OPEN ACCESS
Indonesian Journal of Health Research and Development Vol 3 No 3 (2025) https://doi.org/10.58723/ijhrd.v3i3.369
Check for updates
Research Article

Metabolic Profile and Hepatoprotective Efficacy of *Carica papaya* Leaves in CCl_4 Induced Oxidative Stress Albino Mice

Received: February 15, 2025	Revised: June 21, 2025	Accepted: November 12, 2025	Published: November, 29 2025

Maryam Usman Abdulkadir, Hamza Ahmed Pantami, Muhammad Bappa Sani, Aliyu Ibrahim Lawan, Hajjagana Hamza*

Abstract

Background: Hepatic disorder is fatal and require vital medication, orthodox cure is deleterious, so there is need for natural curative agents.
Aims: Bioactive phytochemical analysis and efficacy of *Carica papaya* leaves extracts versus CCL_4 induced oxidative stress in mice.
Methods: The metabolite profile of *Carica papaya* leaves extract was analysed by LC-MS/MS analysis via GNPS. Liver aminotransferases were assessed, liver tissues and homogenates were assessed for histopathology and MDA, catalase, superoxide dismutase, and Glutathione S-Transferase activities, qRTPCR mRNA expression of hepatic tissue Glutathione S- Transferase was performed. Free radical scavenging activity of the extract were evaluated using *invitro* prototypes.
Results: Numerous metabolites were identified from crude extract. Observed weight loss in rats, liver damage through elevating serum variables (marker enzymes and bilirubin), decrease in total protein and albumin. A significant decrease in tissue catalase, GST, and superoxide dismutase activities and marked increase in MDA level ($p<0.05$) were also observed. The extract effectively ameliorated CCl_4 effects with marked decrease in serum variables. GST mRNA expression of hepatic tissue and histopathological results also supported the biochemical findings. Extract exhibited radical scavenging activity with DPPH IC_{50} of 0.56mg/ml, FRAP IC_{50} of 0.52mg/ml, H_2O_2 IC_{50} of 0.58mg/ml and Phosphomolybdenum IC_{50} of 0.62.
Conclusion: *Carica papaya* leaves was beneficial in modulating the alterations induced in liver and serum variables of mice in CCL_4 induced oxidative stress.

Keywords: *Carica papaya*; Hepatoprotective; Metabolic profile

1. INTRODUCTION

The liver plays a crucial role in managing the biochemical processing, neutralization, release, and removal of a wide range of internal and external compounds, including xenobiotics (Al-Seeni *et al.*, 2016). In addition to these vital functions, it serves as a primary barrier against the toxic impacts of pharmaceutical agents, toxins, and microbes, it is essential for preserving cellular homeostasis.

Accordingly, the organ's healthy operation determines the person's overall health (Lawal *et al.*, 2015). Despite these defensive functions, the liver's distinct metabolic functions and close proximity to the gastrointestinal system make it vulnerable to a variety of illnesses and conditions brought on by harmful substances, medications, and other agents (Gan *et al.*, 2025).

If treatment is not received, steatosis may progress to chronic hepatitis, fibrosis, and hepatocellular cancer (Delli Bovi *et al.*, 2021). The orthodox medications that are frequently used to treat and manage hepatic disorders have side effects (Cortés & García, 2022). As a result, a lot of focus has been placed on identifying substitute, less harmful, and more efficient antioxidants and hepato-protective agent from natural products for the management and treatment of liver-related illnesses (Tibiri *et al.*, 2020).

Carbon tetrachloride (CCl_4) is an exogenous compound commonly utilized to induce toxic hepatitis and hepatic injury in experimental animal models. CCl_4-induced liver damage functions as one of the key scientific investigative models used to examine the hepatoprotective potential of various pharmacological agents. A single administration of CCl_4, owing to its

potent hepatotoxic properties, can lead to marked hepatic necrosis and steatosis. Use of plant-based remedies as substitutes for conventional medicine holds deep cultural and traditional significance among indigenous communities in Nigeria (Hamza *et al*., 2024).

Medicinal plants are known to contain a broad spectrum of bioactive phytochemicals, which may exert therapeutic effects either independently, synergistically, or additively. Compounds such as phenolic and polyphenols—including flavonoids, flavones, and related derivatives—are associated with many therapeutic effect, namely cytokine inhibiting, anti-carcinogenic, pathogen- suppressing , and antioxidant properties (Sun & Shahrajabian, 2023).

Carica papaya is a member of the *Caricacaece* family, dicotyledonous polygamous and diploid specie. Both the leaves and fruit of *Carica papaya* are rich in diverse bioactive constituents, which may enhance antioxidant capacity and mitigate lipid peroxidation (Singh *et al*., 2020). Specifically, the leaves contain a variety of phytochemicals which are believed to contribute to cellular protection against oxidative stress. (Khor *et al.,* 2021) and has been used in folklore medicine for centuries as anti-inflammatory, antitumor agents and immune adjuvant for vaccine therapy (Nugroho *et al*., 2017).

The various parts of *Carica papaya* have been widely documented for their ethnopharmacological uses. Traditionally, the leaves have been applied as poultices to alleviate neuropathic pain and elephantoid swellings, and in some tropical indigenous communities, they are smoked as a remedy for asthma (Airaodion *et al*., 2019). In folk medicine, the plant's latex is reputed to relieve dyspepsia and is also utilized topically for treating burns and scalds. Both the seeds and fruits are traditionally recognized for their potent antihelminthic and antiamoebic activities.

In Chinese traditional medicine, most of the *C. papaya* plant parts are employed in the management of gastrointestinal discomfort, microbial infections, and inflammatory conditions (Bhowmick *et al*., 2021). The therapeutic applications of *C. papaya* in traditional medicine are linked to its abundance of bioactive compounds, comprising tannins, steroids, terpenoids, saponins, phenolic compounds, flavonoids, proanthocyanidins, alkaloids, anthraquinones, cardiac glycosides, and antioxidant constituents such as ferric reducing antioxidant power (FRAP) indicators (Ugbogu *et al.,* 2023).

Based on claimed curative value of *Carica papaya* in ethnomedicine and paucity of data regarding pharmacological potentials of this plant. We assumed *Carica papaya* leaf extract may be rich in phytochemicals with therapeutic values and also has the ability to alleviate CCL_4-induced oxidative injury in mice liver by modulating the activities of liver marker enzymes and oxidative stress biomarkers. This hypothesis was tested in an *in vivo* murine model as well as *in vitro* antioxidant experiments.

2. MATERIAL AND METHOD

2.1 Chemicals and Reagents

Silymarin, carbon tetrachloride (CCl4), trichloroacetic acid (TCA), were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA Solvents were of LC-MS grade (Sigma-Aldrich, Germany). All other chemicals used were of analytical grade.

2.2 Sample Collection and Extract Preparation

Fresh, healthy, matured *Carica papaya* leaves were plucked from the plant within Gombe metropolis. The leaf specimen was taxonomically identified and validated at the Department of Plant Sciences, Gombe State University, Gombe, Nigeria, with a previously deposited voucher specimen (No. GSU H253) archived in the departmental herbarium. *Carica papaya* leaves were carefully washed using sterile water to remove external impurities and particles, dried under shade in laboratory setting. Samples were milled in to fine-texture and preserved in a clean, airtight, dark container until further use.

Two hundred (200g) of dried fine powder of the *Carica papaya* leaves was macerated with 1000ml ethanol (70%) at room temperature for 2 weeks and the mixture was passed through a Whatman No. 1 (11 cm) filter paper (Hikmawanti et al., 2021), and the resulting filtrate was concentrated with a rotary evaporator maintained at 40 °C. dark green jelly−like extract gotten was kept in the desiccator until use.

2.3 Sample Preparation and UHPLC-MS/MS Analysis

A 2 mg aliquot of the 70% ethanol extract was reconstituted in 1 mL of LC-MS-grade methanol. The solution was vortexed for 10 minutes, centrifuged for another 10 minutes, and subsequently filtered through a 0.22 µm nylon membrane into a clean glass vial for LC-MS/MS analysis. Chromatographic separation was conducted using a Dionex Ultimate 3000 UHPLC system equipped with a DAD-3000 diode-array detector. A C18 reversed-phase Hypersil GOLD aQ column (100 × 2.1 mm, 1.9 µm particle size) maintained at 30 °C was employed for separation.

Gradient elution was achieved using two mobile phases: Solvent A—comprising 0.1% formic acid and 10 mM ammonium formate in a 70:30 methanol-acetonitrile mixture (500 mL)—and Solvent B—consisting of 0.1% formic acid and 10 mM ammonium formate in 500 mL LC-MS-grade water. The gradient profile was set to 20% A for the initial 5 minutes, followed by a linear increase from 20% to 80% A over the next 25 minutes, at a constant flow rate of 0.2 mL/min. The sample injection volume was 10 µL, and detection wavelengths were set at 210, 310, 410, and 510 nm.

Mass spectrometric detection was performed using a Q-Exactive Focus Orbitrap LC-MS/MS system operated in both positive and negative ESI (electrospray ionization) switching modes, scanning a mass range of *m/z* 100–1500 amu. The ESI source

parameters included a spray voltage of 4.2 kV, with high-purity nitrogen used as both sheath and auxiliary gas at flow rates of 40 and 10 arbitrary units, respectively. The capillary temperature was maintained at 350 °C, while the auxiliary gas heater was set at 10 °C. Data acquisition and initial processing were carried out using Thermo Xcalibur software version 2.2 SP1.48.

MS data were converted to mzXML format using MSConvert, then uploaded to the Global Natural Products Social Molecular Networking (GNPS) platform (Wang et al., 2016) via FileZilla for molecular networking. Network construction used the GNPS Data Analysis Workflow with a precursor ion mass tolerance of 0.02 Da and a fragment ion mass tolerance of 0.02 Da. Fragment ions with intensities below 10 counts were excluded. Molecular networks (MNs) were generated based on a minimum of six matched peaks and a cosine similarity score threshold of 0.7.

The resulting networks were downloaded and visualized using Cytoscape software version 3.7.1 (Institute for Systems Biology, Seattle, WA, USA). The visualized cytoscape was uploaded to the MZmine software where the spectrum was gotten (Pantami *et al*., 2020). The compound identification was validated by comparison with existing literature and available databases such as Human Metabolome Data Base (HMDB), PubChem, METLIN and Mass Bank.

