HEPATOPROTECTIVE EFFECTS OF FRACTIONS OF ADANSONIA DIGITATA LEAVES ON CARBON TETRACHLORIDE (CCL₄)- INDUCED TOXICITY IN WISTAR RATS

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Abstract

Phytochemicals, naturally occurring compounds in plants, offer health benefits to humans. This study aimed to determine the effects of fractions of ethanol extract of Adansonia digitata leaves on carbon tetrachloride (CCL₄)-induced toxicity in wistar rats. The extraction was done using absolute ethanol, followed by fractionation with different solvent combination via column chromatography. Elution of extract was done with solvent system by gradually increasing polarity beginning from n-hexane, chloroform, ethyl acetate, methanol, ethanol and finally water. In total, 22 fractions were collected in 200 mL beaker each. The fractions were subjected to total antioxidant analysis using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Total phenolics and total flavonoids content were analyzed by Folin-Ciocalteu. GC-MS, FTIR and HPLC analysis were also carried out. Thirty-six male albino rats were distributed into six groups of six (n = 6). The negative control group received CCl₄ only. The standard control group was administered 2 CCl₄/kg body weight + 25mg/kg body weight silymarin followed by different doses of ethyl
acetate:ethanol fraction-20 mg/kg, 40 mg/kg and 50 mg/kg for 21 days. Induction and treatment were carried out in the beginning of a new week. The animals were fasted for 24 hours, sacrificed and blood samples were collected for biochemical analysis. The administered fraction led to the statistically insignificant (p> 0.05) and statistically significant (p< 0.05) reduction in the levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) respectively, the reduction in the levels of total protein (TP) and albumin (ALB) were statistically not significant (p> 0.05) in the treated rats as compared to the untreated rats. The phytochemical analysis of the extract revealed the presence of antioxidant and phenolics. From the total antioxidant capacity, fraction 6b (ethyl acetate: ethanol) was selected for GC-MS, FTIR and HPLC analysis. The GC-MS analysis of fraction 6b revealed the presence of 14 compounds from which Bis(2-ethylhexyl) phthalate had the highest percentage constituent (48.30%) while while 2-Methyl-Z,Z-3,13-octadecadienol had the lowest relative abundance of 0.14 %. Dichloroacetic acid, tridecyl ester, d-Glycerodido-heptose, Phthalic acid, ethyl pentyl ester, Trifluoroacetoxy hexadecane, 2-Hexene, 6-nitro-, 2-Tetradecanol, 3,5-Dimethyl-2-octanol, Cyclopropanecarboxylic acid, 7,11-Hexadecadienal, 9-Octadecenamide, Squalene and E, E-1,9,17-Docosatriene were the other compounds found in the fraction. FTIR spectral analysis of the fraction showed peaks of various functional groups. The HPLC results showed the presence of quercetin in the fraction.

