test: Distilled water (500 µl) was added to 2 ml of extract/fraction in a test tube and was vigorously shaken for 2minute. Frothing indicates the presence of saponins.

Flavonoids: In 3 ml of extract/fraction of *Mangifera haden* seed solution, 1 ml of 10% NaOH was added in a test tube. A yellow colouration indicates the presence of flavonoids.

Steroids: Salakowsti test: *Mangifera haden* seed solution (500 µl), has 5 drops of concentrated H_2SO_4 added in the test tube. Red colouration indicates the presence of steroids.

Phlobatannins: A known volume of (500 µl) *Mangifera haden* seed solution, was added 1 ml of (1% HCl) in a test tube. A red precipitate indicates the presence of phlobatannins.

Triterpenes: A known volume of (500 µl) *Mangifera haden* seed solution, has 5 drops of acetic anhydride added with a drop of concentrated H_2SO_4 and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of 250 ml chloroform. A blue green colour indicates the presence of triterpenes.

Phytosterols (Finar 1986): Liberman-Burchard's test: A known volume of (500 µl) *Mangifera haden*seed solution, was added 50 mg which was dissolved in 2ml acetic anhydride. Two drops of conc. H_2SO_4 was added slowly along the sides of the test tube. An array of colour changes showed the presence of phytosterols.

2.3.Animal Studies

2.3.1 Acute Toxicity test

The mean lethal dose (LD_{50}) for ethyl acetate fraction was determined following the method described by Lorke, [15].

2.3.2 Experimental Design

A total of sixty (60) male adult wistar albino rats weighing between 150 g – 250 g were selected and used for the study. They were randomly divided into twelve (12) groups of five (5) wistar albino rats each. A suspension of the fraction was prepared in Tween - 80 and different doses of ethyl acetate fraction of *Mangifera haden* seeds (200, 400, 600 mg/kg body weight) were administered to the animals for 14 consecutive days.

2.3.3 Carbon Tetrachloride (CCl_4) – Induced Hepatotoxicity

Liver injury was induced in rats with a dose of 1.0 ml/kg body weight of CCl_4 (Aromose*et al*., [16]. Carbon tetrachloride (CCl_4) in its undiluted 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.

Preventive Model

Group 1: Normal control (Distilled water only)
Group 2: (Carbon tetrachloride, CCl_4 (1.0 ml/kg body weight)
Group 3: 200 mg/kg body weight of fraction of *Mangifera haden* + (CCl_4)
Group 4: 400 mg/kg body weight of fraction of *Mangifera haden* + (CCl_4)
Group 5: 600 mg/kg body weight of fraction of *Mangifera haden* + (CCl_4)
Group 6: (Standard control): 100 mg/kg body weight of Silymarin + (CCl_4)

Curative Model

Group 1: Normal control (Distilled water only)
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: (Standard Control) CCl_4 + 100 mg/kg body weight of Silymarin

Preventive model:

The animals were administered ethyl acetate fraction of *Mangifera haden* seeds from day 1 to 12, and were injected with a double dose of carbon tetrachloride via intra-peritoneal route on days 13 and 14 before sacrifice.

Curative study:

Carbon tetrachloride was injected as a double dose via intra – peritoneal route on days 1 and 2, the ethyl acetate 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. The liver organs were collected for preparation of tissue homogenate and histological examination.

2.3.4 Liver Profile Test

2.3.4.1 Assay of Alanine Aminotransferase (ALT) Activity

Alanine aminotransferase (ALT) activity was assayed using the method described by Reitman and Frankel, [17].

Method: The blank and sample test tubes were set up in duplicates. Serum (0.1 ml) was pipetted into the sample tubes. To these were added 0.5 ml buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate. The mixtures were thoroughly mixed and incubated for exactly 30 minute at 37^0C and pH 7.4. A known volume 0.5 ml of reagent containing 2, 4-dinitrophenylhydrazine was later added to both tubes. The tubes were mixed thoroughly and incubated for exactly 20 minute at 25^0 C. Sodium hydroxide solution (5 ml) was then added to each tube and mixed. The absorbance of sample was read against the blank after 5 minute at 540 nm. The activity of ALT was obtained from already calibrated table.

2.3.4.2 Assay of Aspartate Aminotransferase Activity

Aspartate aminotransferase (AST) activity was assayed using the method described by Reitman and Frankel, [17].

Method: The blank and sample test tubes were set up in duplicates. A volume, 0.1 ml of serum was pipetted into the sample tubes and 0.5 ml of (reagent 1) was pipetted into both sample and blank tubes.

The solutions were thoroughly mixed and incubated for exactly 30 minute at 37 ^{0}C and pH 7.4. Reagent 2 containing 2, 4-dinitrophenylhydrazine (0.5 ml) was added into all the test tubes

followed by 0.1 ml of sample into the blank tubes. The tubes were mixed thoroughly and incubated for exactly 20 minute at 25^0C and 5.0 ml of sodium hydroxide solution was then added to each tube and mixed. The absorbance was read against the blank after 5 minute at 546 nm. The activity of AST was obtained from already calibrated table.

2.3.4.3 Assay of Alkaline Phosphatase Activity

This was determined according to Englehardt, [18].

Method: The blank and sample test tubes were set up in duplicates and 0.05 ml of sample was pipetted into the sample test tubes. Distilled water (0.05 ml) was pipetted into the blank tube. A known volume (3 ml) of substrate was pipetted into each tube respectively, which was then mixed and the initial absorbance taken at)5 nm. The stop watch was started and the absorbance of the sample and the blank read again three more times at one minute intervals.

Calculation: alkaline phosphatase activity was calculated as follows:

$$\text{Activity of ALP (in U/l)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x } \frac{3300}{1}$$

2.3.5 Other Biochemical Analysis

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 analyses. The serum was used for the determination of alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), (Reitman and Frankel, [17], alkaline phosphatase (ALP) (Englehardt, [18], total bilirubin (Jendrassik and Grof, [19], total protein (Biuret Method), Malondialdehyde concentration (Wallin *et al*., [20], SOD activity (Xin *et al*., [21], Catalase activity (Aebi, [22], Glutathione Peroxidase activity (Wood, [23], and Glutathione activity (King and Wootton, [24]. The concentration of Total Cholesterol was determined using the method of Abell *et al*., [25], High-Density lipoprotein was determined by the method of Toth *et al*., [26], Triacylglycerol was determined by method of Otvos, [27], Low Density lipoprotein was determined by the method of Friedwald *et al.,* [28]. The liver was dissected and excised immediately. A section of the left lobe of the liver was used for histological studies, and the remaining section were frozen quickly and stored at -80^0 C for biochemical analysis.

2.4 Histological Examination of liver

Tissue Preparation

Sections of the liver were collected for histological examination. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hr. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5 µm thick with a rotary microtome, floated in water bath and incubated at 60˚C for 30 minute. The 5 µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minute. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

2.5Statistical 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: Qualitative Phytochemical Composition of Crude extract and Fraction of *Mangifera haden* Seeds

The phytochemical constituents of crude extract and ethyl acetate fraction of *Mangifera haden* seeds as presented in table 1 showed that they contain relative amount of flavonoids, phenolics, saponins, tannins, terpenoids, triterpenes, alkaloids and coumarins. Terpenoids, glycosides and saponins were present in small amount in the crude extract and ethyl acetate fraction. Alkaloids, coumarin, tannin and phenolics were present at moderate amounts while flavonoids and triterpenes were present in high quantity in the crude extract and ethyl acetate fraction.

Table 1: Qualitative phytochemical composition of crude extract and ethyl acetate fraction of *Mangifera haden* seeds

Phytochemical Constituents	Crude Extracts	Ethyl Acetate Fraction
P**henolics**	++	++

Saponins	+	+
Tannins	+	++
Flavonoids	+++	+++
Coumarins	++	++
Triterpenes	+++	+++
Anthocyanins	N.D	N.D
Steroids	N.D	N.D
Glycosides	+	++
Amino acid	N.D	N.D
Phlobatannin	N.D	N.D
Alkaloids	++	++
Terpenoids	+	+

Keys

+ Present in low concentration

++ Present in moderate concentration

+++ Present in high concentration

N.D Not Detected

3.2 Effect of ethyl acetate fraction of *Mangifera haden* seeds on Alanine Aminotransferase (ALT) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 2 shows significant (*P*=0.05) increase in serum ALT levels in the CCl_4 untreated group as compared to that of the normal control group. The ALT values were significantly (*P*=0.05) decreased with the treatment of ethyl acetate fraction at all dose levels (200, 400, 600 mg/kg) as compared to that of the CCl_4 untreated group. The standard drug (silymarin, 100 mg/kg) significantly (*P*=0.05) decreased the serum ALT values when compared to the CCl_4 untreated group. Hence, the reduction in ALT by the fraction was dose dependent.