2.3 Biological Studies

Animal Maintenance

Healthy albino mice of the strain (C5BL/6) were procured from NITR Jos, Plateau state Nigeria. The animals were maintained in clean, small-sized polypropylene cages under controlled laboratory settings that maintained a 12-hour light and dark alternation, a room temperature of 25 ± 2 °C, and a relative humidity level of 55 ± 5%. The animals were provided with standard pelletized feed (vital feds Jos Nigeria), clean tap water and were maintained for one week to acclimatized.

2.4 Experimental Design

A total of thirty (n = 30) adult albino mice were arbitrarily allotted into five groups (n = 6 per group) and treated over a period of four consecutive weeks as follows:

Group I (Normal Control): Received 1% distilled water orally three times per week and intraperitoneal (IP) injections of olive oil at a dose of 0.5 mL/kg body weight (BW) twice weekly.

Group II (CCl_4 Control): Administered IP injections of carbon tetrachloride (CCl_4) at 0.5 mL/kg BW, prepared in a 1:1 (v/v) ratio with olive oil, twice per week to induce hepatotoxicity.

Group III (Silymarin + CCl_4): Treated with silymarin at a dose of 100 mg/kg BW via oral gavage, five times per week, in addition to CCl_4 administration (0.5 mL/kg BW in olive oil, IP) twice weekly.

Group IV (Low Dose Extract + CCl_4): Received the plant extract orally at a dose of 200 mg/kg BW daily. One-hour post-treatment, CCl_4 (0.5 mL/kg BW in olive oil, IP) was administered twice per week.

Group V (High Dose Extract + CCl_4): Administered the plant extract orally at a dose of 400 mg/kg BW daily. After one hour, animals were given CCl_4 (0.5 mL/kg BW in olive oil, IP) twice weekly.

2.5 Biochemical Analyses

Serum level of aminotransferase; ALAT, ASAT, total proteins, total bilirubin, and albumin were analyzed using commercial diagnostic reagent kits (Randox Laboratories Ltd., UK; Agappe Diagnostics Ltd., India), following the manufacturers' instructions.

2.6 Estimation of Oxidative Stress markers

In vivo oxidative stress biomarkers Glutathione S-transferase, Catalase, Superoxide dismutase and Lipid peroxidation (MDA) level in liver tissue was assayed using ELISA kit (Beijing Solarbio Science and Technology Co., Ltd.) following the manufacturers' instructions.

2.7 Estimation of Glutathione S Transferase mRNA expression

The hepatic tissue GST mRNA expression was assayed using qRT PCR kit. QuickExtract RNA Extraction Kitby PrimeScript™ RT reagent kit; United Kingdom was used to target and extract RNA, and qRTPCR kit SolarbioScience and Technology Co., Ltd Beijing was used for GST mRNA expression. All procedures were conducted under standardized laboratory conditions following the manufacturers' instructions.

2.8 Histopathological Examination of Liver

In each Group a representative was Samples were gathered and preserved in 10% neutral buffered formalin at ambient temperature for a duration of 24–48 hours, after which they were systematically treated and enclosed within paraffin wax blocks. A thin section (~5 µm) was prepared and colored following the conventional hematoxylin and eosin (H&E) staining procedure to highlight nuclear and cytoplasmic structures — following the refined methodology described by Peñuelas, Laguna, and Vila (2024), later observed under a light microscope (magnification, x100 and x200) and images were captured.

2.9 Estimation of *In vitro* Antioxidant Markers of *Carica papaya* leaves Extrac

2.9.1 Determination of Ferric Reducing Antioxidant Power (FRAP) (Xiao, 2020).

A volume of 2.5 mL of 0.2 M phosphate buffer (pH 6.6) along with 2.5 mL of a 1% potassium ferricyanide reagent were added to the assay solution (1 mL) and combined. The reaction solution was maintained at 50 °C for 20 minutes, after which 2.5 mL of 10% trichloroacetic acid was introduced and the mixture centrifuged for 10 minutes. Subsequently, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance of all samples was measured at 700 nm,

and results were expressed as milligrams of ascorbic acid equivalents per gram of extract. (mg AAE/g extract).

2.9.2 Determination of Hydrogen Peroxide (H_2O_2) Scavenging Assay (Gülçin, 2025).

A 40 mM hydrogen peroxide (H_2O_2) solution was freshly prepared using a 50 mM phosphate buffer at pH 7.4, and its concentration was verified spectrophotometrically at 230 nm. Sample extracts (20–60 µg/mL) were combined with the H_2O_2 solution and allowed to react for 10 minutes at room temperature. The absorbance was then recorded at 230 nm using a buffer-only blank as the reference.The scavenging capacity was calculated using:

% scavenged (H_2O_2) = [(Ai – At)/Ai] ×100

Where Ai is the absorbance of control and at the absorbance of test.

2.9.3 Determination of Total Antioxidant Capacity (Phosphomolybdic Acid Method) Benjamaa *et al.*, (2024)

The total antioxidant activity was determined through the conversion of molybdenum (VI) to molybdenum (V) by the test extract, leading to the formation of a green-colored phosphomolybdenum complex under acidic conditions. A reagent mixture consisting of 0.6 M sulfuric acid (H_2SO_4), 28 mM sodium phosphate (Na_3PO_4), and 4 mM ammonium molybdate ($(NH_4)_6Mo^{7+}$) was combined with 0.3 mL of the extract at concentrations of 25, 50, and 100 µg/mL, while control tubes contained methanol instead. The mixtures were incubated at 95 °C for 90 minutes, allowed to cool to ambient temperature, and their absorbance was read at 695 nm. Antioxidant results were expressed in ascorbic acid equivalents, using a calibration curve.

2.9.4 Determination of Diphenyl- Picryl Hydrazine (DPPH) Scavenging Activity

The free radical scavenging activity of the sample was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method described by Angeli *et al.* (2023) with minor adjustments. In summary, 1.0 mL of a 0.3 mM DPPH solution prepared in methanol was mixed with 1.0 mL of the sample extract, and the reaction mixture was kept in the dark for 10 minutes. The absorbance was then measured at 517 nm. The ability of extract to scavenge the DPPH radical was assessed using the formula;

$$\frac{Ao-A1}{Ao} \; x \; 100\%$$

Where:

Ao is the absorbance before reaction,

A1 is the absorbance after reaction has taken place.

The concentration of the sample necessary to cause 50% inhibition (IC_{50}) was evaluated by extrapolation from the linear regression analysis

2.10 Statistical Analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS, version 16.0). Variations among experimental groups were considered within one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for several comparisons. Statistical significance was established at *p* < 0.05. Results are stated as mean ± standard error of the mean (SEM).

2.11 Abbreviations and Acronyms

CCL_4-carbon tetrachloride; CP-*Carica papaya*; DPPH 2,2 , diphenyl-1-picryl-hydrazl;H_2O_2-hydrogen peroxide; GST-glutathione S transferase; MDA-malondialdehdye; GNPS-global natural product social molecular network; LC/MS-liquid chromatogramhy/mass spectrometry; qRTPCR-quantitative reverse transcriptase polymerase chain reaction; FRAP-ferric reducing antioxidant power; CH_3CCL_3- trichloromethyl; $CH_3CCL_3O_2$-trichloromethylperoxy; UHPLC-ultra high performance liquid chromatography; NITR-national institute of trypanosomiasis research; IP-intraperitoneal; AST-aspartate amino transferase; ALT-alanine amino transferase;

2.12 Ethical approval

The ethics of the animal study was authorized by the Gombe State University (GSU) Institutional Animal Care and Use Committee (IACUC/GSU-R02/2023).

3. RESULT AND DISCUSSION

3.1 Results

3.1.1 Metabolite Profiling of *Carica papaya* leaves Extract.

Table 1 summarizes the metabolites identified in the sample, along with their corresponding chemical structures and molecular formulas. The chromatographic peaks representing each metabolite are indicated numerically in Figure 1. Liquid chromatography–mass spectrometry (LC–MS) analysis was performed in positive ionization mode, and compound identification was achieved by interpreting the full MS data in conjunction with MS/MS fragmentation spectra.

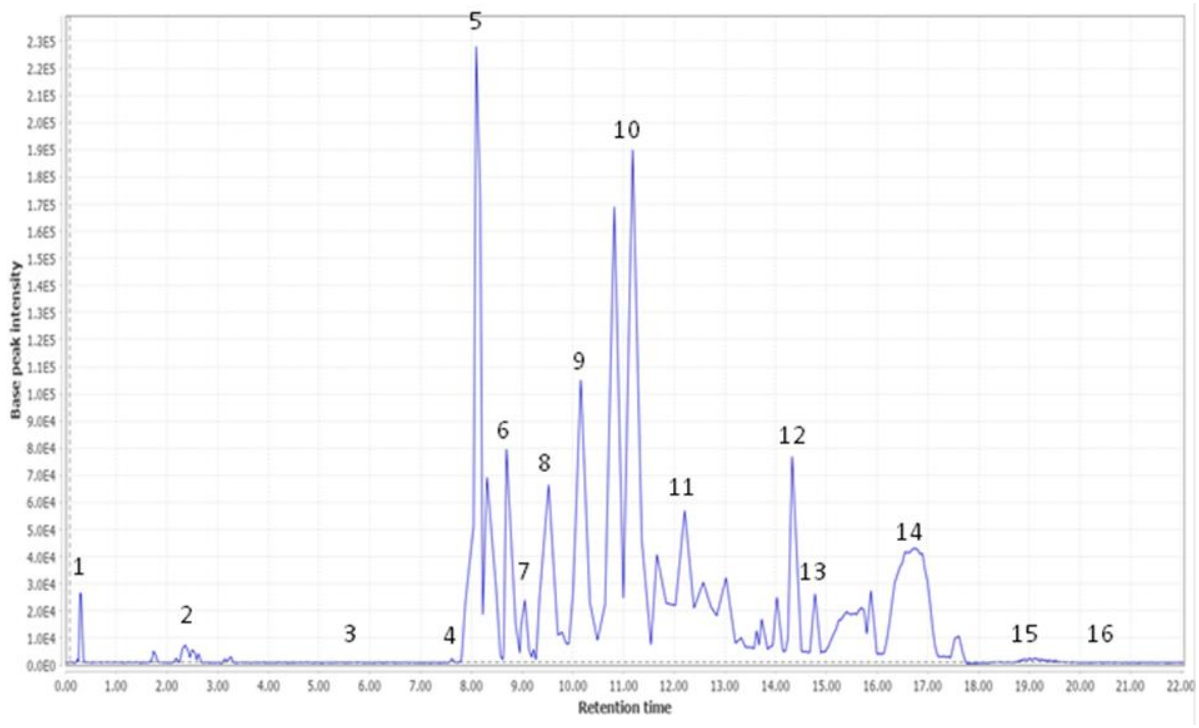

Figure 1. A chromatogram of (Dionex) LCMS of *Carica papaya* leaves extract in positive node. Number refers to the identified compound listed in Table 1.

