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Full Length Research

Ameliorative effects and structure-function relationship of *Chromolaena odorata* (Linnaeus) King and Robinson leaf extract on carbon tetrachloride (CCl_4)-induced hepatotoxicity in wistar rats

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ABSTRACT: *Chromolaena odorata* (*C. odorata*), has been used as a medicinal herb for various conditions, including malaria, inflammations, and more. This study aimed to assess the ameliorative effects and structure-function relationship of *Chromolaena odorata* leaf extract on Carbon Tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats. The leaves of *C. odorata* were collected, washed and air-dried for one week at room temperature before ethanolic crude extraction and fractionation using column chromatography. Some of the fractions underwent GC-MS, FTIR and HPLC analyses. Forty-two male Wistar rats were randomly divided into 7 groups (n=6). The animals were administered the n-hexane fraction of the extract for twenty-one days before being euthanized for further examination. GC-MS analyses were conducted on selected fractions of the extract. Fractions were found to contain bioactive compounds like Nonyl octacosyl, Methyl stearate, etc. The FTIR analyses revealed peaks representing various functional groups. The HPLC findings indicated the presence of important compounds. Histopathological examination showed degrees of impairment in all groups except group 1, displaying a normal liver architecture. Results of the liver marker enzymes showed a significant increase ($p<0.05$) in ALT and AST levels across all test groups, except for group 3, where no significant change was observed compared to the normal control. Total protein exhibited a marked decrease in all test groups relative to the control, while albumin levels remained unchanged in all test groups compared to the control. Globulin levels significantly decreased ($p<0.05$) in groups 2, 4, 5, and 6, whereas groups 3 and 7 displayed no significant alteration compared to the normal control across all test groups. With exception of Catalase, which decreased non-significantly, Superoxide Dismutase and Catalase, decreased significantly in most of the test groups. The study suggests that the n-hexane fraction of ethanolic leaves extract of *C. odorata* may possess ameliorative effects on the liver, as evidenced by the reduction in liver function enzymes of the test groups when compared with the negative control.

Keywords: Antioxidants, Carbon Tetrachloride, *Chromolaena odorata*, liver, medicinal.

INTRODUCTION

Medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. The plant-based, traditional medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.,* 2007). The

medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Okigbo *et al.,* 2008). According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.,* 2000).

The medicinal properties of plants are attributed to their phytochemical constituents, such as alkaloids, tannins, flavonoids, and various phenolic compounds, which exert specific physiological effects on the human body (Phan *et al.,* 2001). Additionally, scientific studies have highlighted that plant leaves serve as significant reservoirs of antioxidants, antimicrobial agents, and phytochemicals with therapeutic benefits (Obadoni *et al*., 2002).

Chromolaena odorata, found in tropical Africa, has gained recognition as a medicinal herb for a range of conditions, including malaria, dysentery, toothaches, and fevers (Taiwo *et al.,* 2000). *C. odorata* is also known by various other names such as Armstrong's weed, baby tea, bitter bush, butterfly weed, Christmas bush, devil weed, eupatorium, French weed, Jack in the bush, king weed, paraffin bush, paraffin weed, Siam weed, turpentine weed, triffid weed, communist weed, hagonoy, etc. (McFadyen, 2004; Vaisakh and Pandey, 2012). In Nigeria, *C. odorata* is commonly known as *Akintola* or *Awolowo* in Yoruba, *Obialofulu* in Igbo, *Obiarakara* in Hausa and *Anagba-agwu* in Idoma. Traditionally, its decoction is consumed to alleviate coughs and colds, or applied in baths to address skin ailments. Its effectiveness in treating diarrhea, malaria fever, toothaches, diabetes, skin conditions, dysentery, and colitis has been extensively documented (Akinmoladun *et al.,* 2007). In folk medicine, the plant's aqueous leaf extract is utilized as an antiseptic for wound care. Additionally, fresh leaf juice is applied to staunch bleeding from cuts and nosebleeds (Phan *et al.,* 2001).

Despite its widespread use across Africa, including Nigeria, its healing properties have been embraced without a comprehensive understanding of its overall impact on health. Therefore, it is imperative to explore the bioactive compounds present in ethanolic leaf extracts of *Chromolaena odorata* and assess their effects on the liver of consumers, employing male albino rats as a model for investigation.

MATERIALS AND METHODS

Plant collection and preparation

Fresh leaves of *C. odorata* were gathered from its native environment on the grounds of the Federal University Wukari, Taraba State, Nigeria. These leaves were dried naturally at 23°C and then pulverized using an electric blender.

Experimental animals

Male Wistar rats, aged 8 weeks, were utilized for the study. A total of 42 rats were housed in cages under standard laboratory conditions (temperature: 25±2°C; light/dark cycle: 12 hours) and acclimatized for two weeks in the laboratory prior to the start of the experiment. Throughout the acclimatization and experimental periods, the rats were provided with daily feedings of grower's mash (Vital Feeds Company, Jos, Nigeria) and access to clean tap water *ad-libitum*.

Equipment/apparatus

Wooden mortar and pestle, Digital analytical weighing balance (Ohaus: PA-1000), Thermostatic water cabinet (Model: HH-W420), UV-Visible light spectrophotometer, Auto-chemistry analyzer (Landwind LW E60B), GCMS (QP201Ultra system), Centrifuge (800D), Refrigerator (HTF-146F), Rotary evaporator (RE400), Automatic haematology analyzer (Sysmex KX-21N Kobe), HPLC analytical 3D (TurboSquid 1657559) and FTIR (530; 7800~350cm^{-1}).

Reagents/chemicals

Reagents used include: Carbon Tetrachlordie (CCl_4), Formalin, Chloroform, Follin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH). All other chemicals used were of analytical standard.

Extraction with ethanol

The crude extraction followed the procedure outlined by Yakubu *et al*. (2014). Exactly 200 g of the powdered sample was immersed in approximately 1000 millilitres of ethanol for 48 hours. The mixture obtained underwent initial filtration using cheesecloth and Whatman filter paper (No 1). The resulting filtrate was concentrated using a rotary evaporator at 68°C to obtain the desired concentrate.

Fractionation of ethanolic extract

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

Packing of column

The column was packed following the method described by Yakubu *et al*. (2014). Glass wool was placed at the bottom of the glass column using a glass rod. A slurry was

prepared by dissolving 235 grams of silica gel (G60-200 mesh size) in 235 millilitres of n-hexane. The chromatographic column (30 mm diameter by 400 mm height) was filled with the silica gel slurry, allowing the solvent to flow freely into a conical flask below. The setup was deemed satisfactory when the solvent drained freely without carrying any silica gel or glass wool into the tap. After the packing process, the tap was closed, and the column was allowed to stabilize for 24 hours. Subsequently, the clear solvent at the top of the silica gel was allowed to drain down the silica gel meniscus.

Elution

The elution process followed the method outlined by Yakubu *et al*. (2014). A total of 2 grams of the extract was dissolved in 15 millilitres of absolute ethanol, and the solution was applied to a chromatographic column (30 mm diameter by 40 mm height). Phytochemicals were eluted from the plant material using solvent combinations of varying polarities, as detailed below: 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 using DPPH

The antioxidant activity of the plant extracts and the resulting fractions from ethanol extract against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was assessed spectrophotometrically at 517 nm, with Trolox used as a standard, following the procedure outlined by Singleton *et al*. (2002). Each fraction was measured for absorbance in triplicate. The total antioxidant activity capacity (TAC) was determined as mg/mL of Trolox equivalent (TE) using the regression equation derived from the calibration curve.

Procedure

A solution of 39.4 mg DPPH in 1 liter of 80% methanol was prepared to achieve a concentration of 0.1 mM. Subsequently, 2 mL of the DPPH solution was pipetted into a cuvette, followed by the addition of 100 µL of the sample. The mixture was thoroughly mixed for 30 seconds, and the absorbance was measured at 517 nm within 30 seconds against the reagent blank.

Determination of Total Phenol Content (TPC)

The total phenol content was determined colorimetri-cally at 765 nm following the method outlined by Lachman *et al*. (2000), using Folin-Ciocalteu reagent and expressed in terms of gallic acid equivalent (GAE). Specifically, 0.2 mL of the sample was added to a test tube containing 1.0 mL of Follin reagent and left to stand for 8 minutes. Subsequently, 0.8 mL of sodium carbonate solution was added, and the mixture was incubated at room temperature for 2 hours. The reactions were carried out in duplicate, and the absorbance of the sample at 765 nm was measured using a spectrophotometer against the reagent blank. The results were reported as GAE per gram of extract.