Table 2: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Alanine Aminotransferase (ALT) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	ALT (IU/dl) Ethyl acetate Fraction (Preventive Model)	ALT (IU/dl) Ethyl acetate Fraction (Curative Model)
Group 1	61.73 ± 2.49^{a}	62.69 ± 4.60^{a}
Group 2	72.52 ± 1.69^{b}	71.05 ± 9.31^{b}

Group 3	61.27 ± 6.28[a]	59.25 ± 2.67[a]
-Group 4	58.76 ± 2.40[a]	61.16 ± 5.89[a]
Group 5	58.29 ± 3.35[a]	61.06 ± 1.60[a]
Group 6	59.90 ± 2.74[a]	59.50 ± 8.64[a]

(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.

3.3: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Aspartate Aminotransferase (AST) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 3 reveals that CCl_4 untreated rats (Group 2) was significantly (*P*=0.05)higher in serum AST activity as compared to that of the normal control group. Treatment with the dose of (200, 400, 600 mg/kg) of ethyl acetate fraction significantly(*P*=0.05) decreased the serum AST activity when compared to that of the CCl_4 untreated group (group 2). The standard drug (silymarin, 100 mg/kg) significantly (*P*=0.05)lowered serum AST activity when compared to that of the CCl_4 untreated group (group 2).

Thus, at the dose of 400 mg/kg ethyl acetate fraction of *Mangifera haden* seeds (curative model) exhibited the greatest significant reduction of serum AST activity at (251.85 IU/dl) when compared to other doses in both model.

Table 3: Effect of Ethyl acetate Fraction of *Mangifera haden* Seeds on Aspartate Aminotransferase (AST) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	AST (IU/dl) Ethyl-acetate fraction (Preventive Model)	AST (IU/dl) Ethyl-acetate fraction (Curative Model)
Group 1	260.65 ± 5.87[a]	261.08 ± 3.71[a]
Group 2	279.48 ± 6.59[b]	280.01 ± 5.15[b]
Group 3	252.00 ± 8.10[a]	262.66 ± 7.25[a]
Group 4	259.71 ± 5.90[a]	251.85 ± 5.46[a]
Group 5	255.81 ± 11.21[a]	255.35 ± 24.86[a]
Group 6	257.41 ± 4.74[a]	246.04 ± 10.67[a]

(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).

3.4: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Alkaline phosphatase (ALP) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 4 shows that serum ALP activity of CCl_4 – intoxicated rats (Group 2) was significantly (*P*=0.05) higher than that of the normal control group. Treatment with ethyl acetate fraction of *Mangifera haden* seeds at doses of 200, 400 and 600 mg/kg significantly(*P*=0.05) decreased the serum ALP activity as compared to that of the untreated group. The standard drug (silymarin, 100 mg/kg) significantly (*P*=0.05) reduced serum ALP activity when compared to the CCl_4 untreated group.

At 400 mg/kg ethyl acetate fraction (preventive model) exhibited the most significant (*P*=0.05)reduction of serum ALP when compared to plant's fraction in both model.

Table 4: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Alkaline Phosphatase (ALP) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	ALP(IU/dl) Ethyl acetate fraction (Preventive Model)	ALP (IU/dl) Ethyl acetate fraction (Curative Model)
Group 1	45.97 ± 1.29^{a}	$45.75 \pm 1.57^{a,b}$
Group 2	47.99 ± 1.24^{b}	47.78 ± 1.06^{b}
Group 3	44.64 ± 0.79^{a}	45.06 ± 1.24^{a}
Group 4	44.24 ± 2.43^{a}	43.36 ± 2.36^{a}
Group 5	44.34 ± 1.33^{a}	44.58 ± 1.78^{a}
Group 6	44.23 ± 1.17^{a}	44.50 ± 1.75^{a}

(Results are expressed as Means ± SD; n = 5)

Mean values having different letters as superscripts down the column are considered significant at (*P*=0.05)

3.5: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Total Bilirubin (TB) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 5 shows that total bilirubin (TB) concentrations significantly(*P*=0.05) increased at the administration of CCl_4 in the CCl_4 untreated group when compared to that of the normal control group. Oral treatment of the rats with the doses of 200, 400, and 600 mg/kg ethyl acetate fraction of *Mangifera haden* seeds caused a significant (*P*=0.05) decrease in the levels of serum TB as compared to the CCl_4 –untreated group. The standard drug (silymarin, 100 mg/kg)

significantly (*P*=0.05)decreased total bilirubin concentrations when compared to CCl_4 untreated group.

At the treatment of the animals with 200 mg/kg ethyl acetate fraction of *Mangifera haden* seeds (curative model) exhibited the highest protection against total bilirubin in the membrane of the animals.

Table 5: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Total Bilirubin (TB) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment group	Total Bilirubin Ethyl Acetate Fraction (mg/dl) (Preventive Model)	Total Bilirubin Ethyl Acetate Fraction (mg/dl) (Curative Model)
Group 1	$0.68 \pm 0.07^{c,d}$	$0.64 \pm 0.02^{b,c}$
Group 2	0.71 ± 0.02^{d}	0.74 ± 0.09^{c}
Group 3	$0.56 \pm 0.10^{a,b}$	0.50 ± 0.11^{a}
Group 4	0.52 ± 0.12^{a}	$0.58 \pm 0.87^{a,b}$
Group 5	$0.58 \pm 0.06^{a,b,c}$	0.71 ± 0.08^{c}
Group 6	$0.64 \pm 0.05^{b,c,d}$	$0.57 \pm 0.09^{a,b}$

(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: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Total Protein (TP) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 6 shows that the total protein of CCl_4 untreated group was significantly (*P*=0.05) decreased compared to that of the normal control group. Treatment of the animals with the doses of 200, 400, 600 mg/kg significantly(*P*=0.05) increased total protein of the animals as compared to that of the CCl_4 untreated group. Administration of ethyl acetate fraction (preventive and curative model) at the dose of (200, 400 mg/kg), significantly (*P*=0.05) increased serum total protein while treatment with 600 mg/kg, caused significant (*P*=0.05) decreased in serum total protein (4.74 mg/dl) of the animals as compared to that of the

CCl_4untreated group. At the treatment of the animals with standard drug (silymarin, 100 mg/kg), significantly(*P*=0.05) increased serum total protein when compared to that of the CCl_4 untreated group.

The dose of 400 mg/kg of ethyl acetate fraction of *Mangifera haden* seeds (preventive model) exhibited the most significant(*P*=0.05) increase in serum total protein when compared to other doses in both model including the standard drug.

Table 6: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Total Proteins (TP) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	Total Protein(mg/dl) Ethyl Acetate fraction (Preventive Model)	Total Protein (mg/dl) Ethyl acetate fraction (Curative Model)
Group 1	7.02 ± 0.10^{d}	$6.64 \pm 0.19^{b,c}$
Group 2	4.98 ± 0.19^{b}	4.80 ± 0.03^{a}
Group 3	6.27 ± 0.23^{c}	6.68 ± 0.19^{c}
Group 4	6.99 ± 0.11^{d}	$6.53 \pm 0.11^{b,c}$
Group 5	4.74 ± 0.14^{a}	6.33 ± 0.50^{b}
Group 6	6.97 ± 0.12^{d}	6.73 ± 0.19^{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).

3.7: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Malondialdehyde (MDA) concentrations of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 7 shows that the concentrations of serum MDA significantly (*P*=0.05)increased in the CCl_4 untreated group as compared to that of normal control group. The elevated level of serum MDA was significantly(*P*=0.05) decreased by the treatment of rats with 200, 400 and 600 mg/kg of ethyl acetate fraction of *Mangifera haden*seeds as compared to the CCl_4 administeredgroup. In this study, the standard drug (silymarin, 100 mg/kg) produced a significant (*P*=0.05) reduction in MDA concentrations when compared to the CCl_4 untreated group. This is comparable to the normal control group.

At 200 mg/kg of ethyl acetate fraction (curative model) exhibited the best protective efficacy against lipid peroxides at 3.14 mg/dl when compared to that of CCl_4 untreated group.