Table 1. Metabolites identified in *Carica papaya* leaves extract using LCMS.

Peak No	m/z [M^+H^+]	MS/MS Fragments (*m/z*)	Molecular Formula	Identification
		PHENOLIC ACID		
12	279.1565	194, 196, 218, 262, 268, 280, 281, 291, 305	$C_{16}H_{22}O_4$	(S)-1- Methyhexyl Caffeate
		FLAVONOIDS		
14	425.3782	123, 135, 137, 149, 189, 191, 203, 215, 407, 425	$C_{24}H_{24}O_7$	8-C-Methylvelloquercetin trimethyl ether
		ALKALOIDS		
2	365.1087	335, 217, 189, 175, 161	$C_{20}H_{16}N_2O_5$	10-Hydroxycamptothecin
5	240.1990	190, 220, 222, 230, 238, 240, 241, 244, 248, 266	$C_{14}H_{25}NO_2$	Pseudodistamine
13	425.2076	184, 425, 426, 427, 825, 826, 827, 838, 839	$C_{24}H_{28}N_2O_5$	Jerantine A acetate
		TERPENES GLYCOSIDES		
9	363.1642	173, 319, 345, 363, 364, 385, 407, 429, 451, 473	$C_{16}H_{26}O_9$	Patriscabroside I

Peak No	m/z [M^+H^+]	MS/MS Fragments (*m/z*)	Molecular Formula	Identification
			FATTY ACIDS	
1	226.9544	130, 143, 205, 209, 223, 225, 226, 227	$C_6H_4CL_2O_5$	2, 5- dichloro-4-oxohex-2-enedioate
8	307.1707	147, 217, 241, 245, 307, 308, 326, 329, 330, 343	$C_{15}H_{27}ClO_4$	Chlorosphaero-lactylate B
16	217.1052	155, 166, 173, 217, 218, 235, 254, 257, 258, 273	$C_{10}H_{16}O_5$	Ophiocerin D
11	763.3803	404, 619, 745, 763, 764, 780, 783, 785, 786, 829	$C_{38}H5_0O_6$	Dichapetalin U
			AMINO ACIDS	
4.	205.0970	205.0853, 206.0890	$C_{11}H_{12}N_2O_2$	L-Tryptophan
			PEPTIDE DERIVATIVES	
10	419.2278	229, 347, 401, 419, 420, 436, 441, 442, 457, 483	$C_{21}H_{30}N_4O_5$	IIe-Thr-Trp
			ORGANIC ACIDS	
3	217.1052	173, 217, 218, 235, 236, 252, 254, 257, 258, 273	$C_{10}H_{16}O_5$	4-oxosebacic acid
6	217.1052	173, 217, 218, 235, 254, 273	$C_{10}H_{16}O_5$	Decarestrictine D
15	158.9581	158, 162,	$C_2H_7O_2PS_2$	Dimethyldithio-Phosphate
			UNIDENTIFIED	
7	345.1080	181, 217, 323, 324, 345, 346, 361, 387, 409, 495	$C_{17}H_{16}N_2O_6$	Venezuline

3.1.2 Effect of *Carica papaya* leaves Extract on Serum AST, ALT, Total Protein, Total Bilirubin and Albumin in CCl_4 Induced Albino Mice

Administration of 0.5mL/kgbodyweight carbon tetrachloride (CCl_4) in olive oil, twice per week caused a significant (*p* < 0.05) increase in serum AST levels in the CCl_4-exposed group compared to the normal control. However, silymarin (100 mg/kg b. wt) and the extract at doses of 200 mg/kg and 400 mg/kg b. wt markedly reduced AST activity by 73.6%, 59.5%, and 61.0%, respectively, relative to the CCl_4 group (Table 1a).

Similarly, CCl_4 exposure produced a marked (*p* < 0.05) elevation in serum ALT activity was observed compared to the normal control. Subsequent treatment with silymarin (100 mg/kg b. wt) and the extract at doses of 200 mg/kg and 400 mg/kgb. wt produced a significant (*p* < 0.05) decline in ALT levels when compared with the CCl_4-treated group. (Table 1b).

CCl_4 intoxication caused a marked decline in serum total protein compared to the normal control. In contrast, administration of silymarin (100 mg/kg b.wt.) and the extract at 200 mg/kg and 400 mg/kg b.wt. significantly ($p < 0.05$) restored total protein levels (Table 1c).

A significant ($p < 0.05$) reduction in serum albumin was also observed in the CCl_4 group in compared to the normal control (Table 1e). Treatment with silymarin and the extract at both doses significantly increased serum albumin in compared to the CCl_4 group ($p < 0.05$).

Furthermore, serum total bilirubin levels were significantly elevated ($p < 0.05$) following CCl_4 administration. Post-treatment with silymarin (100 mg/kg b.wt.) and the extract (200 mg/kg and 400 mg/kg b.wt.) significantly reduced total bilirubin concentrations in compared to the CCl_4 group ($p < 0.05$) (Table 1d).

Table 2.0: Depicting effect of *Carica papaya* leaves on Liver parameters.

Groups	AST (U/L) (a)	ALT (U/L) (b)	TP (g/L) (c)	TB (mg/dl) (d)	ALB (g/L) (e)
Control	$38.00^{a} \pm 4.58$	$36.99^{a} \pm 2.46$	$27.65^{a} \pm 0.63$	$13.84^{a} \pm 0.46$	$14.04^{a} \pm 0.32$
CCl_4	$175.65^{b} \pm 3.39$	$167.88^{b} \pm 8.87$	$20.49^{b} \pm 0.74$	$20.89^{b} \pm 0.49$	$8.87^{b} \pm 0.06$
Silymarin+CCl_4	$46.61^{c} \pm 5.70$	$49.00^{c} \pm 7.11$	$28.77^{a} \pm 0.69$	$14.41^{a} \pm 0.85$	$14.28^{a} \pm 0.34$
CCl_4+200 mg/kg bwt	$71.00^{d} \pm 7.86$	$49.78^{c} \pm 4.72$	$29.66^{a} \pm 2.50$	$8.89^{c} \pm 1.69$	$14.53^{a} \pm 0.52$
CCl_4+400 mg/kg bwt	$68.40^{d} \pm 7.89$	$48.84^{c} \pm 2.86$	$31.02^{a} \pm 6.68$	$15.19^{a} \pm 3.51$	$16.18^{c} \pm 1.60$

(AST, ALT, Total Protein, Bilirubin and Albumin) in CCl_4 Induced albino mice. All values are represented as ± standard error of mean (SEM) of six different replicates. Values with different superscript are significantly different (P< 0.05) down the column.

3.1.3 Effect of *Carica papaya* Extract on Antioxidant Biomarkers (GST, CAT, SOD, and MDA) in CCl_4-Induced Albino Mice

Intraperitoneal administration of 0.5 mL CCl_4 in olive oil (1:1, v/v) to mice resulted in a notable ($p < 0.05$) reduction in hepatic glutathione S-transferase (GST) activity in comparison with the normal control group. Treatment with silymarin (100 mg/kg b.wt.) and *Carica papaya* extract at doses of 200 mg/kg.b.wt and 400 mg/kg b.wt. markedly ($p < 0.05$) restored GST activity relative to the CCl_4 group (Table 2a).

Catalase (CAT) activity was also markedly reduced ($p < 0.05$) in the CCl_4-treated group in comparison with the control. Treatment with silymarin or the extract at both doses markedly increased CAT activity ($p < 0.05$) relative to the CCl_4 group (Table 2b).

Similarly, CCl_4 exposure caused a notable ($p < 0.05$) decline in superoxide dismutase (SOD) activity in comparison with the normal control. Administration of silymarin and the extract (200 mg/kg and 400 mg/kg b.wt.) markedly increased SOD activity ($p < 0.05$) in comparison with the CCl_4 group (Table 2c).

In contrast, malondialdehyde (MDA) levels were markedly elevated ($p < 0.05$) in the CCl_4 group relative to the control. Post-treatment with silymarin and both doses of the extract resulted in a notable ($p < 0.05$) decrease in MDA concentrations in comparison with the CCl_4 group (Table 2d).

Table 3. Effect of *Carica papaya* leaves on in vivo antioxidant biomarkers in CCl_4-induced albino mice. All data are presented as ± standard error of mean (SEM) for six replicates. Values within the same column bearing different superscript letters significantly discrete ($p \leq 0.05$) according to Tukey's post hoc test.

Groups	GST (U/g) (a)	CAT (U/g) (b)	SOD (U/g) (c)	MDA (nmol/g) (d)
Control	$1.81^{a} \pm 0.08$	$704.80^{a} \pm 8.89$	$93.62^{a} \pm 1.08$	$102.47^{a} \pm 1.10$
Ccl_4	$0.17^{b} \pm 0.01$	$229.01^{b} \pm 4.65$	$28.09^{b} \pm 2.18$	$242.17^{b} \pm 3.82$
Silymarin+ccl_4	$0.66^{c} \pm 0.02$	$478.45^{c} \pm 1.97$	$63.66^{c} \pm 2.91$	$121.03^{c} \pm 1.13$
CCL_4+200mg/kg bwt	$0.86^{d} \pm 0.06$	$519.05^{d} \pm 15.24$	$79.47^{d} \pm 4.32$	$138.55^{c} \pm 1.63$
CCL_4+400mg/kg bwt	$0.91^{d} \pm 0.02$	$551.55^{d} \pm 10.71$	$86.64^{d} \pm 5.21$	$162.67^{d} \pm 1.91$

3.1.4 Effect of *Carica papaya* Leaves on GST mRNA Expression in CCl_4-Induced Albino Mice

Administration of 0.5 mL CCl_4 in olive oil (1:1, v/v) to mice caused a notable ($p < 0.05$) downregulation of glutathione S-transferase (GST) mRNA expression compared to the typical contro group. In contrast, silymarin at dose of 100 mg/kg b.w or *Carica papaya* leaves at doses of 200 mg/kg and 400 mg/kg b.wt. markedly ($p < 0.05$) upregulated GST mRNA expression relative to the CCl_4 group (Table 3).