**Keywords:** Carbon Tetrachloride, Toxicity, Liver, Antioxidants, Adansonia Digitata, and Oxidative Stress.

**INTRODUCTION**

The liver, a vital and intricate organ within the body, plays a crucial role in metabolizing, secreting, detoxifying, and excreting xenobiotics (Ubhenin *et al.*, 2016). According to Hong *et al.* (2009), the liver is highly metabolically active and serves as a primary target for drugs and pathogens that can potentially initiate cellular damage. Liver injury commonly stems from various factors such as viral infections, exposure to xenobiotics, toxic substances, prolonged drug therapy, environmental pollutants, chronic alcohol consumption, and chemotherapy drugs (Ubhenin *et al.*, 2016). Certain hepatotoxic chemicals induce significant damage to liver cells by promoting lipid peroxidation and oxidative stress. The absence of safe hepatoprotective medications, researchers and practitioners in traditional medicine have shifted focus toward herbal remedies for managing different liver disorders.
(Ubhenin et al., 2016). According to Bansal et al. (2005), mitigating the impact of reactive metabolites using antioxidants can help prevent liver damage. Natural polyphenolic compounds found in plants are often regarded as liver protectants due to their inherent antioxidant and anti-inflammatory properties. Notable examples include resveratrol, quercetin, curcumin, and silymarin (Haddad et al., 2011). The hepatotoxicity model induced by carbon tetrachloride (CCl₄) is widely employed to assess the hepatoprotective effects of both drugs and plant extracts (Suja et al., 2002; Ubhenin et al., 2016). Kim et al. (2010) and Adewale et al. (2014) emphasized that CCl₄, a common industrial solvent, is notorious for inducing liver injury via free radical mechanisms. Ozturk et al. (2003) highlighted that CCl₄ not only targets the liver but also impacts other organs such as the lungs, heart, testes, kidneys, and brain. Adansonia digitata, commonly known as Baobab, is a tropical plant native to Africa and belongs to the Malvaceae family. This plant holds significant importance for the livelihoods of individuals in arid regions of Africa, where it is referred to as "Kuka" by the Hausas and "Ose" by the Yorubas in Nigeria. Adansonia digitata has been reported to possess various pharmacological effects (Al-Qarawi et al., 2003; Oyewopo et al., 2015). This research is therefore aimed at determining the hepatoprotective effects of Adansonia digitata against hepatotoxicity induced by Carbon tetrachloride in wistar rats.

METHODS

Plant Collection and Preparation

The fresh leaves of Adansonia digitata were collected within Wukari Local Government Area of Taraba State, Nigeria from December 2022 to March 2023. The plant materials were dried in the laboratory at room temperature and pulverized using traditional mortar and pestle.

Crude Extraction with Ethanol

The crude extraction was carried out in accordance with the method reported by Yakubu et al. (2014). Exactly 200 g of the pulverized sample was soaked in about 1000 ml of ethanol for 48 hours. The extract was first filtered using a filtered cloth of which the filtrate obtained was further filtered under reduced pressure using filter paper, to obtain the final filtrate. The filtrates were concentrated at room temperature to obtain the desired concentrate.
Fractionation of Ethanolic Extract

The ethanol extract was subjected to column chromatography using silica gel stationary phase. The column was eluted using varying solvent combinations of increasing polarity as the mobile phase.

Packing of column

The packing of the column was done according to the method of Yakubu et al. (2014). In the packing of the column, the lower part of the glass column was stocked with glass wool with the aid of glass rod. 235 g of silica gel (G60-200 mesh size) was dissolved in 235 ml of n-Hexane to make the slurry. The chromatographic column (30 mm diameter by 400 mm height) was packed with silica gel and was allowed free flow of the solvent into a conical flask below. The set up was seen to be in order when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed 24 h to stabilize after which, the clear solvent at the top of the silica gel was allowed to drain down the silica gel meniscus.

Elution

The method of Yakubu et al. (2014) was adopted for the elution. The extract (2 g) was dissolved in 15 ml absolute ethanol after which the solution was applied to the chromatographic column (30 mm diameter by 400 mm height). Phytochemicals were eluted from the plant material using solvent combinations of different polarities, as follows: n-hexane (100:0); n-hexane:chloroform (50:50); chloroform (100:0); chloroform:ethyl acetate (50:50); ethyl acetate (100:0); ethyl acetate:ethanol (50:50); ethanol (100:0); ethanol:methanol (50:50); methanol (100:0); methanol:distilled water (50:50); distilled water (100:0).

Determination of Total Antioxidant Activity Capacity (TAC)

The scavenging action of the plant extracts and the resulting fractions from ethanol extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined spectrophotometrically at 517 nm using Trolox as standard according to the method described by Singleton et al. (2002). The absorbance was measured in triplicate for each fraction. Total antioxidant activity capacity (TAC) was calculated as mg/ml of Trolox equivalent (TE) using the regression equation from the calibration curve.
Total Phenolic Content (TPC) of Fractions of Ethanolic Extract of *Adansonia digitata* leaves

The data for Total Phenolics Content showed that fraction 7b (2177.33 mg/ml) had the highest Phenolics content followed by fraction 10b and 6b with the Phenolics content of 2116.00 mg/ml and 2070.00 mg/ml respectively. The lowest Total Phenolic Content was observed in fraction 4b (35.67 mg/ml).