Table 7: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Malondialdehyde (MDA) concentrations of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	Malondialdehyde (MDA) Ethyl acetate Fraction(mg/dl) (Preventive Model)	Malondialdehyde (MDA) Ethyl Acetate Fraction(mg/dl) (Curative Model)
Group 1	3.26 ± 0.08^{a}	3.37 ± 0.08^{c}
Group 2	4.59 ± 0.10^{c}	4.74 ± 0.16^{d}
Group 3	3.29 ± 0.20^{a}	$3.14 \pm 0.09^{a,b}$
Group 4	3.16 ± 0.17^{a}	$3.25 \pm 0.10^{b,c}$
Group 5	3.50 ± 0.18^{b}	3.34 ± 0.12^{c}
Group 6	$3.33 \pm 0.06^{a,b}$	3.10 ± 0.06^{a}

(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).

3.8: Effect of ethyl acetate fraction of *Mangifera haden* Seeds on Superoxide Dismutase (SOD) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 8 shows that the serum SOD activity of CCl_4 untreated group (group 2) was significantly(*P*=0.05) decreased compared to that of the normal group for both the preventive and curative models. The activities of serum SOD were significantly (*P*=0.05) increased by treatment with ethyl acetate fraction of *Mangifera haden* seeds at (200, 400, 600 mg/kg) doses. Also, the standard drug (silymarin, 100 mg/kg) significantly(*P*=0.05) increased the serum SOD activity when compared to that of the CCl_4 untreated group (group 2).

The administration of 200 mg/kg ethyl acetate fraction of *Mangifera haden* seeds (curative model), exhibited the most significant (*P*=0.05)increase in SOD activity (12.47 IU/dl) when compared to all the treated groups including the standard drugs.

Table 8: Effect of Ethyl acetate Fraction of *Mangifera haden* Seeds on Superoxide Dismutase (SOD) of CCl_4-Intoxicated Rats (Preventive and Curative Model)

Treatment group	SOD(IU/dl) Ethyl acetate fraction (Preventive Model)	SOD(IU/dl) Ethyl acetate fraction (Curative Model)
Group 1	12.64 ± 0.17^{b}	12.63 ± 0.36^{b}
Group 2	8.88 ± 1.13^{a}	8.14 ± 1.42^{a}

Group 3	12.27 ± 0.56[b]	12.47 ± 0.33[b]
Group 4	11.93 ± 0.62[b]	11.79 ± 0.56[b]
Group 5	12.40 ± 0.33[b]	11.99 ± 0.67[b]
Group 6	12.42 ± 0.48[b]	12.16 ± 0.68[b]

(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).

3.9: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Catalase (CAT) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 9 shows that there was significant (*P*=0.05)reductionof serum CAT activity of CCl_4 untreated group compared to that of the normal control group for both the preventive and curative models. The decreased activities of CAT activity were significantly (*P*=0.05)increased by treatment with ethyl acetate fraction of *Mangifera haden* seeds at 200, 400 and 600 mg/kg compared to that of the CCl_4 untreated group. Also, the standard drug (silymarin, 100 mg/kg) significantly(*P*=0.05) increasedserum CAT activity when compared to that of the CCl_4 untreated group.

At the administration of 400 mg/kg ethyl acetate fraction of *Mangifera haden* seeds (curative model) exhibited the most significant (*P*=0.05)increase in serum CAT activity among the treated groups in both models.

Table 9: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Catalase (CAT) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	CAT(IU/dl) Ethyl acetate fraction (Preventive model)	CAT (IU/dl) Ethyl acetate fraction (Curative Model)
Group 1	4.75 ± 0.16[d]	4.81 ± 0.13[d]
Group 2	3.09 ± 0.20[a]	3.06 ± 0.22[a]
Group 3	4.33 ± 0.16[c]	4.09 ± 0.06[b]
Group 4	4.02 ± 0.20[b]	4.42 ± 0.16[c]
Group 5	4.11 ± 0.17[b,c]	4.36 ± 0.14[c]
Group 6	4.78 ± 0.16[d]	4.48 ± 0.17[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).

3.10: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Glutathione Peroxidase (GPx)activity of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 10 shows that serum GPx activity of CCl_4 untreated group (group 2) significantly(*P*=0.05) lowered compared to that of the normal control group for both preventive and curative model. Treatment of the animals with doses of 200, 400, 600 mg/kg of ethyl acetate fraction significantly (*P*=0.05) increased serum GPx activity compared to that of the CCl_4 untreated group (group 2). The standard drug (silymarin, 100 mg/kg) significantly (P<0.05) increased serum GPx activity compared to that of the CCl_4 untreated group (group 2).

Treatment with 400 mg/kg ethyl acetate fraction of *Mangifera haden* seeds (curative model) showed the most significant(*P*=0.05) increase in Glutathione Peroxidase (GPx) activity compared to all groups in both models.

Table 10: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Glutathione Peroxidase (GPx) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Groups	GPx(IU/dl) Ethyl acetate fraction (Preventive Model)	GPx(IU/dl) Ethyl acetate fraction (Curative Model)
Group 1	98.26 ± 0.47^{d}	96.65 ± 2.39^{d}
Group 2	71.98 ± 1.34^{a}	70.94 ± 0.97^{a}
Group 3	$94.42 \pm 1.61^{c,d}$	92.04 ± 3.45^{b}
Group 4	89.15 ± 1.04^{b}	$95.92 \pm 3.25^{c,d}$
Group 5	$90.64 \pm 7.94^{b,c}$	$92.58 \pm 3.36^{b,c}$
Group 6	98.17 ± 0.69^{d}	96.70 ± 1.70^{d}

(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).

3.11: Effect of ethyl acetate fraction of *Mangifera haden* Seeds on Glutathione (GSH) of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 11 shows that serum GSH activity of CCl_4 untreated group was significantly (*P*=0.05)decreased compared to that of normal control group. However, the activities of serum GSH were significantly (*P*=0.05) increased by treatment with ethyl acetate fraction of *Mangifera*

*haden*seeds at (200, 400, 600 mg/kg) when compared to that of the CCl_4 untreated group. The standard drug (silymarin, 100 mg/kg) significantly (*P*=0.05) increased the serum GSH activity as compared to CCl_4 untreated group.

At 400 mg/kg administration of ethyl acetate fraction (curative model) showed the most significant(*P*=0.05) increase in GSH activity more than all the doses in both model.

Table 11: Effect of ethyl acetate fraction of *Mangifera haden* seeds on Glutathione (GSH) of CCl_4-intoxicated rats (Preventive and Curative Model)

Treatment Group	GSH (mg/dl) Ethyl acetate fraction (Preventive Model)	GSH (mg/dl) Ethyl acetate fraction (Curative Model)
Group 1	0.67 ± 0.11^{c}	0.69 ± 0.09^{d}
Group 2	0.28 ± 0.06^{a}	0.37 ± 0.03^{a}
Group 3	0.54 ± 0.05^{b}	0.48 ± 0.04^{b}
Group 4	0.52 ± 0.03^{b}	$0.61 \pm 0.05^{c,d}$
Group 5	0.53 ± 0.06^{b}	$0.54 \pm 0.10^{b,c}$
Group 6	0.65 ± 0.06^{c}	0.64 ± 0.06^{d}

(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).

3.12: Effect of ethyl acetate fraction of *Mangifera haden* seeds on the lipid profile of CCl_4-intoxicated rats (Preventive and Curative Model)

Table 12a and 12b shows the effect of ethyl acetate 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 pre-treated and post-treated with fractions (group 3 - 5) 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). Oral administration of the standard drug (silymarin, 100 mg/kg) significantly(*P*=0.05) decreased the levels of TC, TAG, LDL-C and significantly increased (*P*=0.05) the HDL-C. These values were comparable to that of the normal control group.

Table 12a: Effect of ethyl acetate fraction of *Mangifera haden* seeds on the lipid profile of CCl_4-intoxicated rats (Preventive Model)

Treatment Group	TC (mg/dl)	TAG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Group 1	5.61 ± 0.05^{c}	0.95 ± 0.05^{d}	3.04 ± 0.06^{b}	2.37 ± 0.06^{d}
Group 2	7.37 ± 0.06^{e}	1.39 ± 0.03^{e}	5.84 ± 0.10^{e}	1.25 ± 0.04^{a}
Group 3	6.10 ± 0.04^{d}	0.84 ± 0.03^{c}	3.64 ± 0.17^{d}	$2.29 \pm 0.14^{c,d}$
Group 4	5.49 ± 0.07^{b}	0.71 ± 0.05^{a}	3.22 ± 0.05^{c}	2.12 ± 0.03^{b}
Group 5	6.07 ± 0.04^{d}	$0.74 \pm 0.03^{a,b}$	3.67 ± 0.05^{d}	2.25 ± 0.05^{c}
Group 6	5.37 ± 0.07^{a}	0.77 ± 0.05^{b}	2.91 ± 0.09^{a}	$2.31 \pm 0.03^{c,d}$

(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).