Procedure

Samples (2 mL, triplicates) were introduced into test tubes; 1.0 mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (Systronics UV-vis spectrophotometer). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

Estimation of Total Flavonoids Content (TFC)

The flavonoid content was assessed using the aluminium chloride colourimetric method developed by Ordonez *et al*. (2006), with quercetin used for generating the calibration curve. Specifically, 0.5 mL of the diluted sample was added to a test tube containing 1.5 mL of methanol. Subsequently, 0.1 mL of 10% aluminium chloride solution and 0.1 mL of potassium acetate were added. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm, and the flavonoid concentration in the sample was determined from the calibration curve. In the blank, an equal amount of distilled water was substituted for the 10% aluminum chloride solution. The total flavonoid content was expressed as mg/mL quercetin equivalent (QE).

Procedure

A solution of aluminum chloride (10%) was prepared by dissolving 10 grams of aluminum chloride in 100 milliliters of distilled water. Similarly, potassium acetate (1 M) was prepared by dissolving 98.15 grams in 1 liter of methanol. To a test tube, 1.5 milliliters of methanol was added, followed by 0.1 milliliters of the 10% aluminum chloride ($AlCl_3$) solution and 0.1 milliliters of 1 M potassium acetate (CH_3COOK). Subsequently, 0.5 milliliters (500 µL) of the diluted sample was added to the test tube, which was then incubated at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. In the blank, an equivalent amount of water replaced

the 10% aluminum chloride solution. The concentration of flavonoids in the sample was determined from the calibration curve.

Gas Chromatography-Mass Spectrometric (GC-MS) analysis

GC-MS analysis of the samples was conducted using a GC Clarus 500 Perkin Elmer system, which includes an AOC-20i autosampler and a gas chromatograph interfaced with a mass spectrometer (GC-MS) instrument. Approximately 2 µL of the ethanolic leaves extract of *C. odorata* fractions was utilized for GC-MS analysis of various compounds under the following conditions: an Elite-1 fused silica capillary column (30×0.25 mm×ID×1 µm of the capillary column, composed of 100% Dimethylpolysiloxane) operated in electron impact mode at 70 eV; helium (99.999%) served as the carrier gas at a constant flow rate of 1 mL/min, with an injection volume of 0.5 µL (split ratio of 10:1); injector temperature set at 250°C; and ion-source temperature at 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10-200°C/min, then 5-280°C/min, ending with a 9 min isothermal period at 280°C. Mass spectra were recorded at 70 eV, with a scan interval of 0.5 sec and fragments ranging from 45-450 Da. The eluted components were detected in the mass detector. The spectrum of the unknown component was compared with the spectra of known components stored in the NIST 23 (2023) mass spectral library to determine the name, molecular weight, fatty acids, volatile compounds, and other useful components analyzed in the GC-MS study (Revathi *et al*., 2014).

Fourier Transform Infrared Spectrophotometric (FTIR) analysis

The dried powder from various solvent extracts of each plant material was employed for FTIR analysis. Each dried extract powder (10 mg) was encapsulated in a 100 mg KBr pellet to create translucent sample discs. The powdered sample of each plant specimen was then analyzed using an FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}.

High Performance Liquid Chromatography (HPLC) analysis

HPLC rapid analysis was conducted using an Agilent1260-Infinity Quaternary LC system with quaternary pump (G131B) tagged autosampler (G1367E), assembled with a thermostat Diode Array Detector (DAD) connected to a high-accuracy computer system (HP Intel i3 processor). The system utilized a noise level of <± 0.6 µAU and a revolutionary 6 cm flow cell, resulting in up to 10 times higher sensitivity compared to other methods. Data processing for quercetin, trolox, testosterone and gallic acid was performed using Agilent Open LAB ChemStation version C.01.05 (Agilent, USA), based on the chromatogram's area and retention time.

Experimental animals and design

Male albino rats, aged 8 weeks, were utilized for the study. A total of 42 rats were housed in cages under standard laboratory conditions (temperature: 25±2°C; light/dark cycle: 12 hours) and acclimatized for two weeks in the laboratory prior to the start of the experiment. Throughout the acclimatization and experimental periods, the rats were provided with daily feedings of grower's mash from Vital Feeds Company Nigeria and ad-libitum access to clean tap water.

Animal groupings and treatments

Seven groups of male rats (n = 6 per group) were utilized as treatment groups. Each treatment group received different doses of the crude leaf extract for three weeks. Group 1 animals served as the Normal Control, receiving unrestricted access to food and water throughout the treatment period. Group 2 served as hepatotoxic controls (CCl_4 group), also provided with *ad libitum* food and water, in addition to carbon tetrachloride (25 mg/kg body weight in a 1:1 mixture with liquid paraffin). Group 3, the positive control, received *ad libitum* food and water, along with carbon tetrachloride (25 mg/kg body weight in a 1:1 mixture with liquid paraffin) and Sylimarin (25 mg/kg body weight of the animal). Groups 4-7 were administered ad libitum food and water, carbon tetrachloride (25 mg/kg body weight in a 1:1 mixture with liquid paraffin), and n-hexane fractions of ethanolic leaf extract of *C. odorata* at doses of 20 mg/kg, 50 mg/kg, 100 mg/kg, and 150 mg/kg body weight of the animals, respectively.

Induction of hepatotoxicity

The protective effect of *C. odorata* leaf extract was assessed in experimental rats subjected to hepatotoxicity induced by CCl_4 (2 mL/kg). CCl_4 was administered intraperitoneally as a single dose, following the method described by Kamisan *et al*. (2014). Over the course of three weeks, each group (2-7) received intraperitoneal injections of CCl_4 once per week, totaling three injections over the span of 21 days. Hepatotoxicity was induced in all rats (except the rats of Group 1).

Blood collection and analysis

Blood was collected from experimental rats at the end of the treatment period. Following an overnight fast, the animals were anesthetized using chloroform vapour. Incisions were then made into their thoracic cavity, and blood samples were obtained via cardiac puncture using a 10 mL syringe. The blood samples were transferred into tubes and allowed to clot for fifteen minutes before being centrifuged at 4000 rpm for 10 minutes. The serum supernatants were collected into sterile plain bottles and were used for biochemical assays while the whole blood samples were collected using EDTA sample bottles for evaluation of haematological indices.

Tissue homogenization

The livers of Wistar albino rats in each group were harvested with utmost care and placed in normal saline (to clean it from any tissue remnants). Following weighing, the liver tissues were homogenized in a phosphate buffer at a pH of 7.4, with a ratio of 1:10 (w/v). Subsequently, the homogenate was centrifuged at 12,000×g for 60 minutes. The resulting supernatant, referred to as the post-mitochondrial fraction, was utilized for the assessment of Thiobarbituric Acid Reactive Substances (TBARS), Superoxide Dismutase (SOD), and Catalase (CAT) activities.

Estimation of Thiobarbituric Acid Reactive Substance (TBARS)

The Thiobarbituric Acid Reactive Substances (TBARS) assay is frequently employed as a general measure of lipid peroxidation in biological fluids. It is commonly regarded as a reliable indicator of oxidative stress levels within a biological sample, given that the sample has been adequately managed and preserved (Jesús and Chad, 2020).

Principle

In the assay, lipid peroxidation products, particularly malondialdehyde (MDA), react with thiobarbituric acid (TBA), resulting in the formation of MDA-TBA2 adducts known as TBARS. This reaction produces a red-pink coloration, which can be quantitatively measured spectrophotometrically at 532 nm (Jesús and Chad, 2020).

Procedure

One milliliter of 14% trichloroacetic acid was dispensed into a test tube, followed by the addition of 1 mL of thiobarbituric acid (0.67%). Subsequently, 50 µL of the tissue homogenate was introduced into the mixture. The resulting solution was then subjected to incubation at 80°C for 30 minutes in a water bath. Afterward, rapid cooling was achieved by placing the mixture in ice for 5 minutes, followed by centrifugation at 3000 x g for 10 minutes. The spectrophotometric measurement of Malondialdehyde (MDA) was conducted at 535 nm, and the level of lipid peroxidation was determined using the molar extinction coefficient of malondialdehyde.