Table 4. Depicting the effect of *Carica papaya* leaves on Glutathione S Transferase mRNA expression in CCl_4 induced albino mice. The relative mRNA level of GST is directly proportional to gene expression. All values are represented as ± standard error of mean (SEM) of six different replicates (P<0.05). Values with different superscripts across column are statistically different (P ≤ 0.05).

Groups	Relative GST mRNA Expression Level
Control	$12.165^{b} \pm 0.663$
CCl_4	$85.730^{a} \pm 4.062$
Silymarin + CCl_4	$41.702^{c} \pm 5.515$
CCl_4 + 200mg/kg bwt	$35.512^{c} \pm 3.004$
CCl4 + 400 mg/kg bwt	$32.290^{c} \pm 6.216$

3.1.5 Effect of *Carica papaya* leaves on *In Vitro* Antioxidant Markers

A. DPPH Radical Scavenging Activity of *Carica papaya* Leaves Extract

The percentage inhibition of DPPH radicals was evaluated at extract dosage of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. As shown in Figure 4.8, a concentration-dependent rise in scavenging activity was noted, with higher concentrations exhibiting greater radical quenching capacity. Linear regression analysis of the dose–response curve yielded an IC_{50} value of 0.56 mg/mL (Table 5), indicating strong antioxidant potential. The relationship between concentration and scavenging percentage was statistically significant ($p < 0.05$), confirming that the extract's activity against DPPH radicals was both potent and dose-dependent (Figure 2)

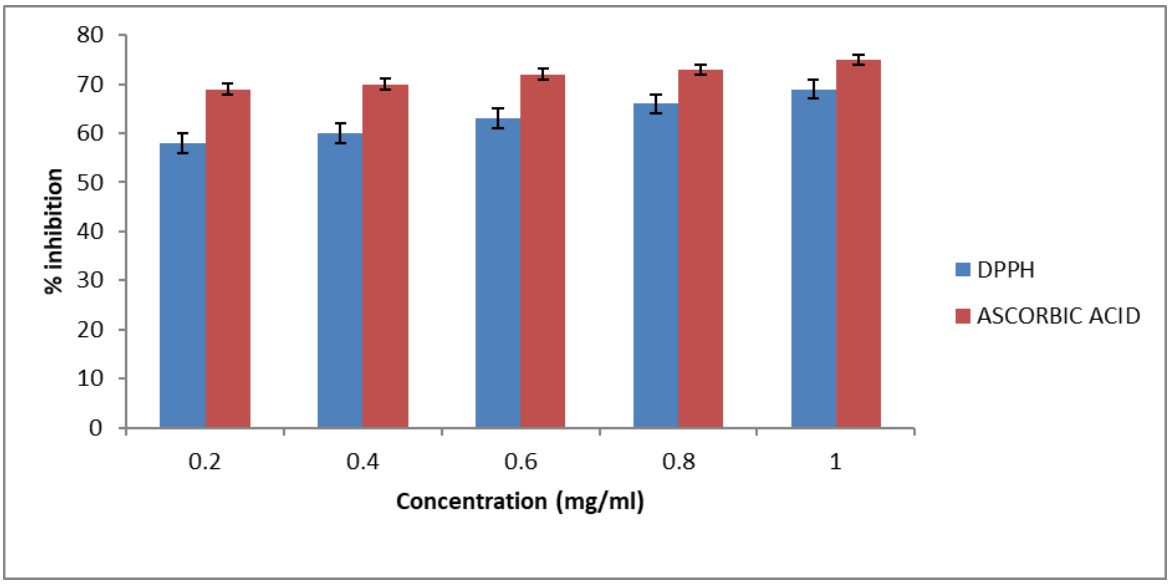

Figure 2. Histogram showing DPPH free radicals scavenging activity of *Carica papaya* leaves extract. Data represent are SEM of three independent experiments.

B. Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity of *Carica papaya* Leaves Extract

The hydrogen peroxide scavenging potential of *Carica papaya* leaves extract and ascorbic acid across various concentrations is presented in Figure 3.0. Both the extract and ascorbic acid exhibited a concentration-

dependent increase in scavenging activity, indicating that hydroxyl radical quenching capacity improved with increasing concentration. The extract demonstrated potent activity, with IC_{50} value of 0.58 mg/mL (Table 5), which was statistically comparable ($p < 0.05$) to that of ascorbic acid. These findings suggest that *C. papaya* extract showed an effective hydroxyl radical scavenging efficacy close to the standard antioxidant used.

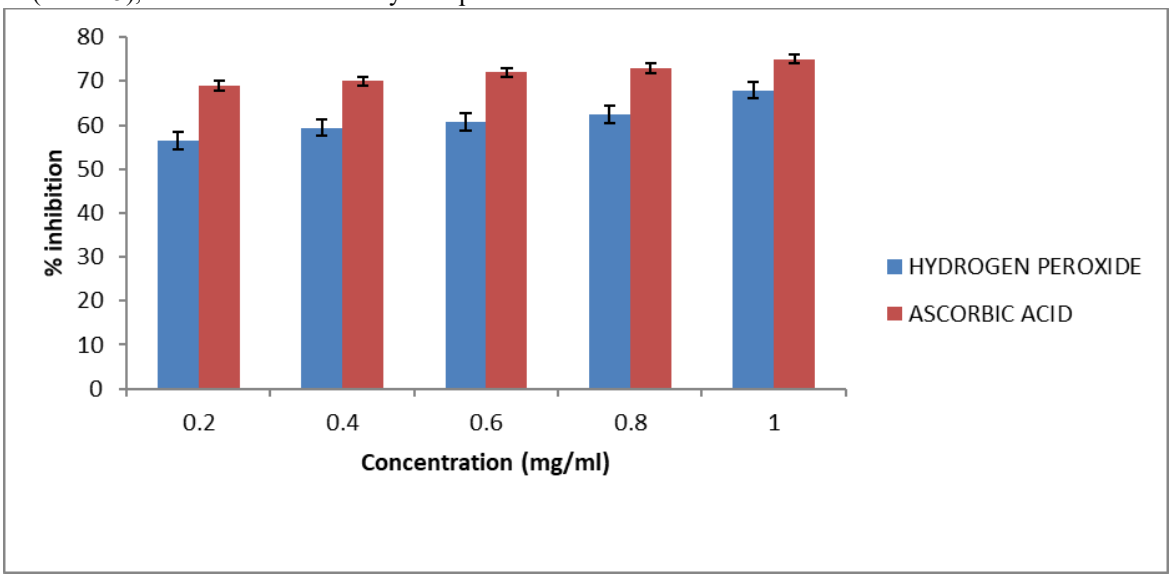

Figure 3. Histogram showing Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity of *Carica papaya* leaves extract.

C. Phosphomolybdenum Method

The percentage scavenging effect of the extract on phosphomolybdenum showed a corresponding rise with increasing extract concentrations. as shown in Figure 4.0. Its IC_{50} value was found to be 0.58 mg/ml compared to that of standard Ascorbic acid 0.50mg/ml Table 5.

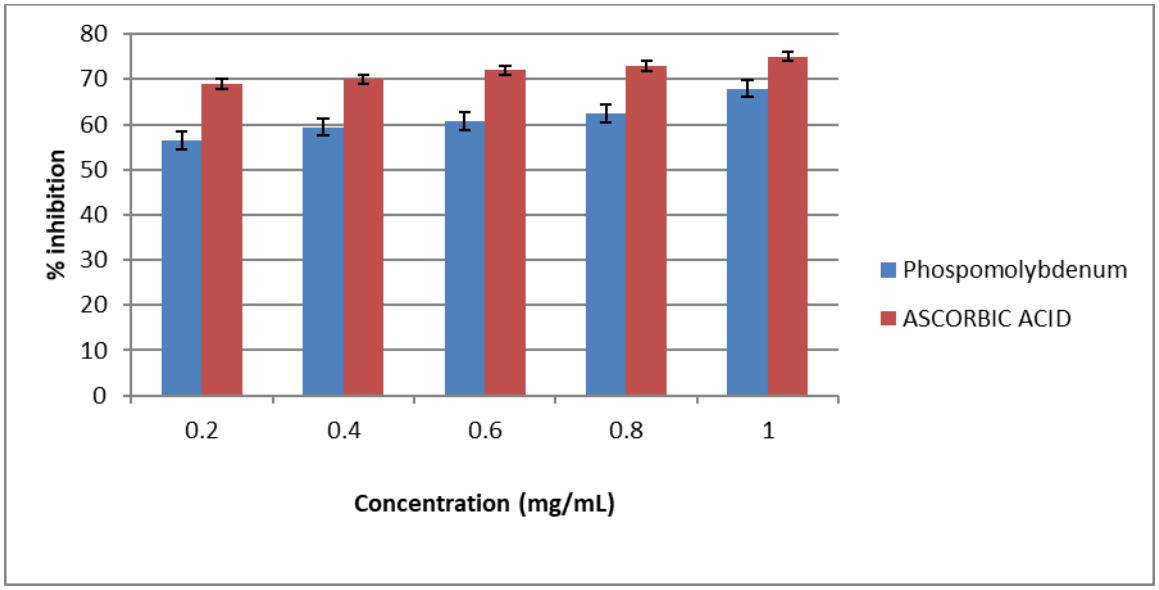

Figure 4. Histogram showing Total antioxidant capacity of *Carica papaya* leaves extract.

D. Ferric Reduction-Antioxidant Power (FRAP) Assay

The reducing power of the *Carica papaya* extract exhibited a progressive enhancement with increasing concentrations. as shown in Figure 5.0. From the analysis, ethanolic extract of *Carica papaya* at a concentration of 1 mg/ml produced the maximum inhibitory effect. The percentage inhibition of the extract of at 1 mg/ml was found to be 66% compared to that of standard ascorbic acid 75% and its IC_{50} value was found to be 0.62mg/ml compared to that of standard ascorbic acid 0.50 mg/ml (Table 5).