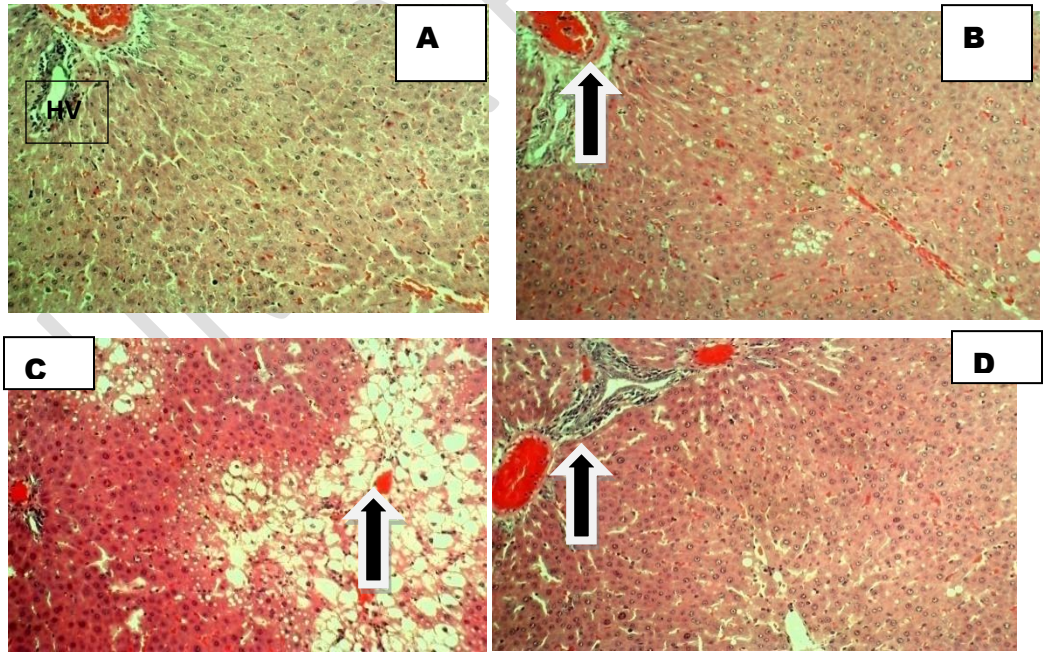
Table 12b: Effect of ethyl acetate fraction of *Mangifera haden* seeds on lipid profile of CCl_4-intoxicated rats (Curative Model)

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).

3.13 Histological Examination



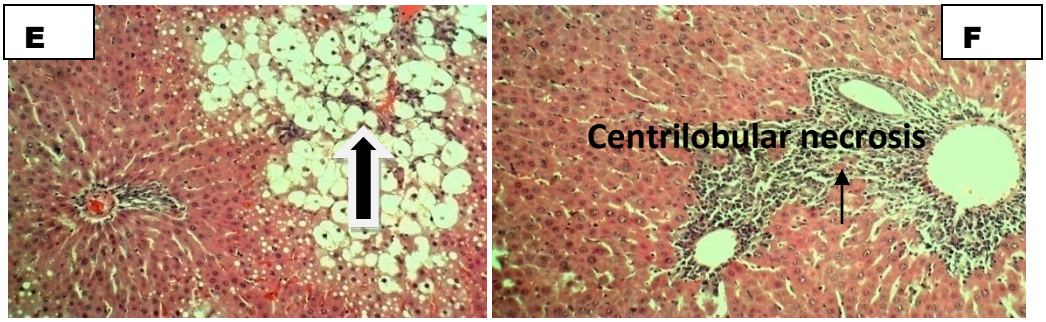


Figure 1: Histological studies of ethyl acetate fraction of *Mangifera haden* seeds (Preventive Model).

PLATE A - E. Tranverse section of the liver stained with haematoxylin and eosin, X400 magnification.

Figure 1: Photomicrographs showing the effect of ethyl acetate fractions of *Mangifera haden* seeds and silymarin on liver histology of CCl_4 intoxicated rats (Preventive Model); Stains: Haematoxylin and eosin; Magnification: X 400**.**

Plate A shows the liver section of normal control rats with normal portal triad, normal hepatic histomorphology, numerous normal hepatic lobules, containing normal hepatocytes arranged in radiating interconnecting cords around the central veins (V) and numerous sinusoids.

Plate B shows liver section of rats intoxicated with carbon tetrachloride with visible distortion in the liver architecture, a mild, multifocal and random, vacuolar degeneration of the hepatocytes.

Plate C shows the liver section of rats treated with 200 mg/kg *Mangifera haden* seeds fraction and CCl_4 (1.0 ml/kg) with normal portal triad, marked hepatocellular degeneration and necrosis of the hepatocytes in the centrilobular and mid-zonal areas of the hepatic lobules (Black arrow).

Plate D reveals the liver section of rats treated with 400 mg/kg b.wt fraction of *Mangifera haden* seeds and carbon tetrachloride (1.0 ml/kg) showing fibrin deposition and numerous mitotic bodies which indicates repair and regeneration of the hepatocytes.

Plate E showing liver section of rats treated with 600 mg/kg b.wt fraction of *Mangifera haden* seeds and carbon tetrachloride (1.0 ml/kg) with moderate hepatic damage and inflammatory cells within the portal tract.

Plate F shows the liver section of rats treated with silymarin (100 mg/kg) and carbon tetrachloride (1.0 ml/kg) with normal portal triad, a marked hepatocellular degeneration and necrosis of the hepatocytes in the centrilobular and mid-zonal areas of the hepatic lobules was observed.

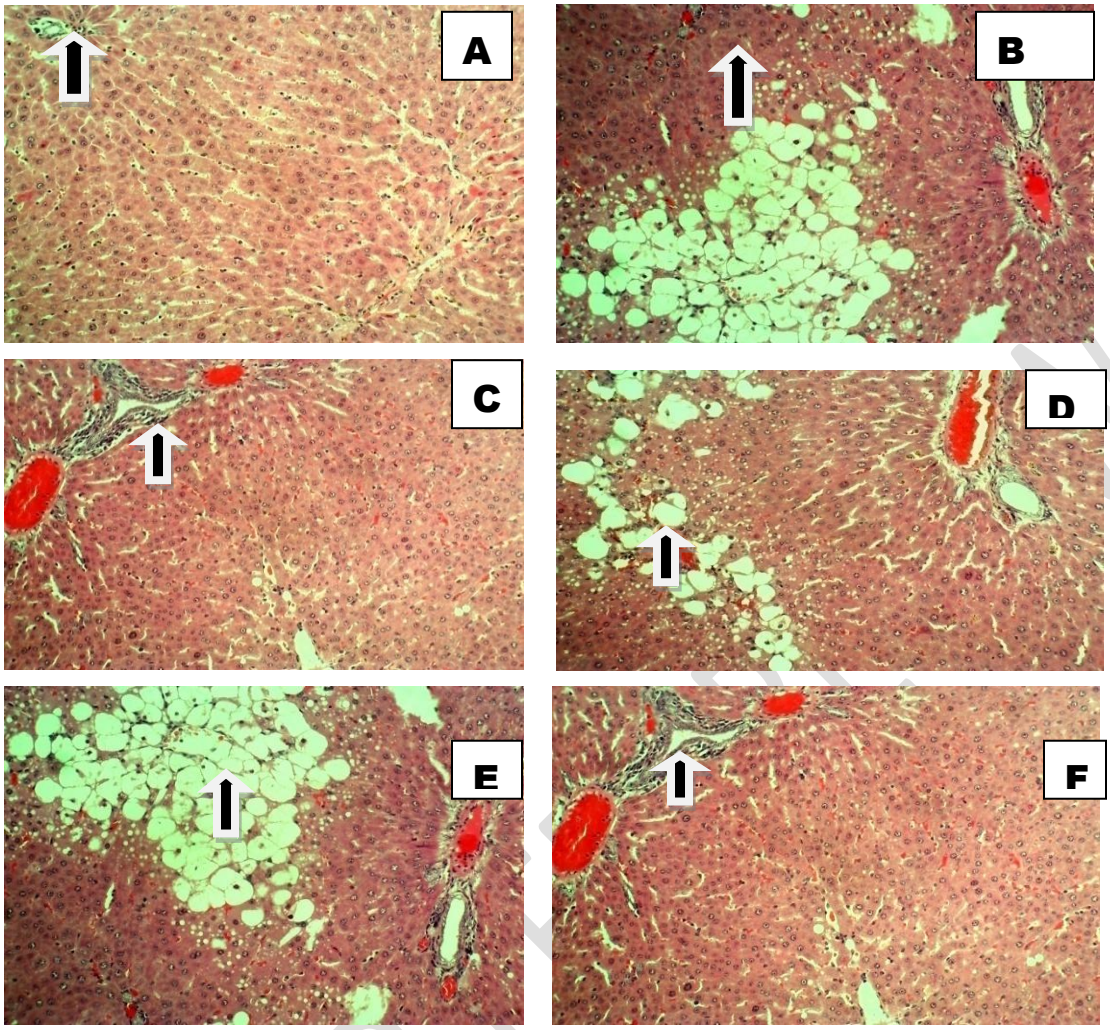


Figure 2: Histological studies of ethyl acetate fraction of *Mangifera haden* seeds (Curative Model)

Figure 2: Photomicrographs showing the effect of ethyl acetate fractions of *Mangifera haden* seeds and silymarin on liver histology of CCl_4 intoxicated rats (curative model);

Stains: Haematoxylin and eosin; Magnification, X400.