Experimental animals

Total of 36 Male healthy looking albino rats (8 weeks old) were procured from the animal house of Federal University and were kept in cages under standard laboratory conditions (25±2°C; 12h light/dark) and acclimatized for a period of two (2) weeks in the laboratory before the commencement of the experiment. They were fed daily with grower mash from Vital Feeds Company Nigeria and clean tap water *ad-libitum*.

Treatment Groups

Six groups of male rats were used (n = 6) as treatment groups. Each treatment group was administered various doses of the crude extract for three weeks Animals of the 1st group were kept as the normal control; the 2nd group (hepatotoxic control) group was administered the vehicle (Olive Oil/ CCl₄ – 1:1 ratio) at 2 mL/kg. Rats of 3rd, 4th and 5th groups were administered *A. digitata* extract at doses of 20mg/kg, 40 and 50mg/kg respectively. The 6th group were kept as standard and were prophylactically treated with silymarin at 25mg/kg The CCl₄ was injected, while *A.digitata* extract and silymarin were dosed orally. Induction and treatment were carried out in the beginning of a new week (3 times in 21 days).

Induction of Hepatotoxicity

The hepatoprotective activity of *A. digitata* extract was done in experimental rats using CCl₄ (2ml/kg) as a hepatotoxic agent (by the administration of a single intraperitoneal dose of CCl₄ as described by Kamisan et al. (2014). Hepatotoxicity was induced in all rats (except the rats of Group I).

Blood Collection and Analysis

The animals were anaesthetized with chloroform; incisions were made into their thoracic cavity and blood samples were collected by cardiac puncture using a 10ml syringe and dispensed into tubes and allowed to clot for fifteen minutes after which it was centrifuged.
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for 10 minutes at 4000 rpm. Serum was separated from the clot using Pasteur pipette for the serum biochemical analysis. The serum activities of liver marker enzymes ALT and AST, total protein (TP), albumin (ALB) were assayed using auto-analyzer (Cobas C111 Chemistry Analyzer).

Statistical analysis:
The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23. One-way ANOVA was used to analysed the data. The mean and standard deviations were calculated.

RESULTS
Total Antioxidant Capacity (TAC) of Fractions of Ethanolic Extract of *Adansonia digitata* leaves

The result for Total Antioxidant Capacity (TAC) revealed that fraction 6b had the highest antioxidant capacity (84.72 mg/ml) followed by fraction 1b (78.82 mg/ml) and 7a (78.72 mg/ml), fraction 10a and 9a were also observed to have approximately the same total antioxidant capacity of 76.76 mg/ml and 76.94 mg/ml respectively. The lowest Antioxidant Capacity was recorded in fraction 3a (29.27 mg/ml) as shown in Figure 1.

![Figure 1: Total antioxidant activity of fractions of ethanolic extract of *Adansonia digitata* leaves](image-url)
Total Phenolic Content (TPC) of Fractions of Ethanolic Extract of *Adansonia digitata* leaves

The data for Total Phenolics Content showed that fraction 7b (2177.33 mg/ml) had the highest Phenolics content followed by fraction 10b and 6b with the Phenolics content of 2116.00 mg/ml and 2070.00 mg/ml respectively. The lowest Total Phenolic Content was observed in fraction 4b (35.67 mg/ml) as shown in Figure 2.

**Figure 2:** Total Phenolic content of fractions of ethanolic extract of *Adansonia digitata* leaves

Fraction: 1 = n-hexane (100:0); fraction 2 = n-hexane:chloroform (50:50); fraction 3 = chloroform (100:0); fraction 4 = chloroform:ethyl acetate (50:50); fraction 5 = ethyl acetate (100:0); fraction 6 = ethyl acetate:ethanol (50:50); fraction 7 = ethanol (100:0); fraction 8 = ethanol:methanol (50:50); fraction 9 = methanol (100:0); fraction 10 = methanol:distilled water (50:50); fraction 11 = distilled water (100:0).
Gas Chromatography-Mass Spectroscopy (GC-MS) profiling fractions of ethanolic extract of *Adansonia digitata* leaves

With available data obtained from the total antioxidant activity, fraction 6b (ethyl acetate: ethanol) was selected for GC-MS analysis.

