***Original Research Article***

***Carpobrotus edulis biosafety and potency against common pancreatic insufficiency biomarkers***

**Abstract**

*Carpobrotus edulis*, a succulent plant indigenous to Southern African climes, has been utilized in traditional ethnomedicine for a wide array of ailments, including bacterial infections, digestive disorders, and inflammatory disorders. Pancreatic insufficiency, characterized by impaired enzyme production and subsequent malabsorption, may benefit from therapies that address inflammation, oxidative stress, and infection. This study explores the pharmacologically active secondary metabolites, the pharmacological activities as well as the safety of hydro-ethanolic extracts from *C. edulis* plant parts to validate the traditional uses. Phytochemical screening was done using various classical techniques, Total phenolic content was evaluated using the Folin-Ciocalteu method. Acute and sub-acute toxicity profiles were done using OECD guidelines. Anti-inflammatory activity was evaluated using the egg albumin test with diclofenac as a standard and the antibacterial tests were evaluated using the agar well diffusion method. The metabolomics studies revealed the presence of numerous pharmacologically active metabolites The total phenolic content was high, at 279.7 mg GAE/g. Antibacterial analysis demonstrated a mean zone of inhibition of 18.7±1.5 mm at 100mg/ml, and estimated MIC values lie between 12.5 mg/ml and 25 mg/ml. The anti-inflammatory activity of the lyophilized leaf extract of *C. edulis* showed significant inhibition and was comparable to the standard. In acute toxicity assessments, no mortality or adverse physiological conditions were observed at doses up to 5000 mg/kg body weight. Sub-acute toxicity investigations showed no significant differences in weight gain, food and water consumption, and no histopathological changes were observed in major organs. However, hematology and biochemistry revealed statistically significant alterations, warranting further investigation. Based on the foregoing it is concluded that lyophilized *Carpobrotus edulis* at controlled doses presents a safe, efficacious alternative for the management of pancreatic inefficiency end points

**Key words**: *Carpobrotus edulis*, pancreatic insufficiency, Secondary metabolites, antibacterial, anti-inflammation, anti-oxidant and toxicity

# Introduction

## *Carpobrotus edulis*

*Carpobrotus edulis*, commonly known as sea fig or Hottentot fig, is a perennial ground-creeping plant native to South Africa that has naturalized in various regions worldwide[1,2](#5f4d4ddb046c2541465cc79e558e78d0). Its adaptability to coastal environments, characterized by sandy soils and harsh conditions, has allowed it to naturalise across all continents[2,3](#53fca0c717deb18ac139628c1fcdd05a). The plant's morphology is distinguished by succulent leaves, vibrant flowers ranging from yellow to pink, and edible fruits, contributing to its appeal as an ornamental species and a source of nutrition (Figure 1) [2](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Traditionally, *C. edulis* has been utilized in Zimbabwean ethnomedicine for a wide array of ailments[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Its leaves and flowers are used to make decoctions for bacterial and fungal infections, while the acerbic juice from the leaves serves as a remedy for throat and mouth infections[4](#38a24be4dca31cd3230279d3cfe819f5). Furthermore, traditional uses extend to treating dysentery, stomach cramps, burns, and skin ailments[3](#53fca0c717deb18ac139628c1fcdd05a). Some research suggests its potential in managing diabetes and hypertension, further highlighting its therapeutic versatility[4](#38a24be4dca31cd3230279d3cfe819f5). The traditional use of *C. edulis* to treat digestive disorders, such as dysentery and stomach cramps, provides a rationale for investigating its potential role in pancreatic insufficiency. Pancreatic insufficiency often leads to malabsorption and digestive discomfort, mirroring some of the symptoms traditionally addressed by *C. edulis*[3](#53fca0c717deb18ac139628c1fcdd05a). Phytochemical investigations have revealed a rich composition of bioactive compounds, including flavonoids, phenols, anthraquinones, saponins, cardiac glycosides, alkaloids, and tannins[4](#38a24be4dca31cd3230279d3cfe819f5). These compounds are believed to be responsible for the plant's diverse pharmacological activities, including antibacterial, antioxidant, anti-inflammatory, and antidiabetic effects[5](#a31d280e1cc73610cd1bfa01f1a896cf). Sabiu et al.[3](#53fca0c717deb18ac139628c1fcdd05a) identified a number of phenolics. Studies have demonstrated the ability of *C. edulis* extracts to inhibit α-glucosidase, an enzyme involved in carbohydrate metabolism, suggesting a potential mechanism for its antidiabetic properties[3](#53fca0c717deb18ac139628c1fcdd05a). Despite the traditional uses and promising pharmacological activities of *C. edulis*, there remains a need for extensive research to fully elucidate its therapeutic potential and ensure its safe application[6](#d73ec6c53d6bcec7882fec6ac0484899).