Plate A shows liver section of normal control rats with normal hepatic histomorphology, numerous portal tracts dividing them into lobules.

Plate B shows the liver section of rats intoxicated with carbon tetrachloride with visible distortion in the liver architecture, a marked hepatocellular degeneration and necrosis of the hepatocytes in the centrilobular and mid-zonal areas of the hepatic lobules. The affected hepatocytes showed a mixed micro – vesicular and macro-vesicular lipidosis (fatty degeneration of the hepatocytes).

Plate C shows the liver section of rats induced with CCl_4 (1.0 ml/kg) and treated with 200 mg/kg *Mangifera haden* seeds fraction showing an area of necrosis with numerous mitotic cells that indicates hepatocyte regeneration.

Plate D shows the liver section of rats treated with CCl_4 (1.0 ml/kg) and 400 mg/kg *Mangifera haden* seeds fraction showing areas of necrosis of the hepatocytes, fibrin deposition and few mitotic bodies indicating repair of the hepatocytes.

Plate E shows the liver section of rats induced with CCl_4 (1.0 ml/kg) and treated with 600 mg/kg *Mangifera haden* seeds fraction showing macrovesicular lipidosis, areas of necrosis of the hepatocyte and inflammatory cells within the portal tract which is an indication of injury.

Plate F shows the liver section of rats induced with CCl_4 (1.0 ml/kg) and treated with silymarin (100 mg/kg) revealed fibrin deposition with normal portal triad.

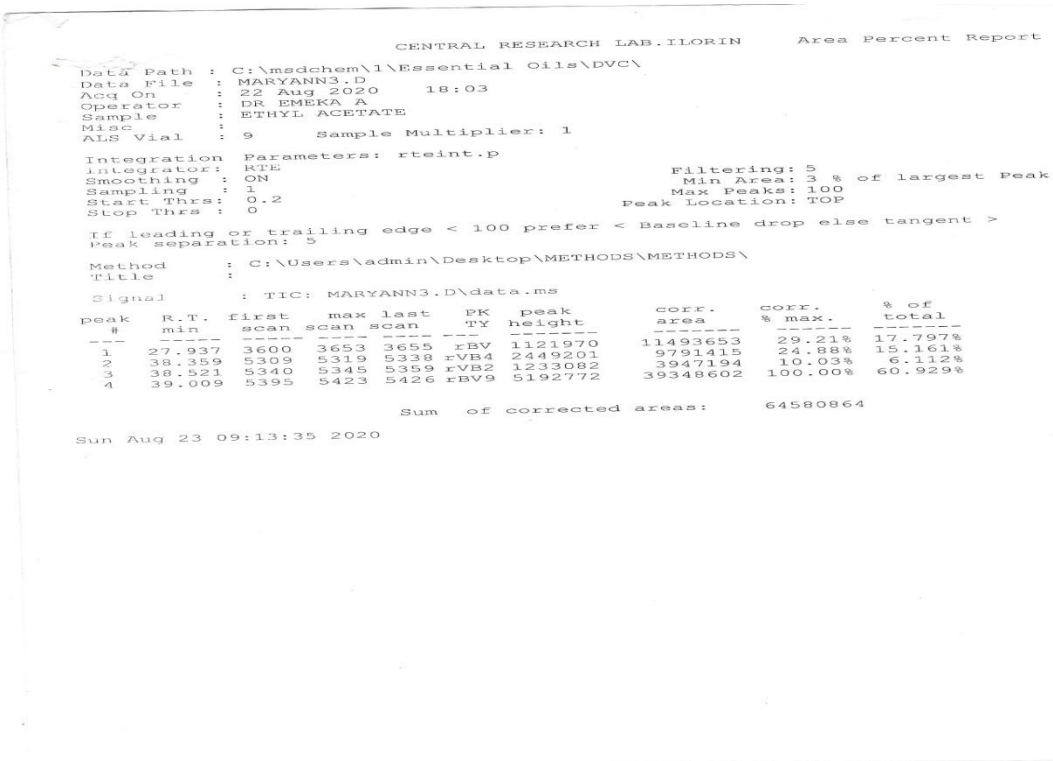
3.14: Phytocompounds detected in ethyl acetate fraction using GC-MS

These phytocompounds (1, 2, 3 – Benzenetriol, 9- octadecenoic acid – (Z) methyl ester and octadecanoic acid) were detected in ethyl acetate fraction of *Mangifera haden* seeds (Table 13).

Table 13: Phytocompounds detected in ethyl acetate fraction using GC-MS

Name of compound	Peak Area %	RT	Molecular Formula	Molecular Weight
1,2,3 – Benzenetriol	17.80	27.937	$C_6H_6O_3$	126.11
9-octadecenoic acid; (Z) – methyl ester	15.16	38.521	$C_{19}H_{36}O_2$	296.50
Octadecanoic acid	60.93	39.009	$C_{19}H_{38}O_2$	298.50

RT; Retention Time



CENTRAL RESEARCH LAB.ILORIN Area Percent Report

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Data File : MARYANN3.D
Acq On : 22 Aug 2020 18:03
Operator : DR EMEKA A
Sample : ETHYL ACETATE
Misc :
ALS Vial : 9 Sample Multiplier: 1

Integration Parameters: rteint.p
integrator: RTE
Smoothing : ON
Sampling : 1
Start Thrs: 0.2
Stop Thrs : 0

Filtering: 5
Min Area: 3 % of largest Peak
Max Peaks: 100
Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : C:\Users\admin\Desktop\METHODS\METHODS\
Title :

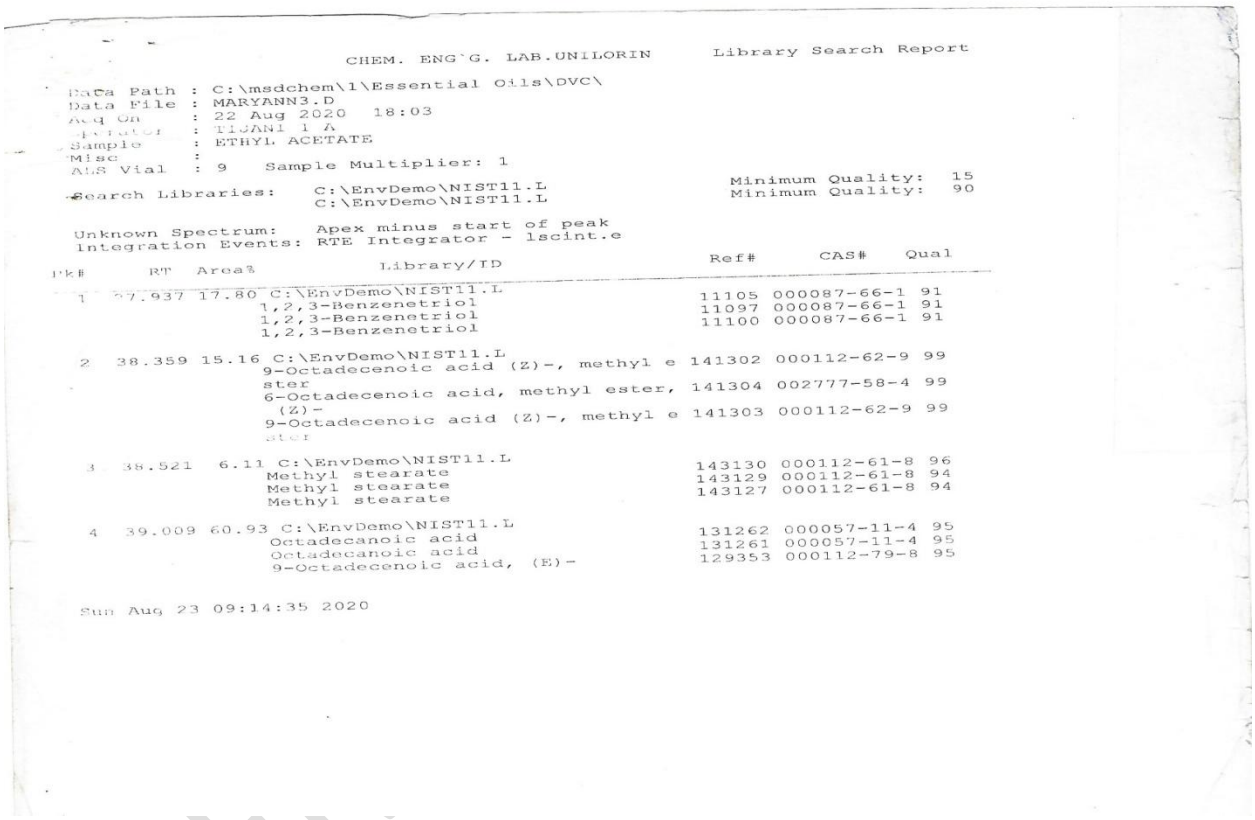
Signal : TIC: MARYANN3.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	27.937	3600	3653	3655	rBV	1121970	11493653	29.21%	17.797%
2	38.359	5309	5319	5338	rVB4	2449201	9791415	24.88%	15.161%
3	38.521	5340	5345	5359	rVB2	1233082	3947194	10.03%	6.112%
4	39.009	5395	5423	5426	rBV9	5192772	39348602	100.00%	60.929%