The GC-MS analysis of ethyl acetate: ethanol fraction of ethanol extract of *Adansonia digitata* leaves revealed the presence of 14 compounds from which Bis(2-ethylhexyl) phthalate had the highest percentage constituent (48.30%). Dichloroacetic acid, tridecyl ester, d-Glycero-d-ido-heptose, Phthalic acid, ethyl pentyl ester, Trifluoroacetoxy hexadecane, 2-Hexene, 6-nitro-, 2-Tetradecanol, 3,5-Dimethyl-2-octanol, Bis(2-ethylhexyl) phthalate, Cyclopropanecarboxylic acid, 7,11-Hexadecadienal, 9-Octadecenamide, Squalene, 2-Methyl-Z,Z-3,13-octadecadienol and E, E-1,9,17-Docasatriene were the compounds found in the fraction. The fourteen (14) peaks, names, retention times, chemical structures and molecular formulae, peak area and biological activities of these compounds are shown in (Figure 3 and Table 1).

![Figure 3: GC-MS peak scan for ethyl acetate:ethanol fraction of *Adansonia digitata* leaves](image-url)
**Table 1:** GC-MS results of ethyl acetate:ethanol fraction of ethanol extract of *Adansonia digitata* leaves

<table>
<thead>
<tr>
<th>S/N</th>
<th>Retention time</th>
<th>Name of compound</th>
<th>Chemical structure/Molecular formula</th>
<th>Peak area (%)</th>
<th>Biological activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.424</td>
<td>Dichloroacetic acid, tridecyl ester</td>
<td>C_{15}H_{28}Cl_{2}O_{2}</td>
<td>0.44</td>
<td>Inhibit the activity of enzyme 1 dehydrogenase kinase</td>
<td>Nisha <em>et al.</em> (2023)</td>
</tr>
<tr>
<td>2</td>
<td>19.327</td>
<td>d-Glycero-d-id-o-heptose</td>
<td>C_{7}H_{14}O_{7}</td>
<td>0.83</td>
<td>Sugar moiety</td>
<td>Dharmalingam and Nazni (2013)</td>
</tr>
<tr>
<td>3</td>
<td>20.104</td>
<td>Phthalic acid, ethyl pentyl ester</td>
<td>C_{16}H_{20}O_{4}</td>
<td>22.3</td>
<td>Antimicrobial, insecticidal</td>
<td>Huang <em>et al.</em> (2021)</td>
</tr>
<tr>
<td>4</td>
<td>21.432</td>
<td>Trifluoroacetox y hexadecane</td>
<td>C_{16}H_{33}F_{3}O_{2}</td>
<td>1.98</td>
<td>Antibacterial activity</td>
<td>Maria <em>et al.</em> (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>---</td>
<td>---</td>
<td>----------------------------------------------------------------</td>
<td>---</td>
<td>----------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>23.17</td>
<td>2-Hexene, 6-nitro-</td>
<td>1.17</td>
<td>Not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>25.11</td>
<td>2-Tetradecanol</td>
<td>0.59</td>
<td>Used as Yakubu et al. (2021)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td>ingredient in cosmetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>28.20</td>
<td>3,5-Dimethyl-2-</td>
<td>1.53</td>
<td>Activity not known</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>octanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>30.34</td>
<td>Bis(2-ethylhexyl)</td>
<td>48.3</td>
<td>Antimicrobial, antifungal, antitumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>phthalate</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>32.78</td>
<td>Cyclopropanecarboxylic acid</td>
<td>7.27</td>
<td>Antiviral, antitumor</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Salaun and Baird (1995)
Table 1 continue