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Figure 1 Carpobrotus Edulis Plant

## Pancreatic insufficiency

Pancreatic insufficiency is a debilitating condition characterized by the impaired ability of the pancreas to produce and secrete adequate digestive enzymes. This leads to maldigestion, malabsorption, and a cascade of adverse nutritional consequences. Exocrine pancreatic insufficiency affects an estimated 5-10% of the global population, with higher prevalence in regions with increased incidence of chronic pancreatitis, such as Southern Africa, where the condition affects up to 15% of the population[7](#643e5cf70494850d7232a4de9c6d0cfe). This disorder arises from reduced pancreatic juice secretion, impacting digestive enzyme activity, notably pancreatic lipase[8](#8b9e10a6f5021e844c93138e75f12926). This deficiency hinders the breakdown of fats, proteins, and carbohydrates, resulting in nutrient loss, weight loss, steatorrhea, and gastrointestinal distress[9](#58e8142166c565a51818244318830753). The etiologies of pancreatic insufficiency are diverse, encompassing chronic pancreatitis, cystic fibrosis, pancreatic cancer, and surgical resections of the pancreas[7](#643e5cf70494850d7232a4de9c6d0cfe). Accurate identification of the underlying cause is crucial for effective diagnosis and treatment strategies to mitigate the effects of pancreatic insufficiency, which can lead to serious complications such as malnutrition, osteoporosis, and vitamin deficiencies[7](#643e5cf70494850d7232a4de9c6d0cfe). Given the limitations in highly accurate or specific testing, the clinical manifestations of EPI are often nonspecific, leading to potential misdiagnosis and suboptimal management[10](#3a3fb76bb96582580d78be7092e5a020). Pancreatic enzyme replacement therapy represents a cornerstone of treatment, aiming to replenish deficient enzymes and restore digestive function[11](#4258108f16ce9ef16c63bf6f93c97bfa). The diagnosis of EPI can be achieved through direct or indirect measurement of pancreatic enzyme output, or by documenting maldigestion in general[10](#3a3fb76bb96582580d78be7092e5a020).

## Bacterial infection and pancreatic insufficiency

Bacterial infections, particularly those involving *Escherichia coli*, can exacerbate pancreatic insufficiency through various mechanisms. *E. coli*-induced inflammation can disrupt pancreatic enzyme production and secretion, further compromising digestive function[13](#945694f7e4b7bffb49ccbb8c3bf50cf6). Furthermore, bacterial overgrowth in the small intestine, a common consequence of pancreatic insufficiency, can lead to nutrient malabsorption and exacerbate symptoms[14](#fec646e022f77747d338b4a1fea58b09). The uncontrolled proliferation of *E. coli* and other bacteria can also result in the degradation of pancreatic enzymes, diminishing their effectiveness in digesting food. Addressing bacterial infections and restoring microbial balance is crucial in managing pancreatic insufficiency and alleviating associated complications[15](#f283cd552c6ea7a715a81dc6903491b9). The disruption of the gut microbiome due to pancreatic insufficiency can create an environment that promotes the growth of pathogenic bacteria, such as *E. coli*.[16](#0807bda57c23b9f05f2701abe18fac89). This bacterial overgrowth can further compromise digestive function, leading to a vicious cycle of malnutrition and exacerbation of pancreatic insufficiency symptoms[17](#e82fe2ceedcee9f55240e736e494c602). Employing strategies to modulate the gut microbiome, such as the use of probiotics or targeted antimicrobial therapy, may help restore the balance of beneficial and pathogenic bacteria, thereby improving overall digestive health and mitigating the effects of pancreatic insufficiency.