Sum of corrected areas: 64580864

Sun Aug 23 09:13:35 2020

Figure 3: Peak identification analysis of ethyl acetate fraction of *Mangifera haden* seeds



CHEM. ENG`G. LAB.UNILORIN Library Search Report

Data Path : C:\msdchem\1\Essential Oils\DVC\
Data File : MARYANN3.D
Acq On : 22 Aug 2020 18:03
Operator : TIJANI I A
Sample : ETHYL ACETATE
Misc :
ALS Vial : 9 Sample Multiplier: 1

Search Libraries: C:\EnvDemo\NIST11.L Minimum Quality: 15
C:\EnvDemo\NIST11.L Minimum Quality: 90

Unknown Spectrum: Apex minus start of peak
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	37.937	17.80	C:\EnvDemo\NIST11.L			
			1,2,3-Benzenetriol	11105	000087-66-1	91
			1,2,3-Benzenetriol	11097	000087-66-1	91
			1,2,3-Benzenetriol	11100	000087-66-1	91
2	38.359	15.16	C:\EnvDemo\NIST11.L			
			9-Octadecenoic acid (Z)-, methyl e	141302	000112-62-9	99
			ster			
			6-Octadecenoic acid, methyl ester,	141304	002777-58-4	99
			(Z)-			
			9-Octadecenoic acid (Z)-, methyl e	141303	000112-62-9	99
			ster			
3	38.521	6.11	C:\EnvDemo\NIST11.L			
			Methyl stearate	143130	000112-61-8	96
			Methyl stearate	143129	000112-61-8	94
			Methyl stearate	143127	000112-61-8	94
4	39.009	60.93	C:\EnvDemo\NIST11.L			
			Octadecanoic acid	131262	000057-11-4	95
			Octadecanoic acid	131261	000057-11-4	95
			9-Octadecenoic acid, (E)-	129353	000112-79-8	95

Sun Aug 23 09:14:35 2020

Figure 4: GC-MS analysis of ethyl acetate fraction of *Mangifera haden* seeds

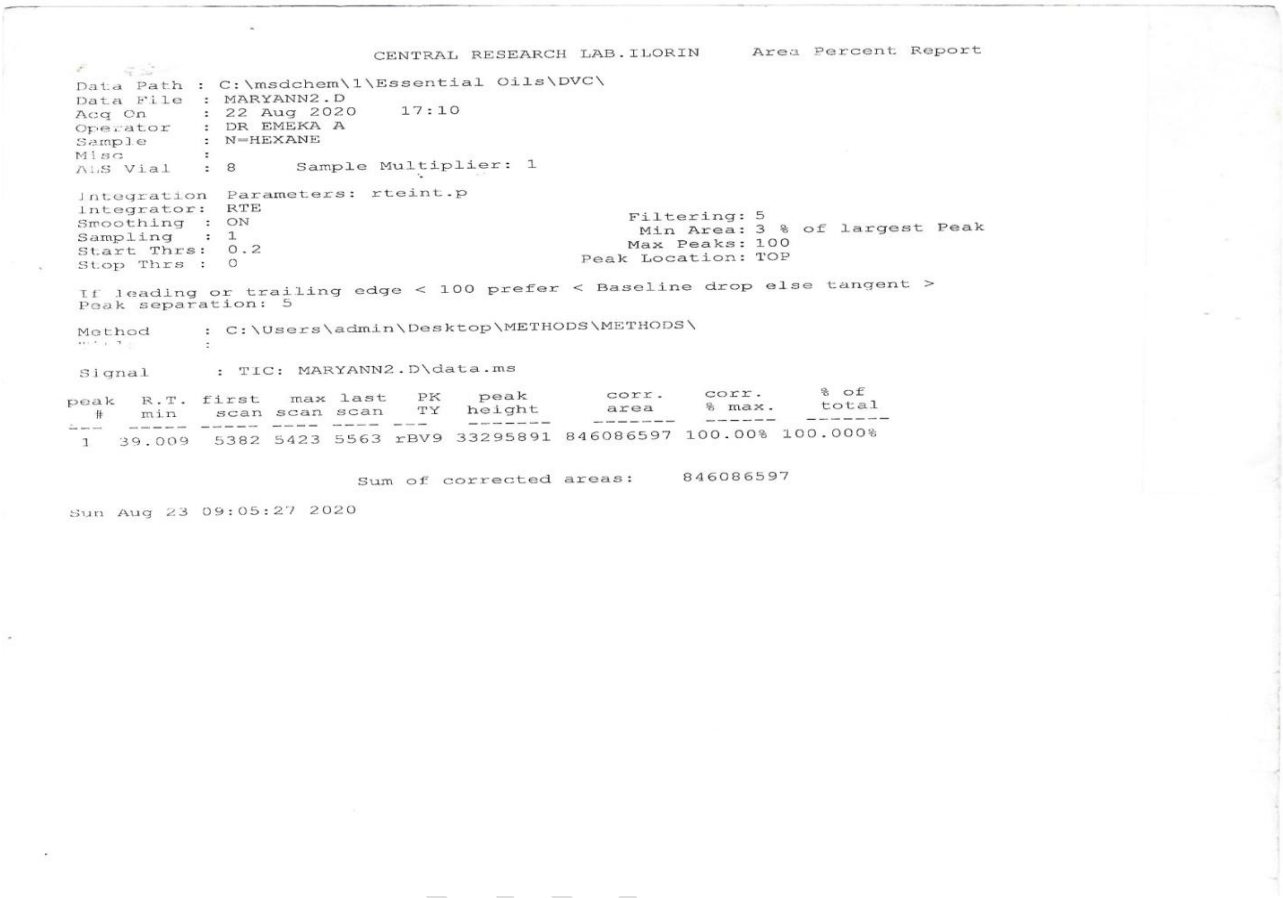
3.15: Phytocompounds detected in n-hexane fraction using GC-MS

The phytocompounds identified in n-hexane fraction of *Mangifera haden* seeds using GC-MS were octadecanoic acid and (Z)-Oleic acid (Table 14).