Calculation

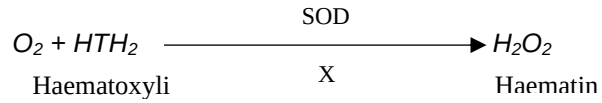
Molar extinction of MDA = $1.56 \times 10^5\ M^{-1}cm^{-1}$
MDA concentration = Absorbance/$1.56 \times 10^5\ M^{-1}cm^{-1}$

Estimation of Superoxide Dismutase (SOD) Activity

Superoxide Dismutase activity was assessed following the method described by McCord and Fridovich (1969).

Principle

The assay method relies on observing the auto-oxidation rate of hematoxylin. In the presence of Superoxide Dismutase (SOD), the auto-oxidation of hematoxylin (measured by absorbance at 560 nm) is slowed down due to SOD activity at pH 7.8. The percentage of inhibition is directly correlated with the amount of SOD present within a defined range.



Procedure

A volume of 920 µL of 0.05 M phosphate buffer at pH 7.8 was dispensed into clean test tubes, followed by the addition of 40 µL of the sample (tissue homogenate), labeled as Test Sample A1 (repeated for Samples A2 to A7). Additionally, a reagent test (blank without sample) was prepared by adding 40 µL of assay buffer (0.05 M phosphate buffer, pH 7.8) into another clean test tube. The mixtures were then shaken and incubated for 2 minutes at room temperature. Subsequently, 40 µL of hematoxylin was swiftly added to both the sample test and reagent test tubes (blank), initiating the auto-oxidation reaction. After the addition of hematoxylin, the absorbance of the sample and reagent test (blank) was measured at 560 nm every 30 seconds for 5 minutes against distilled water using a spectrophotometer. The activity of Superoxide Dismutase (SOD) was determined by assessing the ratios of auto-oxidation rates in the presence and absence of the sample.

The SOD activity in the sample was determined using the following calculation:

$$Absorbance\ _{Reagent\ test}(A_R) = Absorbance\ _{Reagent\ test\ 2} - Absorbance\ _{Reagent\ test\ 1}$$

$$Absorbance\ _{Sample\ test}\ (A_S) = Absorbance\ _{Sample\ test\ 2} - Absorbance\ _{Sample\ test\ 1}$$

$$\%\ SOD\ inhibition = \left(1 - \frac{A_S}{A_R}\right) \times 100$$

$$SOD\ activity\ (U/ml)\ = \left(1 - \frac{A_S}{A_R}\right) \times 100\ \times 1.25$$

Estimation of Catalase (CAT) activity

Catalase activity was assessed following the method outlined by Abei (1974).

Principle

Catalase functions to neutralize hydrogen peroxide (H_2O_2) by converting it into water and molecular oxygen.

$$2H_2O_2 \xrightarrow[\text{Catalase}]{} 2H_2O + O_2$$

(Absorbance at 240 nm) (no absorbance at 240 nm)

The catalase activity in the sample was assessed by monitoring the rate of decrease in absorbance at 240 nm.

Procedure

A volume of 10 µL of serum was added to a test tube containing 2.80 mL of 50 mM phosphate buffer at pH 7.0. The reaction was started by introducing 0.1 mL of fresh 30 mM H_2O_2, and the rate of H_2O_2 decomposition was monitored at 240 nm for 5 minutes using a spectrophotometer. Catalase activity was calculated using a molar extinction coefficient of 0.0411 $mM^{-1}cm^{-1}$.

Catalase activity was evaluated and presented as units per milliliter (U/mL) of the decomposition rate, represented as ΔA240 nm/min, of the sample. ΔA240 nm/min denotes the change in absorbance per minute.

Catalase (U/mL) = (ΔA240 nm/min) / Volume of reaction mixture

Determination of levels of serum biochemical parameters in rats administered n-hexane fraction of ethanolic leaves extract of *C. odorata*

Biochemical assessment

The concentrations of specific biochemical markers, including Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), total protein (TP), albumin (ALB), total cholesterol, triglycerides, creatinine, urea, and electrolytes (Na, K and Cl), were measured utilizing an auto-chemistry analyzer, Landwind LW E60B, manufactured in China.

Determination of levels of hematological indices of rats administered n-hexane fraction of ethanolic leaves extracts *C. odorata*

The determinations of hematological parameters were carried out using automated hematology analyzers (Sysmex KX-21N Kobe Hyogo, Japan).

Preparation of liver homogenate for histological studies

Liver samples were preserved in formalin solution, underwent standard processing, and were embedded in paraffin. Sections measuring 5µm in thickness were then prepared and stained with hematoxylin and eosin (H&E) dye for microscopic analysis. The stained sections were observed and captured under a light microscope, and assessment was conducted by a pathologist based on the degree of hepatic injury severity outlined by El-Beshbishy *et al*. (2010).

Statistical analysis

The data analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 23. A one-way analysis of variance (ANOVA) was employed to analyze the data, and differences between the treated groups and the control groups were assessed using Dunnett's Post Hoc Test, with a significance threshold set at $p<0.05$. The results were presented as mean ± standard deviation and illustrated through tables and charts.

RESULTS

Antioxidant Activity of fractions of ethanolic leaf extract of *Chromolaena odorata*

The findings from the total antioxidant activity assessment indicate that fraction 1b exhibited the highest antioxidant

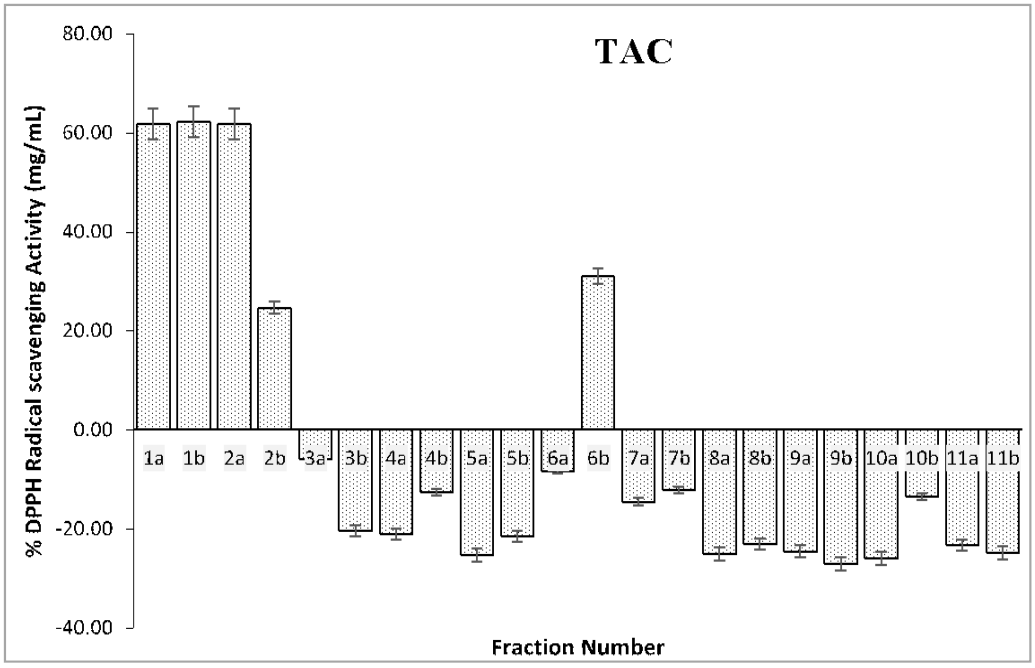


Figure 1. Total antioxidant activity of fractions of ethanolic leaves extract of *Chromolaena odorata.* Fraction: 1 = n-hexane (100:0); fraction 2 = n-hexane:chloroform (50:50); fraction 3 = chloroform (100:0); fraction 4 = chloroform:ethyl acetate (50:50); fraction 5 = ethyl acetate (100:0); fraction 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).

potency at 62.18 mg/mL. Fractions 1a, 2a, 2b, and 6b demonstrated antioxidant activities of 61.66 mg/mL, 61.73 mg/mL, 24.62 mg/mL, and 31.09 mg/mL respectively. Fraction 2b displayed the lowest antioxidant capacity at 31.09 mg/mL, as illustrated in Figure 1.

Total Phenolic **Content (TPC) of fractions of ethanolic leaves extract of *Chromolaena odorata***

The information on overall phenolic content indicates that fraction 2a contained the highest amount at 2166 mg/mL, followed by fraction 6b at 921 mg/mL, and fraction 10b at 915.67 mg/mL. Fraction 1a had the lowest total phenolic content at 23.33 mg/mL, as illustrated in Figure 2.