<table>
<thead>
<tr>
<th>No.</th>
<th>MW</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>p (%)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>33.221</td>
<td>7,11-Hexadecadienal</td>
<td>C₁₆H₂₈O</td>
<td>0.46</td>
<td>It serve as pollinator, also used in assessing insect population (Yakubo et al., 2021)</td>
</tr>
<tr>
<td>11</td>
<td>34.210</td>
<td>9-Octadecenamide</td>
<td>C₁₈H₃₃NO</td>
<td>6.05</td>
<td>Anti-inflammatory activity (Ano et al., 2015)</td>
</tr>
<tr>
<td>12</td>
<td>34.689</td>
<td>Squalene</td>
<td>C₃₀H₅₀</td>
<td>6.23</td>
<td>Antioxidant property (Conforti et al., 2005)</td>
</tr>
<tr>
<td>13</td>
<td>35.281</td>
<td>2-Methyl-Z,Z-3,13-octadecadienol</td>
<td>C₁₉H₃₆O</td>
<td>0.14</td>
<td>Pesticide, herbicide, insecticide (Adeyemi et al., 2017)</td>
</tr>
<tr>
<td>14</td>
<td>36.026</td>
<td>E,E-1,9,17-Docosatriene</td>
<td>C₂₂H₄₀</td>
<td>1.22</td>
<td>Not available</td>
</tr>
</tbody>
</table>
Fourier Transform Infrared Spectroscopy (FTIR) analysis of fractions of *A. digitata* leaves

With available data obtained from the total antioxidant activity, fraction 6b (ethyl acetate:ethanol) was selected for FTIR analyses. The identity of the components of the three fractions were investigated using FTIR spectroscopy by monitoring different functional groups.

The FTIR spectrum shown in Figure 4.7 has bands and wave numbers of 3306 (cm$^{-1}$) to 875 (cm$^{-1}$). Spectrum with frequency 3306 (cm$^{-1}$) showed strong/narrow band, spectrum with frequency 2981 (cm$^{-1}$) showed weak/narrow band, spectrum with frequency 1640 (cm$^{-1}$) showed medium/narrow band, spectrum with frequency 1386 (cm$^{-1}$) showed weak/narrow band, spectrum 1084 (cm$^{-1}$) showed weak/narrow band, spectrum 1043 (cm$^{-1}$) showed medium/narrow band while spectrum 875 (cm$^{-1}$) gave medium/narrow band. The result confirmed the presence of amines, amides, alkanes, alkenes, nitro compound, aliphatic amines as well as aromatics in the fraction as shown in (Figure 4 and Table 2).

![FTIR spectrum of ethyl acetate:ethanol fraction of ethanol extract of *A. digitata* leaves](image)
Table 2: FTIR result of ethyl acetate:ethanol fraction of ethanol extract of *A. digitata* leaves

<table>
<thead>
<tr>
<th>S/N</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Functional group/mode of vibration</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3306.1</td>
<td>N-H stretch</td>
<td>1°, 2° amines, amides</td>
</tr>
<tr>
<td>2</td>
<td>2981.9</td>
<td>C-H stretch</td>
<td>Alkanes</td>
</tr>
<tr>
<td>3</td>
<td>1640.0</td>
<td>-C=C- stretch</td>
<td>Alkenes</td>
</tr>
<tr>
<td>4</td>
<td>1386.0</td>
<td>N=O (R-NO₂)</td>
<td>Nitro compound</td>
</tr>
<tr>
<td>5</td>
<td>1084.7</td>
<td>C-N stretch</td>
<td>Aliphatic amines</td>
</tr>
<tr>
<td>6</td>
<td>1043.7</td>
<td>C-N stretch</td>
<td>Aliphatic amines</td>
</tr>
<tr>
<td>7</td>
<td>875.9</td>
<td>C-H “oop”</td>
<td>Aromatics</td>
</tr>
</tbody>
</table>

High Performance Liquid Chromatography (HPLC) analysis of fractions of ethanol extract of *Adansonia digitata* leaves

With available data obtained from the total antioxidant activity, fractions 6b (ethyl acetate:ethanol) was selected for HPLC analysis. The HPLC fingerprinting of ethyl acetate:ethanol fraction of ethanol extract of *Adansonia digitata* leaves showed seven (7) different peaks with their retention times (min) and absorbances which represent seven (7) different compounds. The chromatogram revealed that quercetin (t<sub>R</sub> = 1.87, peak 4) was the most prominent bioactive compounds in the fraction as shown in Figure 5.
Liver functions of CCl₄–induced rats treated with ethyl acetate: ethanol fraction of ethanol extract of *Adansonia digitata* leaves