## Anti-oxidancy and pancreatic insufficiency

Oxidative stress significantly contributes to the development of pancreatic insufficiency through several key mechanisms. It damages pancreatic tissue, impairs enzyme production, and promotes fibrosis, ultimately reducing the pancreas's ability to produce and secrete digestive enzymes, leading to exocrine pancreatic insufficiency[18](#7121cc97b25c93128e0c07e102ffda97). Reactive oxygen species directly damage acinar cells, impairing enzyme synthesis and secretion, and disrupt mitochondrial function, reducing ATP production and compromising energy-dependent enzyme processes[19](#8edd07e47a5e51acfca296db7516fc97). ROS also activate pancreatic stellate cells, transforming them into myofibroblasts that deposit collagen and replace functional pancreatic tissue with scar tissue, further reducing enzyme production capacity[20](#aed863f2615521988e5d262139db0018). Oxidative stress triggers pro-inflammatory signaling, creating a feedback loop that amplifies tissue damage and fibrosis[21](#08f6e7d1c69a2bbf9d5a59f61485e376). Chronic diseases deplete antioxidants like glutathione and superoxide dismutase, leaving the pancreas vulnerable to oxidative damage[22](#0eba8692fdfa4a9b68bb3589286858a0). ROS can also damage ductal cells, impairing bicarbonate secretion and leading to premature enzyme activation and autodigestion of pancreatic tissue[23](#2e726ed0d2219705a2a45371cf825f49). The relationship between oxidative stress and pancreatic insufficiency highlights the importance of antioxidant defenses and managing risk factors, such as alcohol abuse, smoking, genetic mutations, and chronic pancreatitis, to prevent or slow the progression of pancreatic damage[24](#c7d510b17d326d59957c121f5681452f). Compounds like omega-3 fatty acids have shown promise in suppressing inflammatory pathways and inhibiting oxidative stress in pancreatic acinar cells[25](#a8dee824510325daf850f8ca564586f0). Oxidative stress can overwhelm the cytoprotective molecules in the pancreas, with calcium signaling and reactive oxygen species intricately linked through mitochondrial interactions[12](#b6e5090a1d2cc8fc57d427da43358c22).

## Anti-inflammatory and pancreatic insufficiency

Pancreatic inflammation plays a pivotal role in the pathogenesis of pancreatic insufficiency, contributing to tissue damage, impaired enzyme production, and compromised digestive function[26](#cf7916ea87edd49b45d8c77d229e2b77). Inflammatory mediators, such as cytokines and chemokines, can disrupt the delicate balance within the pancreas, leading to cellular injury and fibrosis[27](#85be994bd204022052d1c7559c7e4f56).Targeting inflammatory pathways represents a promising therapeutic strategy for mitigating pancreatic damage and preserving pancreatic function. Supplementation of omega-3 fatty acids increases anti-inflammatory cytokines and attenuates systemic disease, which suggests that the systemic inflammatory response to pancreatic injury is attenuated[28](#e3ca879b08683bf16ed90ac24d38a680). Natural compounds extracted from plants can inhibit inflammation and oxidative stress in acute pancreatitis by blocking several signaling pathways[28](#e3ca879b08683bf16ed90ac24d38a680), [29](#3670ddb58ef0ad6a5806fba30f88dbc7).

# Materials and Methodology

The materials and methodology employed in this study encompassed a comprehensive range of procedures to investigate the therapeutic potential of *Carpobrotus edulis* leaf extracts in pancreatic insufficiency. The study materials included analytical-grade chemicals and reagents obtained from the University of Zimbabwe, ensuring the reliability and accuracy of the experimental procedures. The study utilized specific equipment and facilities, including spectrophotometers for quantitative phytochemical analyses, which enabled precise measurement of the total phenolic and flavonoid content in the plant extracts. Additionally, cell culture facilities were employed for in vitro anti-inflammatory and antibacterial activity assays, providing a controlled environment for evaluating the biological effects of *Carpobrotus edulis* extracts. Plant specimens were meticulously authenticated at the National Herbarium in Zimbabwe, affirming the correct identification of *Carpobrotus edulis* for subsequent analyses. Ethical approval for the use of animals in this research was obtained from the Joint Research Ethics Committee prior to commencement, adhering to ethical guidelines and ensuring the humane treatment of all animal subjects.

## Plant collection and Preparation

### Plant collection

The *Carpobrotus edulis* leaf plant collection and preparation involved several key steps. The plants were collected in Hatfield, Harare, a suburban area situated in the southern part of Harare in Zimbabwe. The collection was conducted during the dry season to ensure optimal phytochemical content in the leaves[6](#d73ec6c53d6bcec7882fec6ac0484899). The leaves were carefully selected, focusing on mature and healthy specimens, to minimize variations in chemical composition.