Total Flavonoid Content (TFC) of fractions of ethanolic leaves extract of *Chromolaena odorata*

The analytical data obtained showed that fraction 2b (25.67 mg/mL) had the highest total flavonoid content. This is followed by fraction 1b (16.97 mg/mL), fraction 3a (14.54 mg/mL) and fraction 1a (13.11 mg/mL). The data showed the lowest flavonoid content in fractions 10a and 11b (both having total flavonoid content of 0.1 mg/mL) as shown in Figure 3.

GC-MS Phytoconstituents of n-hexane fraction of ethanolic leaves extract of *Chromolaena odorata*

Based on the available data regarding total antioxidant activity, fractions 1a (n-hexane) was chosen for GC-MS Profiling. The analysis results unveiled 20 bioactive compounds, with Aspidospermidine-17-ol, 1-acetyl-19, 21-epoxy-15,16-dimethyl-phthalate being the most prominent, constituting 16.08% of the total. Other compounds include Bis(2-ethylhexyl) phthalate, 1-Hexacosanol, 1-Eicosanol, Hexacosyl nonyl ether, 7-Hexadecenal, (Z)-, 2H-Azepin-2-one, hexahydro-1-methyl, Ethanol, 2-(hexadecyloxy)-, Oxalic acid, allyl hexadecyl ester, Oxalic acid, isobutyl tetradecyl ester, 1-Dodecanol, 2-methyl-, (S)-, Oxalic acid, cyclobutyl hexadecyl ester, 1-Decanol, 2-hexyl-, Oxalic acid, allyl octadecyl ester, Heptadecanoic acid, heptadecyl ester, 1-Dodecanol, 2-octyl-, and Octadecanoic acid, ethenyl ester. The twenty different peaks are displayed in Figure 4 with the biological activities of some of the phytochemicals laid out in Table 1.

Fourier Transform Infrared Spectroscopy (FTIR) analysis of fractions of ethanolic leaves extract of *Chromolaena odorata*

This analysis was conducted on fraction 1a (n-hexane)

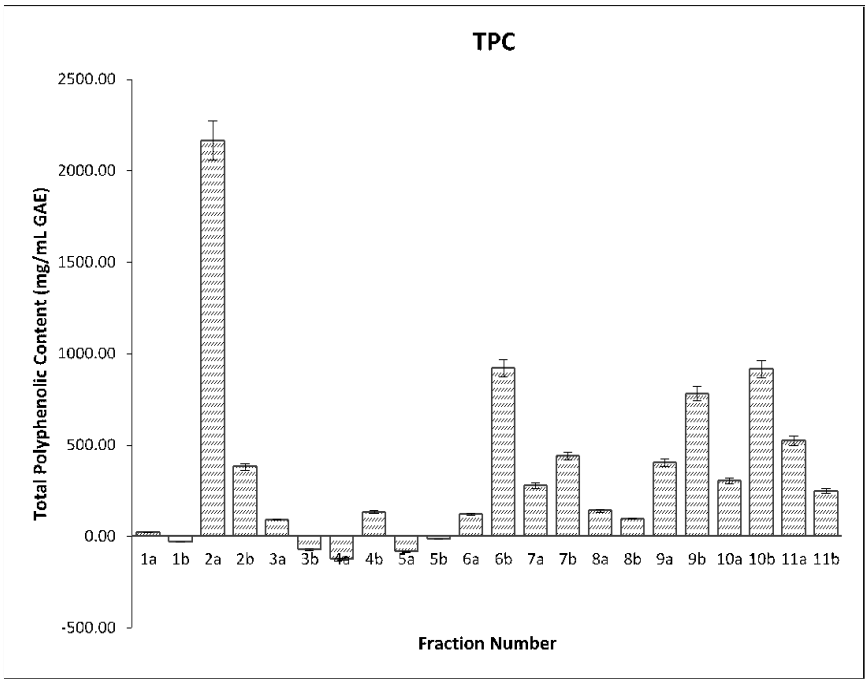


Figure 2. Total polyphenol content of fractions of ethanolic leaves extract of *Chromolaena odorata.* Fraction: 1 = n-hexane (100:0); fraction 2 = n-hexane:chloroform (50:50); fraction 3 = chloroform (100:0); fraction 4 = chloroform:ethyl acetate (50:50); fraction 5 = ethyl acetate (100:0); fraction 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).

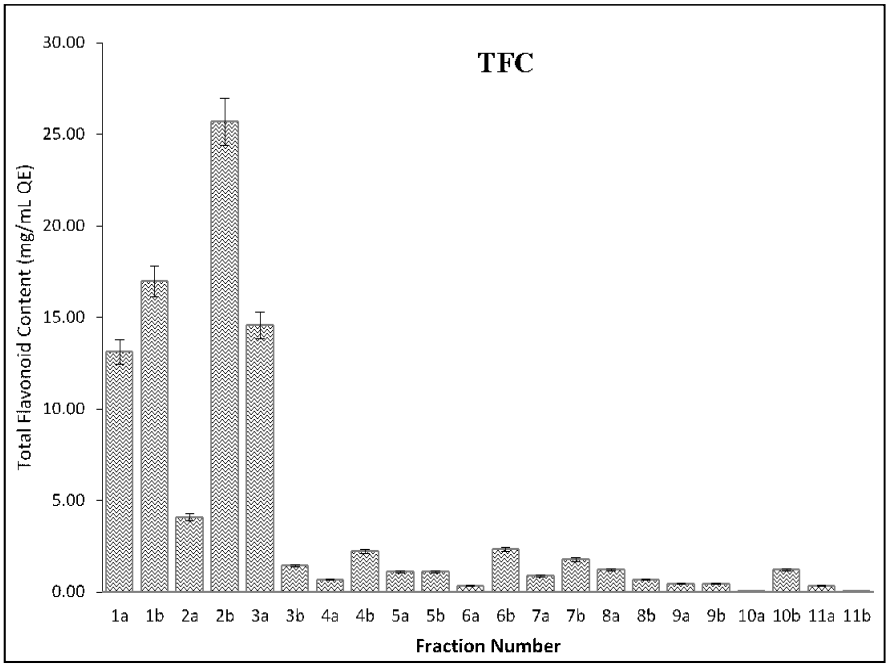


Figure 3. Total flavonoid content of fractions of ethanolic leaves extract of *Chromolaena odorata.* Fraction: 1 = n-hexane (100:0); fraction 2 = n-hexane:chloroform (50:50); fraction 3 = chloroform (100:0); fraction 4 = chloroform:ethyl acetate (50:50); fraction 5 = ethyl acetate (100:0); fraction 6 = 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).

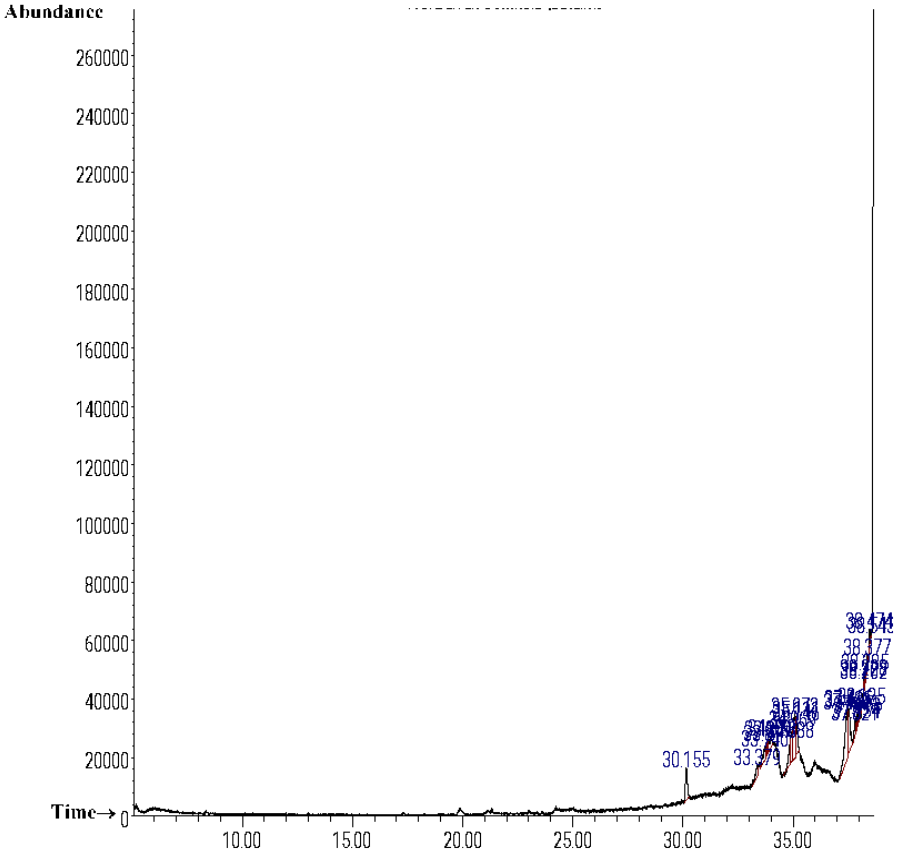


Figure 4. GC-MS peak scan of n-hexane fraction of ethanolic leaves extract of *C. odorata*