The results presented in the Table 3 below revealed an elevated level of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) respectively in the negative control (group 2) which received only CCl₄. The increase was statistically significant ($p<0.05$) when compared with the normal control (group 1) that received water and only feed. The concentrations of Total protein (TP) and Albumin (ALB) showed statistically insignificant ($p>0.05$) increase in CCl₄ treated rats when compared with the normal group (group 1). The administered ethyl acetate: ethanol fraction of ethanol extract of *A. digitata* leaves in group 3, 4, 5 and Silymarin in group 6 showed statistically insignificant ($p>0.05$) reduction in the activities of AST when compared with negative control (group 2). The level of ALT decreased significantly ($p<0.05$) in group 3, 4, 5 and 6 (rats treated with 20, 40 and 50 mg/kg fraction and 25 mg/kg Silymarin) when compared with the negative control (group 2). There was statistically insignificant ($p>0.05$) decrease in the levels of Total protein (TP) and Albumin (ALB) in the treated rats (group 3, 4, 5 and 6) when compared with the negative control (group 2).
Table 3: Concentrations of liver marker enzymes AST, ALT, TP, and ALB in CCl₄-induced rats treated with ethyl acetate: ethanol fraction of ethanolic extract of *A. digitata* leaves

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>AST(U/L)</th>
<th>ALT(U/L)</th>
<th>TP(gm/dL)</th>
<th>ALB(gm/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>51.14±10.93ᵃ</td>
<td>46.82±14.16ᵇ</td>
<td>5.53±2.20ᵃ</td>
<td>3.06±0.38ᵃ</td>
</tr>
<tr>
<td>2</td>
<td>2 ml/kg CCL₄ only</td>
<td>184.33±32.8ᵇ</td>
<td>176.28±17.7₀ᵈ</td>
<td>6.82±0.46ᵃ</td>
<td>3.37±0.33ᵃ</td>
</tr>
<tr>
<td>3</td>
<td>CCL₄+20 mg fraction</td>
<td>155.37±61.07ᵇ</td>
<td>135.61±10.76ᶜ</td>
<td>6.62±0.33ᵃ</td>
<td>3.24±0.30ᵃ</td>
</tr>
<tr>
<td>4</td>
<td>CCL₄+40 mg fraction</td>
<td>124.89±21.32ᵇ</td>
<td>137.98±11.35ᶜ</td>
<td>6.69±0.55ᵃ</td>
<td>3.29±0.26ᵃ</td>
</tr>
<tr>
<td>5</td>
<td>CCL₄+50 mg fraction</td>
<td>180.86±97.69ᵇ</td>
<td>17.02±6.20ᵃ</td>
<td>6.55±0.84ᵃ</td>
<td>2.67±1.35ᵃ</td>
</tr>
<tr>
<td>6</td>
<td>CCL₄+Silymarin (25 mg/kg)</td>
<td>166.44±12.46ᵇ</td>
<td>4.96±2.22ᵃ</td>
<td>5.95±0.76ᵃ</td>
<td>2.79±1.35ᵃ</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± Standard deviation of 6 animals in a group. Values with same letter superscripts are not significantly different from each other at (p>0.05), while values with different letter superscripts are significantly different from each other at p < 0.05.

DISCUSSION

The total antioxidant capacity of ethanolic extract of *Adansonia digitata* leaves in this current research varied from 84.72 – 29.27 mg/ml indicating that the plant had an appreciable amount of bioactive compounds. The results for total antioxidant capacity showed that fraction 6b had the highest antioxidant value and the solvent combination “ethyl acetate:ethanol” (50:50) was the most effective solvent combination for determination of antioxidant activity of ethanolic extract for *Adansonia digitata* leaves agreeing with the research of Kayode *et al.* (2019). Fraction 3a “chloroform” (100:0) showed the least extraction ability. The antioxidant capacity of the *Adansonia digitata* may be due the presence
of phenolic and flavonoid compounds in the plant which are powerful in vitro antioxidant molecules (Londhe et al., 2008; Yakubu et al., 2022).