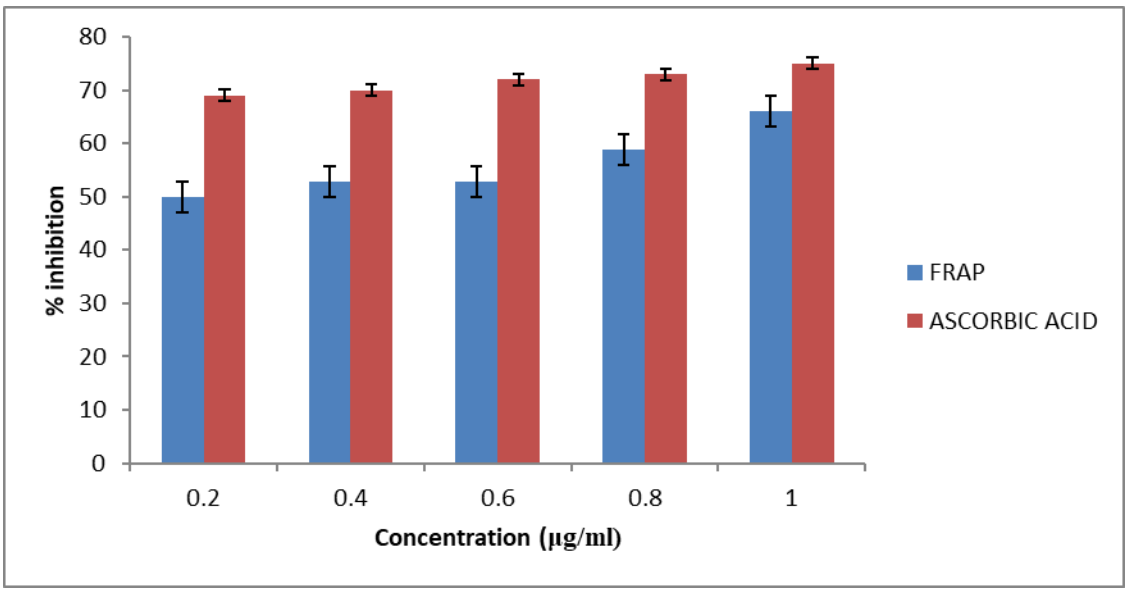

Figure 5. Plot showing Ferric reducing antioxidant power of *Carica papaya leaves* extract.

E. Histopathological Findings

Histological examination of liver sections from the CCl_4-treated group (Figure 6.0b) revealed dispersed focal degenerative changes within the hepatic parenchyma, characterized by pale focal areas, hepatocellular vacuolation, steatosis, cellular degeneration, and empty cells with dark pyknotic nuclei, interspersed with normal foci. In contrast, liver sections from mice administered silymarin (100 mg/kg b.wt.) or *Carica papaya* leaf extract at doses of 200 mg/kg and 400 mg/kg b.wt. exhibited marked improvement in CCl_4-induced hepatic lesions, with reduced vacuolation and degeneration, as shown in Figures 6.0c, 6.0d, and 6.0e, respectively, compared with the CCl_4 group.

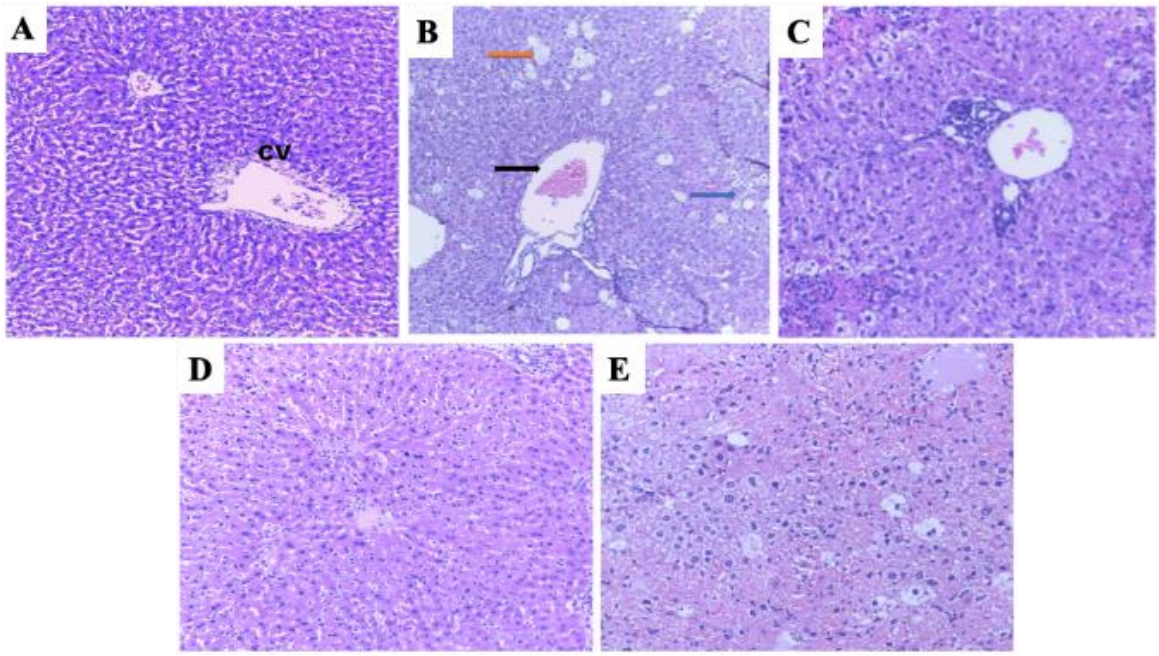

Figure 6. Histological profiles of liver tissues from control and treated groups (H&E stain, magnification ×400). (A) Normal control showing intact hepatic architecture with a clearly defined central vein (CV). (B) CCl_4-treated group displaying steatohepatitis (red arrow), necrosis (blue arrow), and congestion of the central vein (black arrow). (C) Silymarin (100 mg/kg b.wt.) + CCl_4 group indicating improved hepatic structure. (D) *Carica papaya* extract (200 mg/kg b.wt.) + CCl_4 group showing marked lesion recovery. (E) *Carica papaya* extract (400 mg/kg b.wt.) + CCl_4 group showing near-normal hepatic morphology.

3.2 Discussion

Secondary metabolites, often known as phytochemicals, are compounds primarily produced by plants. They have pharmacological effects in treatment and management a variety of illnesses (Mendoza and Escamilla, 2019). Alkaloids, tannins, flavonoids, phenols, saponins, and cardiac glycosides are the most significant phytochemicals with different biological and pharmacological activities. Alkaloid derivatives are used to make anticancer medications such vindesine, vinblastin, vinorelbine, and vincristine (Mondal *et al.,* 2019). For example, tannins exhibit bactericidal activities, flavonoids have robust antineoplastic efficacy against several human malignancies, alkaloids are known to be having anti-inflammatory activities, and saponins have antifungal properties (Jain *et al.,* 2019).

Using LCMS, several metabolites contained in Peak 5 (Table 1 and Figure 1) correspond to Pseudodistamine ((2R)-1-[[(2E,4E)-octa-2,4-dienyl] amino] heptan-2-ol) detected through an ion m/z 240.1990 having a molecular formula ($C_{14}H_{25}NO_2$) is also an alkanolamine.

Peak 13 (Table 1.0 and Figure 1.0) correspond to Jerantine A acetate (methyl (1R,12R,19S)-4-acetyloxy-12-ethyl-5-methoxy-8,16-diazapentacyclo [10.6.1.01,9.02,7.016,19] nonadeca-2,4,6,9,13-pentaene-10-carboxylate) detected through an ion m/z 425.2076 having a molecular formula ($C_{24}H_{28}N_2O_5$).

Fruits and vegetables are rich in some of these bioactive compounds produced such as alkaloids, flavonoids, phenolic compounds, that have antioxidant potentials with pharmacological benefits in minimizing the risk of chronic ailments such as cancer, diabetes, neurodegenerative syndromes, and cardiovascular complications. Aromatic ring to wich one or more -OH substituents are attached. (Hussain *et al.,* 2019). *Carica papaya* leaves is rich in phenolic compounds that provide a variety of pharmacological benefits, including antibacterial, antioxidant, and anticancer effects. In this study, flavonoids, quercetin and cinnamic acid were found in peaks 14 and 12 respectively (Table 1) and this corroborates with earlier studies that reported the presence of P-coumaric acid and quercetin in *Carica papaya* leaves (Hariono *et al.,* 2021).

Since ancient times, flavonoids, a class of polyphenolic chemicals, have been used extensively in the management and treatment of disease due to their proven therapeutic qualities. They have high anti-cancer potentials and robust reducing power properties that stop oxidative cell damage. Additionally, flavonoids inhibit a number of enzymes, including ATPase, phosphodiesterase, lipoxygenase, cyclooxygenase, aldose reductase, and xanthine oxidase (Chitra *et al.,* 2019). Additionally, they modulatory effects on hormones, such as thyroid hormone, androgens, and estrogens, and they have been reported to exhibit pharmacologic effects in both the proliferative and exudative stages of inflammation (Jain *et al.,* 2021).

Also, quercetin derivative 8-C-Methylvelloquercetin 3,5,3'-trimethyl ether was detected. Quercetin has been reported in several studies to offer therapeutic potential on many disorders including diabetes, heart disease, malignancy, and aging. It also up-regulates the body's endogenous antioxidant capacity (Jain *et al.,* 2021). Since phosphorylation at its serine site activates and stabilizes p53, its many phosphorylation sites are crucial for controlling a variety of cellular responses. Methyhexyl Caffeate ([(2S)-heptan-2-yl] (E)-3-(3,4-dihydroxyphenyl) prop-2-enoate) identified is a coumaric acids derivative detected in our extract and this corroborates with earlier report (Sharma *et al.,* 2022). P-coumaric acid, is one of the bioactive substances that reduces oxidative stress-induced liver damage and regulates the immunological response (Zhu *et al.,* 2018; Mehdi *et al.,* 2022).

Furthermore, a Phenoxazine derivative, as actinomycin D contains phenoxazine, which has been shown to have a synergistic anticancer effect on metastatic pancreatic cancer cells (Liu *et al*., 2016).
10-hydroxycamptothecin was identified in this experiment as one of the phytochemicals. Camptothecins have a special anticancer property, it inhibits Topoisomerase I, thereby enhancing cell cycle tumor arrest (Yakkala *et al*., 2023). 10-Hydroxycamptothecin (HCPT), an analog of camptothecin has a wide range of antioxidant and anticancer activity and may possibly inhibits Topoisomerase I activity and this might be ascribed to the antioxidant activity of the extract. Previous study indicates that HCPT reduces cell growth and induces apoptosis in colon cancer cell line, exhibiting dose and time-dependent pattern, (Ma *et al*., 2023).