due to the data made available from the total antioxidant capacity. FTIR spectroscopy was utilized to observe various functional groups present in the fractions. The FTIR spectrum of the fraction showed different absorbance bands from 3339 (cm^{-1}) to 1606 (cm^{-1}). It showed a strong/narrow band at the wavelength of 3339 cm^{-1} that exhibited O-H stretching vibrational mode. At 2940 cm^{-1}, there was a mild/narrow band that resulted from O-H stretching vibrations. The band at the frequency of 2154 cm^{-1} showed a weak/narrow band which resulted from -N=C=O, -N=C=S, -N=C=N-, N=N=N, or -C=C=O stretching vibrations. At the wavelength of 1703 cm^{-1}; there was a mild/narrow band that exhibited C=O stretching vibrational. Peak at the frequencies of 1651 cm^{-1} and 1606 cm^{-1} showed a weak/narrow band that exhibited C=C stretching vibrations. The results revealed the presence of alcohols, carboxylic acids, isocyanates, isothiocyanates, diimides, azides, ketenes, aldehydes and alkenes shown in Figure 5 and Table 2.

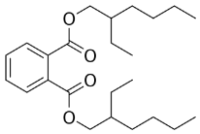
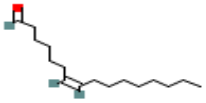
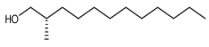
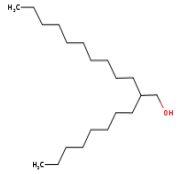
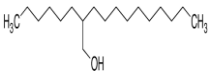
HPLC analysis of fractions from the ethanolic leaves extract of *Chromolaena odorata*

HPLC analysis was carried out on fraction 1a (n-hexane). This selection was based on its total antioxidant activity. The HPLC fingerprinting analysis of the n-hexane fraction extracted from the ethanolic leaves of *C. odorata* displayed ten distinct peaks, each representing a different compound, along with their respective retention time (min) and absorbance. Examination of the chromatogram revealed that Quercetin, present in peak 2 (tR = 1.87, 3.458 ppm), exhibited the highest abundance, followed by Trolox in peak 3 (tR = 2.09, 1.192 ppm), and Testosterone in peak 4 (tR = 2.28, 1.02 ppm), indicating their significance as the most prominent bioactive compounds within the fraction. These findings are illustrated in Figure 6 and Table 3.

Levels of selected liver function parameters in male albino rats induced with CCl_4 and treated with the n-hexane fraction of the ethanolic leaves extract of *Chromolaena odorata*

The outcomes of the selected liver function parameters in Table 4 indicated a significant increase in ALT and AST levels across all test groups, except for group 3, where no significant change was observed compared to the normal control ($p<0.05$). Total protein exhibited a marked

Table 1. GC-MS results of n-hexane fraction of ethanolic leaves extracted from *Chromolaena odorata.*

S/N	Retention Time	Name of Compound	Chemical Structure/ Molecular Formula	Mol. Weight (g/mol)	Peak Area (%)	Biological Function
1	30.15	Bis(2-ethylhexyl) phthalate	 $C_{24}H_{38}O_4$	390.56	7.85	Used as a plasticizer to improve the flexibility and durability of PVC (polyvinyl chloride) products.
2	33.38	1-Hexacosanol	 $C_{26}H_{54}O$	382.71	2.52	Antitumor Effect
3	33.74	1-Eicosanol	 $C_{20}H_{42}O$	298.32	4.48	Used in the food industry as emulsifiers and in Cosmetics
4	33.86	7-Hexadecenal, (Z)-	 $C_{16}H_{30}O$	238.41	1.86	Antifungal, Antibacterial, and Antiviral activity.
5	35.11	1-Dodecanol, 2-methyl-, (S)-	 $C_{13}H_{28}O$	200.36	3.28	Used as surfactants,
5	38.38	1-Dodecanol, 2-octyl-	 $C_{20}H_{42}O$	298.56	2.72	Used as cosmetics such as lipstick, or as an anti-blooming agent in face powder.
6	38.54	1-Dodecanol, 2-hexyl-	 $C_{18}H_{38}O$	270.49	0.19	Used in synthetic detergents, lube additives, pharmaceuticals, cosmetics, rubber, textiles, perfumes, and as a flavouring agent.

decrease in all test groups relative to the control, while albumin levels remained unchanged in all test groups compared to the control. Globulin levels significantly decreased ($p<0.05$) in groups 2, 4, 5, and 6, whereas groups 3 and 7 showed no significant alteration compared to the normal control across all test groups.

Results of MDA, CAT and SOD of CCl_4 -induced rats treated with n-hexane fraction of ethanol extract of *Chromolaena odorata*

The results of liver parameters indices (Table 5) showed that liver CAT decreased non-significantly ($p<0.05$) in all test groups except in group 7 where there was a significant decrease compared to the normal. Liver SOD decreases significantly ($p<0.05$) in group 2, and increases significantly in groups 4, 6 and 7. However, liver SOD decreases non-significantly in groups 3 and 5 compared to normal groups. MDA decreased significantly in test groups 3, 5, 6 and 7 while groups 2 and 4 increased non-significantly compared to the normal.

Histopathological studies

Figure 7 reveals the overall histoarchitecture of the liver of the experimental animals: (**A**) Photomicrograph of liver

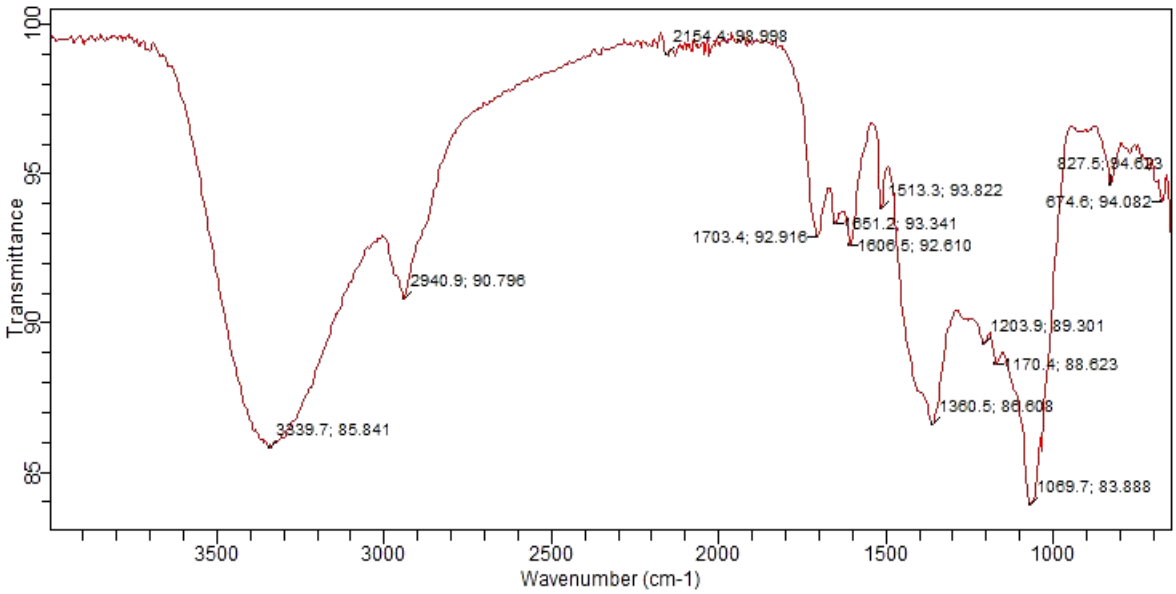


Figure 5. FTIR spectrum of n-hexane fraction of ethanolic leaves extract of *C. odorata.*