The total phenolic content of the fractions of Adansonia digitata ethanolic leaves extract in this present investigation ranged between 2177.33- 35.67 mg/ml with fraction 7b having the highest phenolic content and fraction 4b, the lowest phenolic content. This means that ethanol was the best solvent for the extraction of phenols. Phenolic compounds are the major active natural antioxidants in plants and are very important because their hydroxyl groups confer scavenging capacity (Trease and Evans, 1985; Kayode et al., 2018). The presence of phenol in Adansonia digitata is a clear indication that the plant can be used in pharmaceuticals for the treatment of several disease conditions (Kayode et al., 2018).

The Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of ethyl acetate: ethanol fraction of Adansonia digitata leaves revealed the presence of 14 phytochemicals in this current research work. The peak area percentage showed that Bis(2-ethylhexyl) phthalate had the highest relative abundance of 48.30 % while 2-Methyl-Z,Z-3,13-octadecadienol had the lowest relative abundance of 0.14 %. Others were dichloroacetic acid, tridecyl ester, d-Glycero-d-ido-heptose, Phthalic acid, ethyl pentyl ester, Trifluoroacetoxy hexadecane, 2-Hexene, 6-nitro-2-Tetradecanol, 3,5-Dimethyl-2-octanol, Cyclopropanecarboxylic acid, 7,11-Hexadecadienal, 9-Octadecenamide, Squalene, and E, E-1,9,17-Docasatriene with relative abundance of 0.44%, 0.83%, 1.98%, 1.17%, 0.9%, 1.53%, 7.27%, 0.46%, 6.05%, 6.23%, and 1.22% respectively. Nisha et al. (2023) reported that dichloroacetic acid, tridecyl ester inhibit the activity of enzyme 1 dehydrogenase kinase, this enzyme regulates the activity of hydrogenase in microorganisms, the enzyme is also involved in the phosphorylation of hydrogenase, which is the enzyme that catalyzed the reversible conversion of hydrogen molecule. Dharmalingam and Nazni (2013) reported that d-Glycero-d-ido-heptose has sugar moiety property, the sugar often referring to carbohydrate, serves various essential functions such as energy source, structural support, store information, cellular communication, fuel storage, metabolic intermediates, antioxidant properties as well as osmotic regulation in biological system. Phthalic acid, ethyl pentyl ester had been reported to exhibit antimicrobial and insecticidal properties (Huang et al., 2021). Maria et al. (2020) also started that trifluoroacetoxy hexadecane had antibacterial activity. Yakubu et al. (2021) reported that 2-tetradecanol can be used as ingredient in cosmetics. Bis(2-ethylhexyl) phthalate exhibit antimicrobial, antifungal and antitumor activities (Enenebeaku et al., 2021). Cyclopropanecarboxylic acid have been reported to
possess antiviral and antitumor properties (Salaun and Baird 1995). 7,11-Hexadecadienal serve as pollinator and can also be used in assessing insect population as reported by (Yakubu et al., 2021). 9-Octadecenamide possessed anti-inflammatory activity (Ano et al., 2015). Conforti et al. (2005) reported the antioxidant property of Squalene. 2-Methyl-Z,Z-3,13-octadecadienol exhibit pesticide, herbicide and insecticide activities (Adeyemi et al., 2017).

The Fourier Transform Infrared Spectroscopy (FTIR) results of ethyl acetate:ethanol fraction of ethanol extract of A. digitata leaves in this study revealed the presence of various spectra indicating several functional groups. The FTIR analysis of the fraction confirmed the presence of 1º, 2º amines and amides at peak 3306.1 (cm⁻¹). The peak at 2981.9 (cm⁻¹) represented the presence of alkanes as a functional group. The peak at 1640.0 (cm⁻¹) revealed the presence of alkenes. Peak 1386.0 (cm⁻¹) represented the presence of nitro compound. Peak 1084.7(cm⁻¹) and 1043.7(cm⁻¹) indicated the presence of aliphatic amines while peak 875.9 (cm⁻¹) showed the presence of aromatic compound. The characteristic functional groups are responsible for the medicinal properties of the plant which can be utilized for various pharmaceutical purposes (Muruganatham et al., 2009). Biogenic aromatic amines and other aromatic compounds are well known for their neuroprotective and cycloprotective antioxidant activities which could be exploited to manage a good number of disorders, particularly degenerative diseases (Falodun et al., 2013). Nitro containing compounds are also reported for their pesticides activity (ATSDR, 1990)