Table 14: Phytocompounds detected in n-hexane fraction using GC-MS

Name of compound	Peak Area %	RT	Molecular Formula	Molecular Weight
Octadecanoic acid	100	39.009	$C_{19}H_{38}O_2$	298.0
(Z) – Oleic acid	100	39.009	$C_{18}H_{34}O_2$	282.46

RT; Retention Time



CENTRAL RESEARCH LAB.ILORIN Area Percent Report

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Sample : N-HEXANE
Misc :
ALS Vial : 8 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON
Sampling : 1
Start Thrs: 0.2
Stop Thrs : 0

Filtering: 5
Min Area: 3 % of largest Peak
Max Peaks: 100
Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : C:\Users\admin\Desktop\METHODS\METHODS\
:

Signal : TIC: MARYANN2.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
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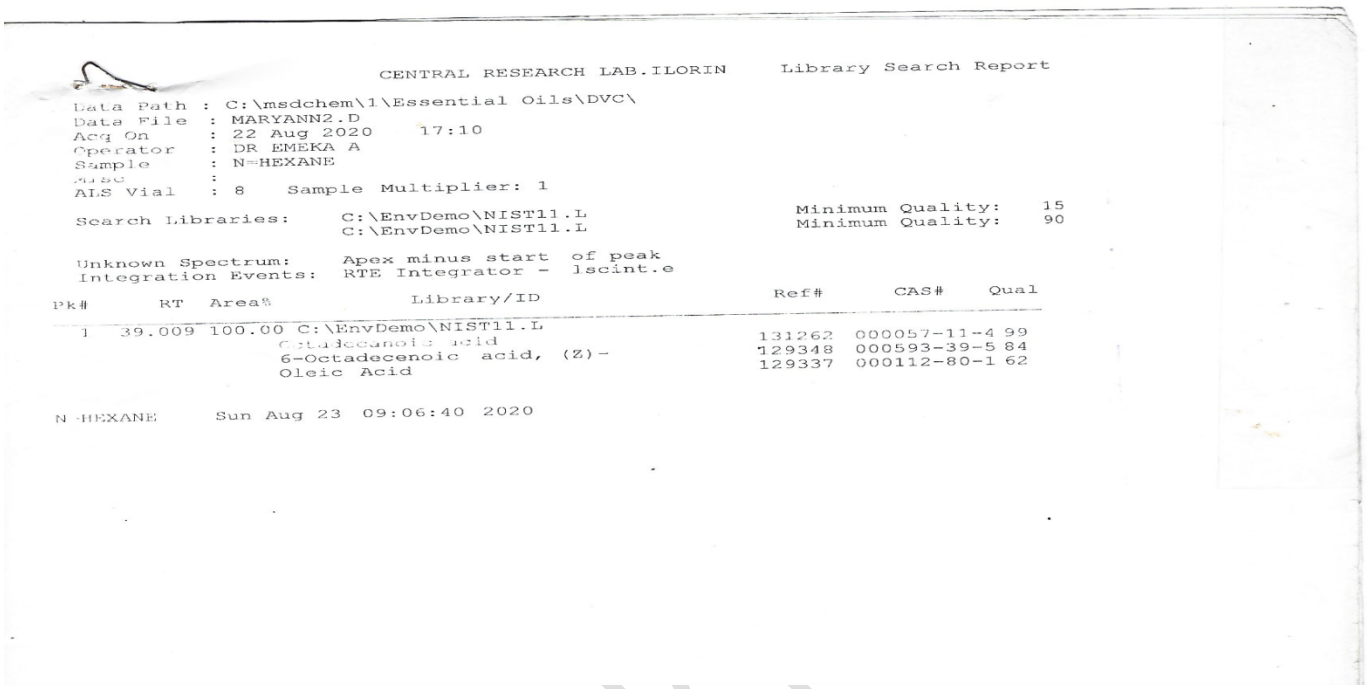
Sum of corrected areas: 846086597

Sun Aug 23 09:05:27 2020

Figure 5: GC-MS analysis of n-hexane fraction of *Mangifera haden* seeds



CENTRAL RESEARCH LAB.ILORIN Library Search Report

Data Path : C:\msdchem\1\Essential Oils\DVC\
Data File : MARYANN2.D
Acq On : 22 Aug 2020 17:10
Operator : DR EMEKA A
Sample : N=HEXANE
Misc :
ALS Vial : 8 Sample Multiplier: 1

Search Libraries: C:\EnvDemo\NIST11.L Minimum Quality: 15
C:\EnvDemo\NIST11.L Minimum Quality: 90

Unknown Spectrum: Apex minus start of peak
Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	39.009	100.00	C:\EnvDemo\NIST11.L			
			Octadecanoic acid	131262	000057-11-4	99
			6-Octadecenoic acid, (Z)-	129348	000593-39-5	84
			Oleic Acid	129337	000112-80-1	62

N-HEXANE Sun Aug 23 09:06:40 2020

Figure 6:Peak identification of compounds in n-hexane fraction of *Mangifera haden* seeds

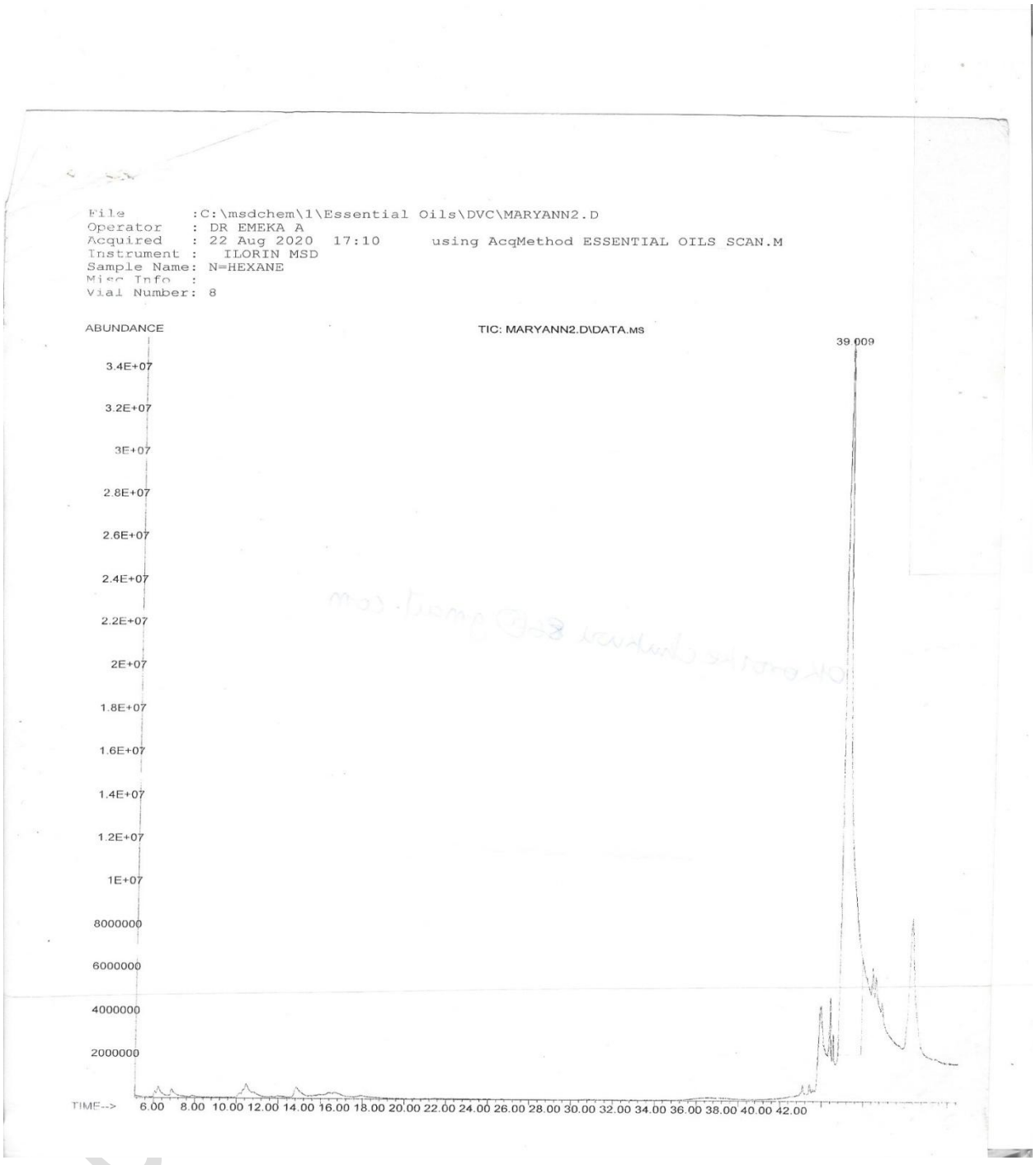


Figure 7: Chromatogram of compounds in n-hexane fraction of *Mangifera haden* seeds

4.0. Discussion

Phytochemical evaluation is one of the excellent tools for evaluating the quality of medicinal plants. Phytochemical screening of crude extract and ethyl acetate fraction of *Mangifera haden* seeds showed that it is rich in terpenoids, glycosides, alkaloids, triterpenes, tannins, saponins, flavonoids, phenolics and coumarins. The detected phytochemical compounds were reported to have medical significance and pharmacological therapy. This is in agreement with the report of Orijajogu*et al*., [29] who documented that *Mangifera indica* seed extract contains alkaloids, flavonoids, tannins, glycosides, phenol and steroids. However, their report did not detect glycosides and saponins. Iwu*et al.,* [30] stated that his findings were consistent with research work as it contains phenols, saponins, tannins, flavonoids, steroids, glycosides and alkaloids, but coumarins, triterpenes and terpenoids were not detected.