Table 2. FTIR result of n-hexane fraction of ethanolic leaves extract of *C. odorata.*

S/N	Wavenumber (cm^{-1})	Functional Group/Mode of Vibration	Inference
1	3339	O-H stretching	Alcohols
2	2940	O-H stretching	Carboxylic acids
3	2154	-N=C=O, -N=C=S, -N=C=N-, N=N=N, -C=C=O stretching	Isocyanates, Isothiocyanates, Diimides, Azides & Ketenes
4	1703	C=O stretching	Conjugated aldehyde
5	1651	C=C stretching	Alkenes
6	1606	C=C stretching	Alkenes

Table 3. Compounds identified by HPLC in different fractions of ethanolic leaf extract of *Chromolaena odorata.*

Fraction	Peak No.	Name of compound	Retention Time (min)	Amount (ppm)	Biological function
n-hexane	2	Quercetin	1.87	3.46	Possesses: anticancer, Anti-oxidant, Antiallergic, Anti-diabetic, Anti-obesity, anti-hyperuricemia and gouty arthritis activities.
	3	Trolox	2.09	1.91	Antioxidant and antitumor.
	4	Testosterone	2.28	1.02	Responsible for the development of primary sexual features, which includes testicular descent, spermatogenesis, enlargement of the penis and testes, and increasing libido.

tissue of normal control rats showing a normal liver consisting of the Portal Vein (PV) with distinct Sinusoids (SN) and Hepatocytes Sheets (HS) radiating from the Central Portal Vein (CPV). (**B**) Photomicrograph of liver tissue of rats induced with 2 mg/kg CCl_4 revealing the CPV with Portal Tract Connective Tissue (PTCT), the normal cyto-architectural direction of sinusoid and hepatocytes sheets towards the portal vein with light congestion of sinusoid and hepatocytes. **(C)** Photomicrograph of liver tissue of rats induced with 2 mg/kg CCl_4 and treated with 25 mL/kg of Silymarin showing dilation of hepatocytes sheets and sinusoid towards the portal vein.

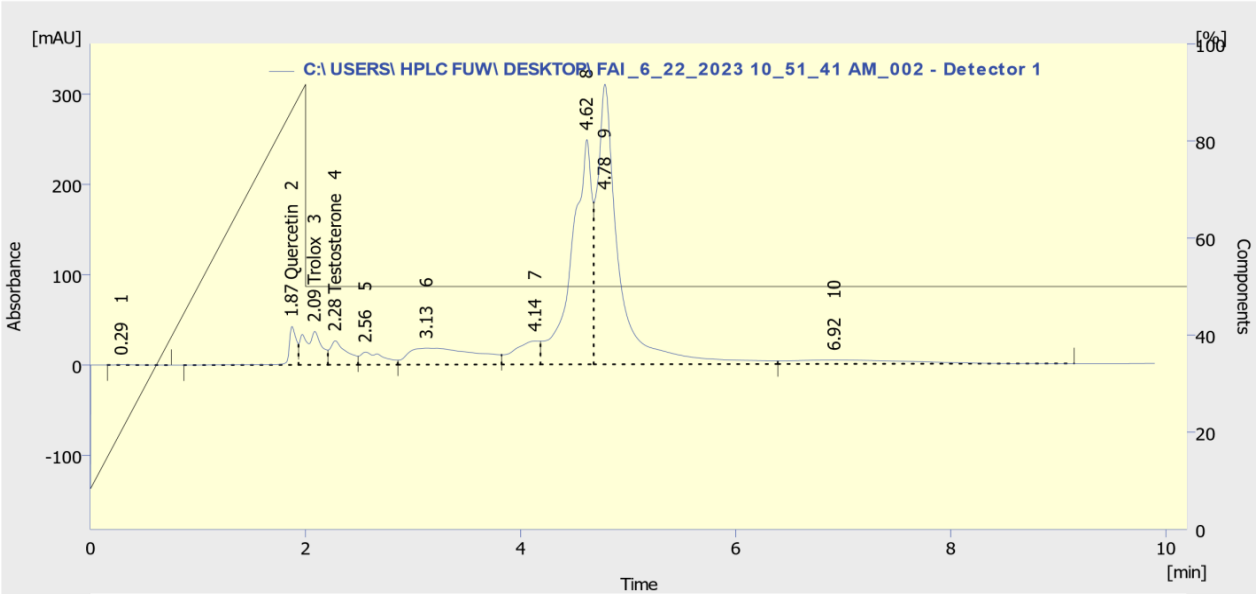


Figure 6. HPLC chromatogram of n-hexane fraction of ethanolic leaves extract of *C. odorata*.

Table 4. Levels of some liver function parameters of CCl_4-induced liver damaged rats treated with n-hexane fraction of ethanolic leaves extracted from *Chromolaena.*

Groups	Treatment	ALT (U/L)	AST (U/L)	TP (gm/dL)	ALB (gm/dL)	GLB (gm/dL)
1	Normal control	48.57 ± 5.61^{a}	57.38 ± 11.65^{a}	8.03 ± 1.25^{c}	3.38 ± 0.24^{a}	4.81 ± 2.47^{b}
2	CCl_4 only	154.26 ± 17.62^{c}	199.22 ± 57.97^{c}	7.37 ± 0.82^{bc}	3.23 ± 1.36^{a}	3.99 ± 0.72^{ab}
3	CCl_4+Silymarin (25 mg/kg)	60.60 ± 21.02^{a}	40.42 ± 7.40^{a}	6.57 ± 0.35^{ab}	3.26 ± 0.23^{a}	3.31 ± 0.22^{a}
4	CCl_4+ 20 mg extract	123.40 ± 26.90^{b}	165.92 ± 17.73^{b}	6.75 ± 0.39^{b}	3.21 ± 0.07^{a}	3.54 ± 0.41^{ab}
5	CCl_4+ 50 mg extract	119.15 ± 16.05^{b}	145.83 ± 9.72^{b}	6.58 ± 0.17^{ab}	3.14 ± 0.50^{a}	3.44 ± 0.51^{ab}
6	CCl_4 + 100 mg extract	116.88 ± 25.38^{b}	130.24 ± 29.30^{b}	6.68 ± 0.41^{b}	3.18 ± 0.13^{a}	3.50 ± 0.39^{ab}
7	CCl_4 + 150 mg extract	114.37 ± 13.7^{b}	147.73 ± 22.94^{b}	5.78 ± 0.36^{a}	3.07 ± 0.09^{a}	3.61 ± 0.41^{a}

Results are expressed in Mean ± Standard deviation of 5 animals in a group corrected to two decimal places. Values with same alphabet superscripts are not significantly different from each other while values with different alphabet superscripts are significantly different from each other at p<0.05. **Key: ALT:** Alanine Transferase, **AST:** Aspartate Transferase, **TP:** Total Protein, **ALB:** Albumin, **GLB:** Globulin.

Table 5. Concentrations of MDA, CAT and SOD of CCl_4 -induced rats treated with n-hexane fraction of ethanolic leaves extract of *Chromolaena odorata.*

Groups	Treatment	Liver CAT (U/mL)	Liver SOD (U/mL)	Liver MDA (nmol/mg protein)
1	Normal control	8.05±1.55^{a}	168.33±1.23^{ab}	5.20±1.74^{c}
2	CCl_4 only	3.56±3.45^{a}	157.33±0.81^{a}	6.17±1.97^{c}
3	CCl_4 + Silymarin (25 mg/kg)	4.77±3.11^{a}	164.00±1.22^{ab}	3.98±1.73^{b}
4	CCl_4 + 20 mg extract	6.49±0.91^{a}	171.00±1.29^{b}	5.55±1.39^{c}
5	CCl_4 + 50 mg extract	6.58±1.86^{a}	163.00±1.00^{ab}	3.08±0.08^{a}
6	CCl_4 + 100 mg extract	6.03±1.34^{a}	170.67±1.04^{b}	3.47±0.83^{b}
7	CCl_4 + 150 mg extract	12.24±2.95^{b}	175.67±1.03^{n}	3.52±1.20^{b}

Results are expressed in Mean ± Standard deviation of 5 animals in a group corrected to two decimal places. Values with same alphabet superscripts are not significantly different from each other while values with different alphabet superscripts are significantly different from each other at P<0.05. **Key: CAT:** Catalase, **MDA:** Malondialdehyde, **SOD:** Superoxide Dismutase.