The High performance Liquid Chromatography (HPLC) analysis of ethyl acetate:ethanol fraction in this present research revealed various chromatogram with retention time and absorbance representing different biological active compounds. Ethyl acetate: ethanol fraction showed seven (7) chromatogram indicating seven (7) different compounds with quercetin as the most prominent compound. The identified phytochemical-quercetin (2,3',4',5,7- pentahydroxylflavone) is a dietary flavonol with numerous pharmacological attributes including antioxidant, antiplatelet, anti-inflammatory, neuroprotective, antimutagenic, anticancinogenic, antiangiogenic, antibacterial, antitumor, antianxiety and hepatoprotective activity (Aluani et al., 2016). Kumar et al. (2010) also reported the anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antidiabetic as well as antiviral activities of quercetin. The various beneficial pharmacological features of quercetin justify its future clinical application (Aluani et al., 2016).
This present investigation revealed significant (p<0.05) increase in the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and insignificant (p>0.05) difference in the level of total protein (TP) and albumin (ALB) in CCl₄-administered rats (group 2) when compared with the normal control (group 1). The significant elevation in the marker enzymes in CCl₄ - administered rats (group 2) in this current study was a confirmation of previous research work on hepatotoxicity of CCl₄ by Patrick-iwuanyanwu et al. (2010). In several experiments involving the induction of liver damage in experimental animals, administered CCl₄ increased the level of marker enzymes (AST and ALT) thereby resulting in significant hepatic damage (Adewale et al., 2014). The increased levels of these biochemical parameters indicated the direct alteration in the hepatic structural integrity (Sa’id et al., 2020). The elevation of marker enzymes (AST and ALT) in rats administered with CCl₄ alone (group 2) observed in this present research was indicative of cellular leakage and loss of functional integrity in the liver (Adewale et al., 2014). The significant increase in the level of AST and ALT in rats administered with CCl₄ alone (group 2) is also an indicator of liver inflammation and necrosis. This means that these enzymes crossed the liver membrane as a result of damage in liver cell membrane (Usunobun et al., 2020). These enzymes are found in the cell cytoplasm and released into circulation once the cellular membrane is damage (Sa’id et al., 2020). The ethanol fraction of ethanol extract of Adansonia digitata leaves administered to group 3, 4 and 5 at different doses (20, 40, 50 mg/kg) and 25 mg/kg of silymarin in group 6 showed no significant (p>0.05) difference in the level of AST when compared with the negative control (group 2). This could be as a result of the dosage used in the experiment which could not mitigate the level of damage caused by CCl₄ or other factors (Ismail et al., 2021). However, the administered fractions and silymarin significantly (p<0.05) decreased the level of ALT in groups 3, 4, 5 and 6 when compared with the negative control (group 2). This indicated that the fractions and silymarin offers hepatic protection and promotes liver integrity and functions (Usunobun et al., 2020). Usunobun et al. (2020) and Abu et al. (2022) also reported the increased in the level of ALT in the liver content of CCl₄ treated rats. The difference in the mean TP and ALB levels for rats treated with CCl₄ in group 2 were not significantly (p>0.05) different when compared with the normal control (group 1). The result agreed with the findings of (Rothschild et al., 1988).
CONCLUSION

Polyphenols have been found to be strong antioxidants which can neutralize free radicals that cause liver damage. In this current research, it was observed that the presence of polyphenols in the plant- *Adansonia digitata* slightly restored the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and albumin (ALB) in serum. The study found out that the plant contained much amount of phenolics, especially flavonoids. Furthermore, the fractions, specifically ethyl acetate: ethanol and its contents may be responsible for the effects elicited.

REFERENCES