ALT, AST, and ALP are liver enzyme markers commonly used to assess liver damage. CCl_4-treated rats produced significant increases in ALT, AST, and ALP, indicating hepatocellular injury. The increase in serum levels of these enzymes has been attributed to impaired structural integrity of the liver, as they are in the cytoplasmic region and are released into the circulation after cell damage. ALT, AST and ALP values were significantly (P = 0.05) decreased with ethyl acetate fraction treatment in a dose-dependent manner at (200, 400, 600 mg/kg) compared to CCl_4 untreated group. This indicates that serum transaminase levels return to normal due to stabilization of the plasma membrane as well as repair of liver tissue damage caused by CCl_4. The findings of Oluwafemi *et al.,*[31] is in tandem with this study who observed that treatment with *Irvingiagabonensis* resulted in significant liver protection as indicated by the reduction of elevated levels of ALT, AST and ALP. Bala *et al.,* [32] supported this study, whose studies showed a slight significant decrease in serum ALT, AST and ALP levels after two weeks of administration of the extract to mice.

Bilirubin is the end product of haemoglobin degradation. It is a very useful clinical indicator of the severity of necrosis. In this study, the concentration of total bilirubin was significantly (P = 0.05) increased in the CCl_4 group showing severe CCl_4-induced damage, which was comparable to the normal control group. Oral treatment of rats with doses of 200, 400 and 600 mg/kg ethyl acetate fraction of *Mangifera haden* seeds caused a significant (P = 0.05) decrease in serum TB levels. In tandem with this study, Nadella and Kumar, [33] demonstrated that *Mangifera indica* leaf extract at doses of 300 and 600 mg/kg significantly (P = 0.05) reduced total bilirubin compared to the CCl_4 group.

Total protein is a biochemical test for measuring the total amount of protein in a serum. It helps diagnose liver disease along with other conditions. Administration of CCl_4 results in the reduction of serum total proteins in animals. Reduced total protein content is a useful index of severity of hepatocellular damage. Treatment of the animals with the doses of 200, 400, 600 mg/kg of ethyl acetate fraction, caused significant (P = 0.05) increase in serum total protein compared to the untreated group (Group 2). Eidangbe*et al*., [34] findings are consistent with this study who observed that "following the administration of 1500 mg/kg ethanolic seed extract of *Dacryodes edulis* for 7 days to 28 days, a remarkable increase in serum albumin and total protein concentrations were observed in the CCl_4 –induced rats compared to CCl_4 –induced but untreated rats".

"Malondialdehyde (MDA) is an end product of lipid peroxidation and a marker of oxidative stress. The degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) concentration in the serum. Elevated levels of serum MDA induced by CCl_4 represent a predictor of atherosclerosis, hepatocellular damage, failure of natural antioxidant defense system to prevent overproduction free radicals and other pathological diseases. The administration of different doses of *Mangifera haden* seeds fraction significantly (P = 0.05) decreased the MDA levels. The observed decrease in MDA levels could be due to the presence of 9-octadecenoic acid (Z)-, methyl ester, oleic acid and HDL-C identified in *Mangifera haden* seeds. HDL is a carrier of antioxidant vitamins such as α-tocopherol (vitamin E), which helps to protect cells against the harmful effects of reactive oxygen species" (Kuyooro*et al*., [35]. HDL is also associated with enzymes such as paraoxonase (PON) and platelet activating factor acylhydrolase (PAF-AH) that may play important roles in the removal of oxidized lipids from other lipoproteins such as low-density lipoprotein (LDL). HDL functions in the transport of cholesterol away from the peripheral tissues to the liver, thus preventing the genesis of atherosclerosis. Oluwafemi *et al*., [31] findings is also in tandem with this study who reported that "the treatment with *Irvingiagabonensis* extract completely ameliorated cadmium – chloride induced increase in lipid peroxidation (LPO)".

Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health and well-being. The activities of the hepatic antioxidant enzymes (SOD, CAT and GPx) and the level of glutathione were found to be drastically decreased in CCl_4 group compared to normal and treated groups. The decreased activities of the antioxidant enzymes and glutathione in CCl_4 – intoxicated rats were augmented more effectively in rats treated with ethyl acetate fractions of *Mangifera haden* seeds at the dose of 200, 400 and 600

mg/kg both for preventive and the curative models. Karuppanan*et al.,* [36] findings is in agreement with this study who reported that the levels of catalase, SOD, CAT, GPx and glutathione significantly (P = 0.05) elevated in methanol *Mangifera indica* (MMI) leaf extracts treated group when compared with the control group, mercuric chloride treated group.

This study revealed that all CCl_4-intoxicated rats exhibited hyperlipidemia as indicated by their increased levels of total cholesterol (TC), triacylglycerols (TAG), low-density lipoprotein (LDL), and decreased high-density lipoprotein (HDL). CCl_4 acts as a surfactant and inhibits the action of lipases to block the uptake of lipoproteins from the circulation by extrahepatic tissues. This results in increased blood lipid concentration, increased cholesterol synthesis in the liver and induction of hyperlipidemia.

"Administration of the ethyl acetate fraction of *Mangifera haden* seeds significantly reduced the levels of TC, TAG, LDL-C and increased the level of HDL-C. The significant reduction in total cholesterol levels observed in the study can be attributed to the increased excretion of cholesterol and its catabolism to bile salts due to the presence of unsaturated fatty acids, which are abundant in oils from *Mangifera haden* seeds. A decrease in cholesterol may indicate increased oxidation of mobilized fatty acids or lipolysis. The hypolipidemic potential of these fractions may be due to their antioxidant and antihyperlipidemic effects due to the phytochemicals present. The underlying mechanism of the lipidaemic-lowering activity of *Mangifera haden* seeds could be the inhibition of lipid absorption due to the presence of saponins and tannins in the ethyl acetate fraction of *Mangifera haden* seeds. It can act by increasing the excretion of cholesterol and bile acids in the stool. Oral administration of saponins from some medicinal plants significantly reduces the level of triacylglycerols and cholesterol in rats" (Kuyooro*et al.,*[35]. This study is consistent with the work of Osorio – Esquivel *et al.,* [37], whose "studies in hypercholesterolemic mice with a diet supplemented with microwave dehydrated mango powder showed a significant decrease in serum total cholesterol, LDL-c, triacylglycerols and a significant increase in HDL-c compared to hypercholesterolemic mice and the conventional group – dehydrated mango powder". Additionally, Gururaja *et al.,* [38] results are similar to this study which studied "Methanol extract of *Mangifera indica* showed significant cholesterol-lowering activity at 90 mg/kg dose and significant decrease in plasma triacylglycerols".

Histological examination revealed distortion in liver architecture, fatty degeneration, mixed microvesicular and macrovesicular lipidosis, hepatocellular degeneration and hepatocyte

necrosis, and inflammatory cells in the portal tract that were induced by the administration of a double dose of CCl_4. *Mangifera haden* and silymarin fractions protected the liver due to the fact that their histological appearance was similar to that of normal control groups, tissue damage and necrosis were less in these treated groups than in the CCl_4 group.

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5.0 Conclusion

The results from animal studies and histological examination reveals that CCl_4 untreated group (group 2) were hepatotoxic. Treatment of the animals with varying doses of ethyl acetate fraction protected the liver cell from CCl_4 intoxication. This could be attributed to the presence of phytochemicals, flavonoids and phenolic compounds including antioxidants identified in the fraction of *Mangifera haden* seeds. On the whole, at the dose of 200 mg/kg ethyl acetate fraction (curative model) exhibited the most effective protection against hepatotoxicity, and brought about repair and restoration of the liver cells integrity compared to other doses of the plant fraction. However, silymarin was found to be more effective drug in protecting the liver cells from damage when compared to some doses of the plant's seeds. But, it has a lot of undesirable adverse effects. Therefore, there is a need to extract and characterize the active phytochemicals and antioxidants in *Mangifera haden* seeds and incorporate it into functional foods or use for drug development. Hence, ethyl acetate fraction of *Mangifera haden* seeds could be explored as an alternative therapeutic agent in the treatment of the liver damage.

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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.

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