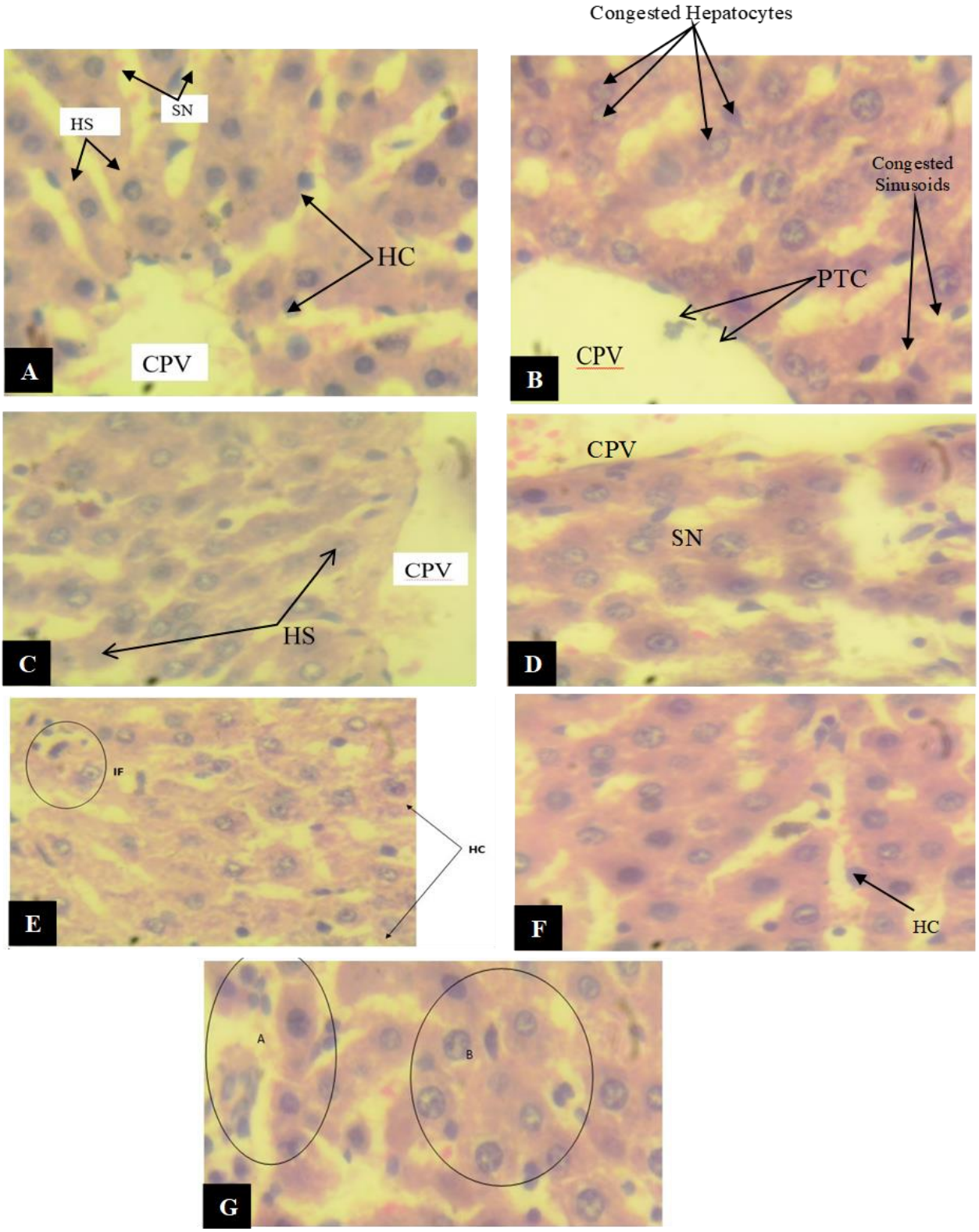


Figure 7. Photomicrograph of liver tissues of rats of Group 1**–A** (Normal), Group 2 **– B** (CCl_4-induced), Group 3 **– C** (CCl_4-induced and treated with Silymarin) , Group 4 - **D** (CCl_4-induced and treated with 20 mg/kg), Group 5-**E** (CCl_4-induced and treated with 50 mg/kg) – Group 6-**F** (CCl_4-induced and treated with 100 mg/kg) and Group 7-**G** (CCl_4-induced and treated with 150 mg/kg) leaves extract of *Chromolaena odorata*).

(D) Photomicrograph of liver tissue of rats induced with 2 mg/kg CCl_4 and treated with 20 mg/kg n-hexane fraction of *C. odorata* leaves showing moderate degree of inflammation (lymphocytic) infiltration around the portal zone. Also observed is cell degeneration with collapse of hepatocytes and sinusoids. **(E)** Photomicrograph of liver tissue of rats induced with 2 mg/kg CCl_4 and treated with 50 mg/kg n-hexane fraction of *C. odorata* leaves showing cyto-degeneration, hepatocytes dilation and inflammation as circled. **(F)** Photomicrograph of liver tissue of rats administered 100 mg/kg n-hexane fraction of *C. odorata* leaves for toxicity studies showing the cyto-architecture of the liver, remaining averagely intact with little degeneration. **(G)** Photomicrograph of liver tissue of rats administered 150 mg/kg n-hexane fraction of *C. odorata* leaves for toxicity studies showing inflammation in Circle A, congested Hepatocytes Sheets and Sinusoids in Circle B, also with congested Central Portal Vein.

DISCUSSION

In this study, the investigation of antioxidant properties of *Chromolaena odorata* leaves vividly revealed that within the substances investigated, there was the presence of phenols and flavonoids. The total antioxidant capacity of the ethanolic extract of *Chromolaena odorata* leaves in this current research was determined and revealed that the plant has a considerable quantity of bioactive compounds. From the results, the total antioxidant capacity of fraction 1b was the highest in value showing that n-hexane (100:0) was the most effective solvent for the determination of the antioxidant activity of the ethanolic extract of *Chromolaena odorata* leaves. The antioxidant capacity of *C. odorata* may be due to the presence of phenolics and flavonoid compounds that have the potential ability to neutralize free radicals thus, preventing free-radical related disorders (Yakubu *et al.,* 2022).

The data for the total phenolic content of the fractions of *Chromolaena odorata* leaves extracted in this present work showed that fraction 2a possessed the highest content of phenol and fraction 11b had the best phenolic content. This result reveals that the solvent combination of n-hexane:chloroform (50:50) was the best solvent for the extraction of phenols. The presence of phytochemicals such as phenolics is indicative of its potential biological activity against various diseases (Yakubu *et al.,* 2022; Umaru *et al*., 2018).

The result for the total flavonoid content of the fractions of *Chromolaena odorata* ethanolic leaves extract in this research showed that fraction 2b had the highest flavonoid content followed by fraction 1b. This result implies that the solvent combination, n-hexane:chloroform (50:50) was the best solvent for the extraction of flavonoids from these plants. Flavonoids have been shown to exhibit antioxidant activity by scavenging harmful free radicals and reactive oxygen species (Urquiaga and Leighton, 2000). *C. odorata* antimicrobial mode of action might be due to the presence of flavonoids and tannins that bind to bacterial cell walls and inhibit its biosynthesis (Anyasor *et al.,* 2011).

One key way they contribute to their antioxidant effects is by stabilizing membranes, which involves reducing membrane fluidity and incorporating flavonoids into the membrane's hydrophobic core. Numerous studies have highlighted the importance of antioxidants in preventing and treating disorders related to free radicals (Urquiaga and Leighton, 2000; Harborne and Williams, 2000).

The Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the n-hexane fraction from *Chromolaena odorata* leaves in this study detected twenty (20) phytochemicals. Aspidospermidin-17-ol, 1-acetyl-19, 21-epoxy-15,16-dimethoxy-phthalate exhibited the highest relative abundance at 16.08%, followed by 1-Decanol, 2-hexyl-, and Oxalic acid, isobutyl tetradecyl ester at 11.50% and 10.42% respectively. Other compounds identified include Bis(2-ethylhexyl) phthalate, 1-Hexacosanol, 1-Eicosanol, Hexacosyl nonyl ether, 7-Hexadecenal, (Z)-, 2H-Azepin-2-one, hexahydro-1-methyl, Ethanol, 2-(hexadecyloxy)-, Oxalic acid, allyl hexadecyl ester, 1-Dodecanol, 2-methyl-, (S)-, Oxalic acid, cyclobutyl hexadecyl ester, Oxalic acid, allyl octadecyl ester, Heptadecanoic acid, heptadecyl ester, 1-Dodecanol, 2-octyl-, and Octadecanoic acid, ethenyl ester. These compounds have been reported by various researchers to possess diverse biological functions. For example, 7-Hexadecenal, (Z)- has demonstrated antifungal, antibacterial, and antiviral properties (Devakumar *et al*., 2017). Additionally, (2-ethylhexyl) phthalate serves as a plasticizer to enhance the flexibility and durability of PVCs (Rowdhwal and Chen, 2018). Furthermore, 1-hexacosanol has been noted for its potential as an antitumor agent (El-Mahmood, 2009).