However, within our literature search, this is first time 10-hydroxycamptothecin (HCPT) was detected in *Carica papaya* leaves. Therefore, this may lend credence to the ethnopharmacological benefits of *Carica papaya* leaves in treatment and management of cancer in folklore medicine. The amino acid L-tryptophan ((2S) -2-amino-3-(1H-Indo-3-yl) propanoic acid) was identified in *Carica papaya* leaves. *Carica papaya* leaves extract contains L-tryptophan, which is its source; however, L-tryptophan is a precursor of two vital metabolic pathways, namely kynurenic acid synthesis and serotonin synthesis.

Also, a tripeptide found in this study which corresponds to IIe-Thr-Trp (L-Isoleucyl-L-threonyl-L-tryptophan) detected by an ion m/z 419.2278 with a molecular formula $C_{21}H_{30}N_4O_5$. Tripeptides comprise of three amino acids linked by a peptide bond were considered to be more diverse than monomers of peptide. The molecular weight of antioxidant peptides ranges from 400-650 which correspond to the molecular weight of compound 11 detected. Such

peptides exhibit robust antioxidant activities against *in vitro* antioxidant models (Ozawa *et al.,* 2022).

Adult albino mice were used to weigh the ameliorative effect of *Carica papaya leaves* extract on CCl_4 provoked hepatic damage. CCl_4 was given via intraperitoneal injection twice weekly for 28 days. This procedure is a model that represents reproducible fibrosis, which can also be reversible after discontinuation of the treatment (Scholten *et al*.,2015). Thus, this archetype is often used in study of fibrosis development and analysis of liver repair mechanisms, emphasizing the systematic importance of this model. In many facets CCl_4-induced hepatic fibrosis and cirrhosis in mouse model echoes the display of toxic damage seen with human disease (Brol *et al.,* 2019).

Aminotransferases, alkaline phosphatase and bilirubin were employed clinically to evaluate the liver tissue damage, while the serum total protein is usually estimated to consider the metabolic and functional ability of the liver. Challenging experimental animal model with CCl_4 significantly derange serum hepatocellular injury biomarkers such as the amino transferases, bilirubin, albumin and total protein from the normal values.

CCl_4-induced hepatotoxicity occurs due to its enzymatic degradation by cytochrome P450 (CYP2E1) in liver cells, producing trichloromethyl radicals ($CCl_3\cdot$) that trigger lipid peroxidation and subsequent membrane disruption.

This study flaunted that CCl_4 toxicity was associated with marked increased in serum level of aminotransferases and bilirubin, also striking reduction in albumin and total protein were noticed. The marked increased in serum enzyme level may be ascribed to disruption of hepatocytes by CCl_4 whereby the enzyme leaked to the plasma. High level of direct bilirubin was recognized as a biochemical indicator cholestasis and disturbances in biliary secretion. Hence, the markedly increased serum bilirubin concentration observed in the CCl_4-exposed group reflects hepatic injury.

The marked reduction in total protein and albumin perceived in response to CCl_4 treatment, may in part attributable to hypomethylation of cellular components. Thus, mRNA could be dislocated from rough endoplasmic reticulum and the result is assumed to be down-regulation of protein synthesis (Schwarz and Blower, 2016). CCl_4 induced hypoproteinemia and hypoalbuminemia in albino rats have been reported by others (Alamri *et al.,* 2022) which corroborates with our findings in albino mice. However, this scenario was reversed by *Carica papaya* leaves extract in a dose dependent manner which mimics the typical drug silymarin.

In this study, the elevated serum bilirubin was observed in response to CCl_4 toxicity and this is an indicator of hepatocytes damage. Thus, the inability of the liver to conjugate bilirubin with glucoronide result to accretion of unconjugated bilirubin in the blood (Usunobun *et al*., 2020). Also, a notable decline in serum total protein and albumin was observed in CCl_4-induced mice.

Interestingly, *Carica papaya* leaves extract counteracted these alterations by restoring serum levels of aminotransferases, bilirubin, as well as albumin and total protein to almost near normal. This ameliorative effect of the plant extract mimics silymarin, an herbal drug with several bioactivities such as antioxidant, anti-inflammatory, immunomodulatory as well as liver regenerating mechanism (Jaffar *et al*., 2024). In this study, 100mg per kg BW of silymarin was utilized as the standard therapeutic agent for comparison. The antioxidant and hepato-protective properties of the extract may in part attributable to the phytochemical components present. In present investigation, several bioactive compounds were detected including alkaloids, amino acids, flavonoids, tripeptides, phenolic acids.

A quercetin derivative, was detected 8-C-Methylvelloquercetin 3,5,3'-trimethyl ether. Several studies have indicated the therapeutic potentials of quercetin to cure several chronic and degenerative conditions encompassing senescence, neoplastic diseases, metabolic dysregulation such as diabetes, and cardiovascular pathologies and also enhances antioxidant capacity of endogenous antioxidant defence system (Jia *et al.,* 2021). Also, a tripeptide, L-isoleucinyl-L-threoninyl-L-tryptophan was detected, the antioxidant effects of the extract may be ascribed to these molecules acting as antioxidant peptides. Several studies showed that antioxidant peptides, in addition to radical scavenging effects (Uno *et al.,* 2020), these molecules also have potentials to resist radiotoxicity and enhance body's resistance to oxidative stress (Yang *et al.,* 2023). Hence, silymarin and *Carica papaya* leaves extract ameliorated the hepatic damages and restores the functional capacity of the hepatocyte to commence protein synthesis

Oxidative stress resulting from free radical generation represents a major factor in liver dysfunction and the fundamental mechanism behind hepatic tissue damage.

(Allameh *et al*., 2023). Aforementioned report showed that augmented oxidative stress triggers lipid peroxidation in cells, mitochondrial calcium burden, inflammatory reaction, anomalous function of organelles, oxidative DNA impairment and even neoplasia (Hajam, 2022).

The pharmacological potential of a substance is essentially linked to its capacity to alleviate hepatotoxin-induced injury or uphold normal hepatic physiological processes distraught thereof.

The present investigation assessed how the plant extract modulates the endogenous antioxidant defense system. The mechanism of antioxidant enzymes such as glutathione transferase, catalase and superoxide dismutase were blatantly conceded by the CCl_4

toxicity. Reduction of the antioxidant system in CCl_4 treated group might be ascribed to CCl_4 effected cellular free radical generation and the succeeding down-regulation of the antioxidant cellular system (El-Boshy *et al*., 2017), whilst *C,papaya* leaves extract usage exotically annulled the situation. Likewise, GST is a key line of defence and hunts free radicals. Furthermore, GSH-dependent enzymes propose a vital route of defence as they detoxify free radical produced toxins (Liedtke *et al*., 2018). In the present investigation the depletion of Glutathine-S transferase activity in the liver in response to CCl_4 toxicity could be ascribed to augmented usage of GSH during peroxide detoxification or NADPH-linked reduction reactions. Previous research indicates that GSH is fundamental in neutralizing the toxic intermediates of CCl_4, and hepatic damage is initiated when intracellular GSH pools are severely depleted (Conde *et al*., 2022). Furthermore, the CCl_4 exposure drastically increased the oxidative stress, thereby increasing the tissue level of malondialdehyde, a by-product of the lipid peroxidation (Ito *et al*., 2019). In the hepatocytes, CCl_4 is metabolized to unstable free radicals (CCl_3• and CCl_3OO•) which are liable for the increase in malondialdehyde level as a result of peroxidation of PUFA of the cell membrane (Mas-Bargues *et al*., 2021). However, *C. papaya* leaves extract treatment significantly reverses the reduction of endogenous antioxidant enzymes and mitigated the generation of the malondialdehyde, and hence inhibited the oxidative stress in disparity to the CCl_4 induced group.

The *in vitro* antioxidant evaluation validated the extract's capacity to scavenge free radicals. According to earlier studies, the presence of antioxidants in plant extracts is typically determined through their ability to convert ferric cyanide complexes to ferrous ions. By accepting electrons from antioxidant molecules, free radicals are converted into stable products, effectively halting the chain reaction of oxidation (Musa *et al*., 2021).

The histological results corroborate the biochemical findings. Histological observations of the control group showed a normal hepatic parenchymal structure. However, liver sections from the CCl_4-exposed group displayed distinct histopathological lesions denoting acute hepatic damage associated with fibrosis. Liver cell parenchyma indicated distraction of liver lobular make-up with connecting fibrosis. The hepatocytes exhibited widespread macrovesicular steatosis accompanied by inflammatory infiltration, apoptotic features, and observable mitotic activity. An increase in oval cells was noted along with bile ductular hyperplasia, Kupffer cell activation, and evident hepatocellular polyploidy, characterized by hepatocytomegaly, karyomegaly, anisokaryosis, and a higher frequency of binucleated hepatocytes. In contrast, liver sections from the silymarin-treated group demonstrated a marked reduction in the histopathological alterations induced by CCl_4 administration. The hepatic lobular architecture remained largely preserved, and macrovesicular steatosis was minimal, with hepatocytes displaying mild mitotic figures and only moderate Kupffer cell activation.

Ovoid cell increase was detected in portal area. Thus, silymarin showed a robust hepato-protective effects beside the CCl_4-induced liver injury because of its cytoprotective, free radical quenching, and anti-inflammatory properties nearly restored normal cytological architecture of the hepatocytes. The Carica papaya leaves extract mimics silymarin in cytoprotective effects however, the level of fibrosis was prominent with additional disorder of hepatic design in the extract treated group. Treating CCl_4-induced toxicity with *C. papaya leaves* extract partially improved the liver tissues. It displayed slight swelling of hepatic cells and minor fatty alterations. This enhancement is attributed to the antioxidant efficacy of some bioactive metabolites detected such as quercetin derivatives, tripeptides, and alkaloids. Likewise, silymarin also ameliorated the oxidative stress within the hepatocyte and almost repaired the hepatic to normal.

3.2.1 Implications

This finding imputes a full representation of the *Carica papaya* leaves extract. Applying modern LC-MS/MS analysis operated in positive ion mode. Sixteen distinct compounds were characterized, among which are alkaloids, amino acid derivatives, fatty acids, flavonoids, terpene glycosides, phenolic acid derivatives, organic acids, flavonoid and peptide derivatives. The *C papaya* leaves extract showed marked hepatoprotective efficacy in CCl_4-induced hepatic injury model. The extract considerably enhanced the biochemical and histological parameters, while enriched the activity of the endogenous antioxidant biomarkers.

3.2.2 Research Contributions

Carica papaya leaf extract was identified in this study, along with a few bioactive substances. Three substances, peaks 2, 5, and 13, were identified as alkaloids in this investigation. The compound 10-hydroxycamptothecin(19-ethyl-7,19-dihydroxy-17-oxa-3,13-diazapentacyclohenicosa-1(21),2(11),3,5,7,9,15(20)-heptaene-14,18-dione), which has the formula $C_{20}H_{16}N_2O_5$, is represented by Peak 2 (Table 1 and Figure 1). The major metabolites of alkaloids, which are heterocyclic nitrogen compounds, (Jain *et al.,* 2021). They are bioactive metabolites that are used as lead compounds in the process of drugs development.

3.2.3 Limitations

The plant metabolites were identified using global natural product molecular networking data base. Although compounds identification was limited to only LC-MS/MS in this research, only compounds

identified with minimal error *m/z* values of ±0.0001 were selected as confirmed metabolites present in *Carica papaya* leaves extract This was to ensure the metabolites identification was valid even though other spectroscopic methods were not exploited which might have been a limitation to this study.

3.2.4 Suggestions

Bioassay guided isolation of the most hepatoprotective metabolites using solvents of different polarities should be carried out. Histopathological studies of the liver tissues should be accompanied by Transmission Electron Microscopy (TEM) or Scanning Electron Microscopy (TEM) to visualise the tissue lesions.

4. CONCLUSION

Furthermore, the extract showed no obvious adverse effect alongside the mouse models. These identified compounds rationalize the ethnopharmacological potentials of *Carica papaya*.

5. ACKNOWLEDGEMENT

The authors sincerely acknowledge the contributions and support of the following persons and organizations towards the realisation and success, for the conduct of the research and its output: Zurina Binti Zainal of Natural Medicines and Products Research Laboratory, Institute of Bioscience Universiti Putra Malaysia for LCMS analysis; AbdulGaniyu AbdulHakeem Raji of Chemical Pathology Laboratory Federal Teaching Hospital Gombe for serum chemistry analysis; Abubakar Ibrahim of the Department of Human Physiology Gombe State University for animal care and treatment.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization, H.H.; Data curation, M.U.A. and M.B.S.,; Formal analysis, M.B.S., M.U.A., N.A., A.I.L, Funding acquisition, H.H.; Investigation, M.B.S. and M.U.A.; Project supervision: H.H and H.A.P.; Validation, H.H.; Visualization, H.H. and H.A.P.; Writing original draft, H.H., M.U.A and H.A.P.; Writing review & editing, M.U.A. H.H. and H.A.P. All authors have reviewed and approved the final version of the manuscript for publication.

AUTHOR INFORMATION

Corresponding Author

Hajjagana Hamza, Gombe State University, Nigeria
ORCiD: https://orcid.org/0000-0001-8804- 2309
Email: hghamzah@gsu.edu.ng

Author

Maryam Usman Abdulkadir, Gombe State University, Nigeria
ORCiD: https://orcid.org/0009-0007-2434-4659
Email: maryamusman600@gsu.edu.ng

Hamza Ahmed Pantami, Gombe State University, Nigeria
ORCiD: https://orcid.org/0000-0001-6067-4531
Email: hamza3983@gsu.edu.ng

Muhammad Bappa Sani, Gombe State University, Nigeria
ORCiD: https://orcid.org/0000-0008-6817-1558
Email: bappahsani@gmail.com

Aliyu Ibrahim Lawan, Gombe State University, Nigeria
ORCiD: https://orcid.org/0000-0002-7245-1703
Email: ailawan1980@yahoo.com

REFFERENCE

Airaodion, A. I., Okoroukwu, V. N., Ogbuagu, E. O., Ekenjoku, J. A., Ogbuagu, U., & Airaodion, E. O. (2019). Antifertility Effect of Ethanolic Leaf Extract of Carica papaya in Male Wistar Rats Antifertility Effect of Ethanolic Leaf Extract of Carica papaya in Male Wistar Rats. Merit Research Journal of Medicine and Medical Sciences. 7(10), 374-381. https://doi.org/10.5281/zenodo.3506541

Alamri, E.S.; El Rabey, H.A.; Alzahrani, O.R.; Almutairi, F.M.; Attia, E.S.; Bayomy, H.M.; Albalwi, R.A.; Rezk, S.M., 2022. Enhancement of the Protective Activity of Vanillic Acid against CCl4 Hepatotoxicity in Male Rats by the Synthesis of Silver Nanoparticles (AgNPs). *Molecules, 27, 8308.* https://doi.org/10.3390/molecules27238308

Allameh, Abdolamir, Reyhaneh Niayesh-Mehr, Azadeh Aliarab, Giada Sebastiani, and Kostas Pantopoulos (2023). Oxidative stress in liver pathophysiology and disease." *Antioxidants* 12(9), 1653. https://doi.org/10.3390/antiox12091653

Al-Seeni MN, El Rabey HA, Zamzami MA, Alnefayee AM. (2016). The hepatoprotective activity of olive oil and Nigella sativa oil against CCl_4 induced hepatotoxicity in male rats. *BMC Complement Altern Med.* 16(1), 438. https://doi.org/10.1186/s12906-016-1422-4

Angeli, L., Morozova, K., & Scampicchio, M. (2023). A kinetic-based stopped-flow DPPH• method. *Scientific Reports, 13*, 7621. https://doi.org/10.1038/s41598-023-34382-7

Benjamaa, R., Khanchouch, A., Bouriche, H., & Kabouche, Z. (2024). Comparative evaluation of antioxidant activity in *[sample type]* using the phosphomolybdenum assay. *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2024.1345340

Bhowmick, A., Sarkhel, S., & Basak, A. An Evaluative Review on Phytochemicals Present in Carica Papaya, Its Extraction Procedures And In Vivo Effects on Wistar Rat Model. International Research Journal of Modernization in Engineering Technology and Science, *32*(212.7), 348-6. researchgate.net

Brol, M. J., Rösch, F., Schierwagen, R., Magdaleno, F., Uschner, F. E., Manekeller, S., ... & Trebicka, J. (2019). Combination of CCl4 with alcoholic and metabolic injuries mimics human liver fibrosis. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 317(2), G182-G194. https://doi.org/10.1152/ajpgi.00361.2018

Conde de la Rosa, L.; Goicoechea, L.; Torres, S.; Garcia-Ruiz, C.; Fernandez-Checa, J.C., (2022). Role of Oxidative Stress in Liver Disorders. *Livers*, *2*, 283–314. https://doi.org/10.3390/livers2040023

Cortés, M. G., & García, A. G. (2022). Management of Pharmacologic Adverse Effects in Advanced Liver Disease. *Clinical Drug Investigation*, *42*(s1), 57–62. https://doi.org/10.1007/s40261-022-01150-w

Delli Bovi, A. P., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., & Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. *Frontiers in medicine*, *8*, 595371. https://doi.org/10.3389/fmed.2021.595371

El-Boshy, M. E., Abdelhamidb, F., Richab, E., Ashshia, A., Gaitha, M., & Qustya, N. (2017). Attenuation of CCl4 induced oxidative stress, immunosuppressive, hepatorenal damage by fucoidan in rats. *J Clin Toxicol*, *7*(3), 1-7. https://doi.org/10.4172/2167-7972.1000348

Gan, C., Yuan, Y., Shen, H., Gao, J., Kong, X., Che, Z., Guo, Y., & Wang, H. (2025). Liver diseases: epidemiology, causes, trends and predictions. *Signal Transduction and Targeted Therapy*, *October 2024*, 1–36. https://doi.org/10.1038/s41392-024-02072-z

Gulcin, I˙.; Alwasel, S.H. (2025) Fe3+ Reducing Power as the Most Common Assay for Understanding the Biological Functions of Antioxidants. *Processes, 13*, 1296. https://doi.org/10.3390/pr13051296

Hajam, Y.A.; Rani, R.; Ganie, S.Y.; Sheikh, T.A.; Javaid, D.; Qadri, S.S.; Pramodh, S.; Alsulimani, A.; Alkhanani, M.F.; Harakeh, S.; *et al*., (2022). Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. *Cells*, 11 (3), 552. https://doi.org/10.3390/cells11030552

Hamza, H. G., Saleh, A.I, Mohammed, Z. K & Ngadda, H. A. (2014). Effect of Aqueous Extract of *Detarium microcarpum* (Guill & Sperr) on Mycotoxin- Induced Tissue Damage in albino rats. *9*(10), *Committee of Publication ethics (COPE) and Journal donation project (JDP).* 629–633. https://doi.org/10.22271/phyto.2020.v9.i4i.112990

Hariono, M., Julianus, J., Djunarko, I., Hidayat, I., Adelya, L., Indayani, F., Auw, Z., Namba, G., & Hariyono, P. (2021). The future of Carica papaya leaf extract as an herbal medicine product. *Molecules*, *26*(22). https://doi.org/10.3390/molecules26226922

Hikmawanti, N. P. E., Fatmawati, S., & Asri, A. W. (2021, April). The effect of ethanol concentrations as the extraction solvent on antioxidant activity of Katuk (Sauropus androgynus (L.) Merr.) leaves extracts. *IOP conference series: Earth and environmental science*, 755(1), 012060. https://doi.org/10.1088/1755-1315/755/1/012060

Hussain, M. B., Hassan, S., Waheed, M., Javed, A., Farooq, M. A., & Tahir, A. (2019). Bioavailability and metabolic pathway of phenolic compounds. In *Plant physiological aspects of phenolic compounds*. IntechOpen. https://doi.org/10.5772/intechopen.84745

Ito F, Sono Y and Ito T., (2019). Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation. *Antioxidants, 8, 72; 1-28.* https://doi.org/10.3390/antiox8030072