The Fourier Transform Infrared Spectroscopy (FTIR) analysis of the n-hexane fraction of ethanolic leaves extracted from *C. odorata* in this study identified various functional groups of compounds. The presence of alcohol was confirmed by a peak at 3339 (cm-1), while carboxylic acids were indicated by a peak at 2940 (cm-1). Additionally, isocyanates, isothiocyanates, diimides, azides, and ketenes were detected at 2154 (cm-1). Aldehyde compounds were represented by a peak at 1703 (cm^{-1}), while alkene compounds were indicated by peaks at 1651 (cm-1) and 1606 (cm-1). El-Mahmood (2009) reported that 1-Hexacosanol (alcohol) possesses antitumor effects, while Devakumar *et al*. (2017) suggested medicinal uses for 7-Hexadecenal, (Z)-(aldehyde) due to its antibacterial, antifungal, and antiviral properties.

The HPLC analysis of the n-hexane fraction of ethanolic leaf extract of *Chromolaena odorata* leaves in this current study revealed diverse chromatograms displaying retention times and absorbances representing various

biologically active compounds. The n-hexane fraction exhibited ten chromatograms, indicating the presence of ten different compounds, with quercetin being the most abundant, followed by trolox (peak 3). Testosterone was identified as the least abundant compound. Quercetin, a dietary flavonol known as 2,3',4',5,7-pentahydroxyflavone, possesses a range of pharmacological properties such as antioxidant, anti-inflammatory, neuroprotective, antibacterial, antitumor, and anticancer effects (Rauf *et al*., 2018). Nutmakul *et al*. (2022) also reported its effectiveness against hyperuricemia and gouty arthritis. The diverse pharmacological benefits of quercetin justify its potential for future clinical applications (Ozgen *et al*., 2016). Trolox has been documented as an antioxidant and antitumor agent in a study by Zakharova *et al*. (2016).

After induction with CCl_4, the results showed a significant elevation ($p<0.05$) in liver enzymes ALT and AST in group 2 (negative control) compared to the Normal control, indicating hepatic toxicity caused by CCl_4 administration. However, administration of the standard drug Silymarin in group 3 (positive control) led to a significant reduction in ALT and AST levels. The liver, being involved in numerous metabolic functions, is susceptible to xenobiotic injury due to its central role in xenobiotic metabolism. Substances like CCl_4 can cause liver damage. Increased serum enzyme activities suggest cellular leakage and a breakdown of the functional integrity of the liver cell membrane, as reported by El-Demerdash *et al*. (2004) and Imo *et al*. (2015).

Administering various concentrations of the n-hexane fraction of ethanolic leaves extracted from Chromolaena significantly decreased the elevated serum liver enzymes compared to the negative control, thus preventing cellular leakage. This observation suggests a potential protective effect of Chromolaena leaves against liver damage caused by CCl_4. Chromolaena is known for its hypoglycemic, hypotensive, hepatoprotective, and hypolipidemic activities (Annisa *et al*., 2021). Administration of the n-hexane fraction of ethanol leaves extracted from Chromolaena, as observed in groups 4, 5, 6, and 7, resulted in reduced levels of liver enzymes (ALT and AST). This reduction in serum liver enzymes compared to the negative control indicates the effectiveness of the leaf extract in maintaining normal liver function. The results from groups 4, 5, 6, and 7 (Table 4) suggest that increasing the concentration of the leaf extract enhances its protective effect on the liver. The alkaloids, phenolic compounds, and sterols found in *Chromolaena odorata* leaves may contribute to this protective effect. These findings align with the report of Ugwu *et al*. (2023), and Bridget and Usunomena (2022) also documented a significant reduction in liver function enzyme levels with similar plants.

Most of the proteins found in the plasma are synthesized by the hepatocytes and released into circulation. The level of serum proteins serves as a marker of the synthetic function of the liver and is a useful indicator for assessing the severity of liver damage (Giannini *et al*., 2005). In this study, total protein and globulin concentrations decreased significantly after the administration of CCl_4. However, there was no significant change in the level of albumin compared to both the normal group, negative control, and other test groups. This suggests that the leaf extract of Chromolaena contains certain phytochemical compounds that may have a negative impact on liver protein synthesis. There was no significant change in the level of total bilirubin after the administration of CCl_4 compared to the normal control group. However, total bilirubin decreased significantly in groups 3, 4, 5, 6, and 7. There was no significant alteration in the levels of direct and indirect bilirubin except in group 3, where there was a significant increase in indirect bilirubin. Elevated levels of indirect bilirubin in group two may be linked to reduced hepatocyte uptake, impaired conjugation, and reduced hepatocyte secretion of bilirubin. The significant elevation of indirect bilirubin levels in the serum of the CCl_4+Silymarin (25 mg/kg) group (group 2) could be attributed to bile flow obstruction from the bile duct due to severe liver damage caused by CCl_4. Upon administration of Chromolaena leaf extracts, there was a significant decrease and non-significant alteration in bilirubin levels. This finding is consistent with the results reported by Ugwu *et al*. (2023). The observed protective effect in this study may be attributed to the prevention of intracellular enzyme leakage, possibly due to the presence of polyphenols and flavonoids in the leaf extract, as well as their membrane-stabilizing activity, which could be linked to their ability to scavenge free radicals attacking cell membranes (Krier and Ahmed, 2009).

The liver parameter indices revealed that liver CAT decreased non-significantly ($p<0.05$) in all test groups except for group 7, where a significant decrease was observed compared to the normal group. Liver SOD significantly decreased ($p<0.05$) in group 2 but increased significantly in groups 4, 6, and 7. However, liver SOD decreased non-significantly in groups 3 and 5 compared to the normal group. MDA significantly decreased in test groups 3, 5, 6, and 7, while groups 2 and 4 showed non-significant increases compared to the normal group. These results suggest a partial compromise in the defense mechanism against oxidative stress caused by CCl_4. However, the significant decrease in MDA levels indicates effective defense against free radicals in the system, possibly due to noticeable scavenging activity by the combined action of CAT and SOD.

In this study, the histology of the liver was examined. Control rats exhibited a normal liver structure with hexagonally shaped lobules, each containing numerous hepatocytes arranged in radiating rows. Rats induced with CCl_4 displayed mild congestion of sinusoids and hepatocytes, indicating the presence of cirrhosis. Rats induced with CCl_4 but treated with silymarin exhibited

noticeable dilation of hepatocyte sheets and sinusoids toward the portal vein, suggesting the threat of hepatitis and cirrhosis. In rats induced with CCl_4 but treated with 20 mg/kg of the extract, moderate inflammation (lymphocytic) infiltration around the portal zone, cell degeneration, and collapse of hepatocytes and sinusoids were observed, indicating necrosis. The group induced with CCl_4 and treated with 50 mg/kg of the extract showed cyto-degeneration, hepatocyte dilation, and inflammation, suggesting cirrhosis. In the groups designated for toxicity study (groups 6 and 7), rats were given doses of 100 mg/kg and 150 mg/kg, respectively. The liver of rats in group 6 exhibited relatively intact liver architecture with minor degeneration, indicating hepatitis, while the liver of rats in group 7 showed noticeable impairment, inflammation, congested hepatocyte sheets, and sinusoids.

Conclusion

The current study has identified significant phytochemicals within *Chromolaena odorata* leaves, demonstrating their potential applications in medicine and various industries. The diverse phytochemicals present in *C. odorata* extracts endorse its utilization in cosmetics, medicine, pharmacology, and beyond. The administration of these leaf extracts induced changes in serum AST and ALT levels without notable alterations in serum proteins. The liver histoarchitecture was, however, not intact (having some degrees of impairments from test groups 2-7). Based on this, individuals consuming/using *C. odorata* should adhere to proper dosages as recommended by medical professionals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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