Jaffar HM., Al-Asmari F., | Khan FA., Abdul Rahim M., Zongo (2024). Silymarin: Unveiling its pharmacological spectrum and therapeutic potential in liver diseases—A comprehensive narrative review *Food Sci Nutr.* 12:3097–3111. https://doi.org/10.1002/fsn3.4010 3097-3111

Jain, C., Khatana, S., & Vijayvergia, R. (2019). Bioactivity of secondary metabolites of various plants: a review. *Int. J. Pharm. Sci. Res*, *10*(2), 494-504. https://doi.org/10.13040/IJPSR.0975-8232.10(2)

Jain Sb, N,, S. K., Hr, A., Champawat, P. S., Mogra, R., & Maherchandani, J. K. (2021). An overview of papaya: Phytochemical constituents and its therapeutic applications. *The Pharma Innovation Journal*, *10*(9), 45–49. www.thepharmajournal.com

Jia, Hai, Yunchang Zhang, Xuemeng Si, Yuhang Jin, Da Jiang, Zhaolai Dai, and Zhenlong Wu. "Quercetin alleviates oxidative damage by activating nuclear factor erythroid 2-related factor 2 signaling in porcineenterocytes." *Nutrients* 13, no. 2 (2021): 375. https://doi.org/10.3390/nu13020375

Khor, B. K. K., Chear, N. J. Y., Azizi, J., & Khaw, K. Y. (2021). Chemical composition, antioxidant and cytoprotective potentials of Carica papaya leaf extracts: A comparison of supercritical fluid and conventional extraction methods. *Molecules*, *26*(5). https://doi.org/10.3390/molecules26051489

Lawal, B., Shittu, O. K., Oibiokpa, F. I., Berinyuy, E. B., & Mohammed, H. (2017). African natural products with potential antioxidants and hepatoprotectives properties: a review. *Clinical Phytoscience*, *2*(1). https://doi.org/10.1186/s40816-016-0037-0

Liedtke C., Luedde T., Sauerbruch T., Scholten D., Streetz K., Tacke F., Tolba R., Trautwein C., Trebicka J., and Li, Y., Li, Z., Li, C., Ma, X., Chang, Y., Shi, C., He, J., Li, R., Muhammad, I., & Liu, F. (2018). Evaluation of hepatoprotective activity of Syringa oblata leaves ethanol extract with the indicator of glutathione S-transferase A1. *Revista Brasileira de Farmacognosia*, *28*(4), 489–494. https://doi.org/10.1016/j.bjp.2018.05.011

Liu XF, Xiang L, Zhou Q, Carralot J-P., Prunotto M., Niederfellner G and Pastan I., (2016). Actinomycin D enhances killing of cancer cells by immunotoxin RG7787 through activation of the extrinsic pathway of apoptosis. *Proceedings Academy of Sciences,* 113(38)1066-1071. https://doi.org/10.1073/pnas.1611481113

Ma S-C., Zhang J-Q.,Yan T-H Miao M-X., Cao Y-B., Zhang L-C and Li L.Novel. (2023). Strategies to reverse chemoresistance in colorectal cancer. *Cancer Medicine,* 12(10) 11073-11096. https://doi.org/10.1002/cam4.5594

Mas-Bargues C., Escriva C., Dromant M., Borras C., Vina J. (2021). Lipid peroxidation as measured by chromatographic termination of malondialdehyde. Human plasma reference values in health and disease. *Biochem. Biophysics, 709(30).* https://doi.org/10.1016/j.abb.2021.108941

Mehdi, S., Ahmad, F., Lodhi, A. H., Khurshid, U., Khalid, A. A., Sidiq, S. S., Hussain, L., & Baig, M. S. (2022). Protective Effects of p-CA Against Acute Liver Damage Induced by LPS / D-GalN in Wistar Albino Rats. *Molecules*, 3327–3342. https://doi.org/10.2147/DDDT.S380324

Mendoza, N., & Silva, E. M. E. (2018). Introduction to phytochemicals: Secondary metabolites from plants with active principles for pharmacological. *Phytochemicals: Source of antioxidants and role in disease prevention*, 25. https://doi.org/10.5772/intechopen.78226

Mondal, A., Gandhi, A., Fimognari, C., Atanasov, A. G., & Bishayee, A. (2019). Alkaloids for cancer prevention and therapy: Current progress and future perspectives. *European journal of pharmacology*, *858*, 172472. https://doi.org/10.1016/j.ejphar.2019.172472

Musa M. Y., Daja A., Bababe A.B., Modu S., & Hamza HG (2021). Phytochemical analysis and in-vitro antioxidant activity of Xeromphis nilotica stem-bark extract. *BIMA Journal of Science and Technology, 5*(2), 138-147. ajol.info

Nugroho, A., Heryani, H., Choi, J. S., & Park, H. (2017). Asian Paci fi c Journal of Tropical Biomedicine scavenging activity. *Asian Pacific Journal of Tropical Biomedicine*, *7*(3), 208–213. https://doi.org/10.1016/j.apjtb.2016.12.009

Ozawa, H., Miyazawa, T., Burdeos, G. C., & Miyazawa, T. (2022). Biological functions of antioxidant dipeptides. *Journal of Nutritional Science and Vitaminology*, *68*(3), 162-171. https://doi.org/10.3177/jnsv.68.162

Pantami, H. A., Shaari, K., Bustamam, M. S. A., & Ismail, I. S. (2020). Metabolite Profiling of Different Solvent Extracts of the Microalgae Chlorella vulgaris Via 1H NMR-Based Metabolomics. *Current Metabolomics and Systems Biology*, *8*(1), 61–74. https://doi.org/10.2174/2666338408999200819162931

Peñuelas, N., Laguna, A., & Vila, M. (2024). Hematoxylin and Eosin (H&E) Staining Protocol. *protocols.io*. https://doi.org/10.17504/protocols.io.ewov19deklr2/v1

Scholten, D., Trebicka, J., Liedtke, C., & Weiskirchen, R. (2015). The carbon tetrachloride model in mice. *Laboratory animals*, *49*(1_suppl), 4-11. https://doi.org/10.1177/0023677215571192

Schwarz D. S., & Blower, M. D. (2016). The endoplasmic reticulum: structure, function and response to cellular signaling. *Cellular and molecular life sciences*, *73*(1), 79-94. https://doi.org/10.1007/s00018-015-2052-6

Sharma, A., Sharma, R., Sharma, M., Kumar, M., Barbhai, M. D., Lorenzo, J. M., Sharma, S., Samota, M. K., Atanassova, M., Caruso, G., Naushad, M., Radha, Chandran, D., Prakash, P., Hasan, M., Rais, N., Dey, A., Mahato, D. K., Dhumal, S., … Mekhemar, M. (2022). Carica papaya L. Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects. *Oxidative Medicine and Cellular Longevity*. *2022*(1), 2451733. https://doi.org/10.1155/2022/2451733

Singh, P. G., Madhu, S. B., Shailasreesekhar, G. T. S., Basalingappa, K. M., & Sushma, B. V. (2020). In vitro antioxidant, anti-inflammatory and anti-microbial activity of Carica papaya seeds. *Global Journal of Medical Research*, *20*, 19-38. https://doi.org/10.34257/GJMRBVOL20IS2PG19

Sun, W., & Shahrajabian, M. H. (2023). Therapeutic Potential of Phenolic Compounds in Medicinal Plants—Natural Health Products

for Human Health. *Molecules*, *28*(4), 1845. https://doi.org/10.3390/molecules28041845

Tibiri, A., Boria, S., Traoré, T. K., Ouédraogo, N., Nikièma, A., Ganaba, S., ... & Carraz, M. (2020). Countrywide survey of plants used for liver disease management by traditional healers in Burkina Faso. *Frontiers in Pharmacology*, *11*, 563751. https://doi.org/10.3389/fphar.2020.563751

Ugbogu, E. A., Dike, E. D., Ebubechi, M., Etumnu, L. R., Okoro, B. C., Ugbogu, O. C., Adurosakin, O. E., Enyinna, C., Ohaeri, E., & I266weala, E. J. (2023). Pharmacological Research - Modern Chinese Medicine Ethnomedicinal uses, nutritional composition, phytochemistry and potential health benefits of Carica papaya. *Pharmacological Research - Modern Chinese Medicine*, *7*(April), 100266. https://doi.org/10.1016/j.prmcm.2023.100

Uno, S., Kodama, D., Yukawa, H., Shidara, H., & Akamatsu, M. (2020). Quantitative analysis of the relationship between structure and antioxidant activity of tripeptides. *Journal of Peptide Science*, *26*(3), e3238. https://doi.org/10.1002/psc.3238

Usunobun, U., Osaigbovo, J. O., & Okolie, N. P. (2020). Hepatoprotective and antioxidant effect of *Rhaphiostylis beninensis* ethanol root extract on carbon tetrachloride (ccl_4)-induced hepatotoxicity and oxidative stress. *Animal Research International*, *17*(2), 3781 – 3789. https://doi.org/10.5099/aj200300155

Wang, M., Carver, J. J., Phelan, V. V., Sanchez, L. M., Garg, N., Peng, Y., Nguyen, D. D., *et al.* (2016). Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nature Biotechnology, 34*(8), 828. https://doi.org/10.1038/nbt.3597

Xiao, F. (2020). Guidelines for antioxidant assays for food components. *Food Science & Technology*, **10**. https://doi.org/10.1002/fft2.10

Yakkala, P. A., Penumallu, N. R., Shafi, S., & Kamal, A. (2023). Prospects of topoisomerase inhibitors as promising anti-cancer agents. *Pharmaceuticals*, *16*(10), 1456. https://doi.org/10.3390/ph16101456

Yang, Nanxiang, Yongyan Pei, Yan Wang, Limin Zhao, Ping Zhao, and Zhanchao Li. "Identifying the antioxidant activity of tripeptides based on sequence information and machine learning." *Chemometrics and Intelligent Laboratory Systems* 238 (2023): 104845. https://doi.org/10.1016/j.chemolab.2023.104845

Zhu, H., Liang, Q. H., Xiong, X. G., Wang, Y., Zhang, Z. H., Sun, M. J., Lu, X., & Wu, D. (2018). Anti-Inflammatory Effects of p-Coumaric Acid, a Natural Compound of Oldenlandia diffusa, on Arthritis Model Rats. *Evidence-Based Complementary and Alternative Medicine* 2018. 1, 5198594. https://doi.org/10.1155/2018/5198594