### Samples preparation

The leaves were first washed to remove any sand and debris[30,31](#478c248fe30b0cb8fcde76cc6893fd43). Following washing, the leaves were air-dried at room temperature (24°C ±3°C) for 12 days[31](#9bab0014f2164b59f8d56aedb072cf0f). Once dried, the leaves were ground into a powder[31](#9bab0014f2164b59f8d56aedb072cf0f). 300g of the resulting powder was then macerated in 70% ethanol at a ratio of 1 to 6 (w/v) in a volumetric flask [32](#16157d3833c9e1fd37d0f726e4e4fcd8). This suspension was macerated for 48 hours at room temperature, with constant shaking[31](#9bab0014f2164b59f8d56aedb072cf0f). After maceration, the suspension was filtered using Whatman® filter paper (number 4)[31](#9bab0014f2164b59f8d56aedb072cf0f). The filtrate was then freeze-dried for 48 hours[31](#9bab0014f2164b59f8d56aedb072cf0f). The extract was weighed to determine the extract yield. The freeze-dried extract was then stored in an airtight container at 4°C until further use in the experiment[32](#16157d3833c9e1fd37d0f726e4e4fcd8).

## Qualitative phytochemical analysis

Qualitative phytochemical analysis was conducted to identify the presence of various classes of secondary metabolites in *Carpobrotus edulis* extracts, providing insights into the chemical composition and potential bioactivity of the plant[32](#16157d3833c9e1fd37d0f726e4e4fcd8). This phytochemical screening employed standard techniques to determine the presence or absence of relevant phytoconstituents, such as alkaloids, flavonoids, tannins, saponins, and other compounds that may contribute to the observed therapeutic effects[32](#16157d3833c9e1fd37d0f726e4e4fcd8). A series of qualitative tests were carried out on the hydro-ethanolic leaf extracts of *Carpobrotus edulis* to confirm the presence of these phytochemicals of pharmacological interest.

### Preparation of a concentration prior to phytochemical analysis

To prepare for phytochemical analysis, 10g of the freeze-dried hydro-ethanolic extract of *C. edulis* was dissolved in 100ml of distilled water in a 200ml round bottom flask. This solution was then tested using various phyto-screening methods to identify the presence or absence of pharmacologically important phytoconstituents. The extract solution underwent the following qualitative tests.

### Detection of Alkaloids

To detect the presence of alkaloids, Mayer's reagent was employed. To 2 ml of the extract, 1 ml of 2N HCl was added. The solution was then heated gently, cooled and filtered. To the filtrate, few drops of Mayer’s reagent were added. Formation of a creamy or white precipitate indicated the presence of alkaloids[33](#d5c2466646bce2022c8e3f4056d0084f).

### Detection of Flavonoids

To detect the presence of flavonoids, a few drops of diluted NaOH were added to 2 ml of the extract. An intense yellow color was formed in the extract, which turned colorless on addition of few drops of diluted acid, indicating the presence of flavonoids[32](#16157d3833c9e1fd37d0f726e4e4fcd8).

### Detection of phenols

To detect the presence of phenols, Ferric chloride test was employed. To 1 ml of extract, 2 ml of distilled water was added followed by addition of few drops of 10% ferric chloride solution. Formation of a blue or green color indicated the presence of phenols[33](#d5c2466646bce2022c8e3f4056d0084f).

### Detection of tannins

To detect the presence of tannins, a ferric chloride test was employed. To 2 ml of the extract, few drops of 1% ferric chloride solution were added. A blue-black or brownish green coloration indicated the presence of tannins[32](#16157d3833c9e1fd37d0f726e4e4fcd8).

### Detection of Saponins

To detect the presence of saponins, a froth test was employed. 2 ml of the extract was diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. Formation of foam indicated the presence of saponins[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e).

### Detection of Sterols

To detect the presence of sterols, Libermann-Burchard test was employed. To 2 ml of the extract, 2 ml of acetic anhydride was added followed by 1 ml of concentrated sulfuric acid. The solution mixture was observed for a change in color. Formation of a blue-green color indicated the presence of sterols[34](#7a539ac1efdad75488aacbfca9d6f9fd).

### Detection of Terpenoids

To detect the presence of terpenoids, Salkowski test was employed. To 2 ml of the extract, 2 ml of chloroform was added followed by 3 ml of concentrated sulfuric acid carefully to form a layer. A reddish-brown color formed at the interface indicated the presence of terpenoids[35](#4c57850881afa4e90555f6c333226247).

### Detection of Glycosides

To detect the presence of glycosides, a glycoside test was employed. 2 ml of the extract was dissolved in 2 ml of distilled water. Then, few drops of Molisch’s reagent were added followed by 2 ml of concentrated sulfuric acid carefully down the side of the test tube. The mixture was then allowed to stand for two minutes. Then, 5 ml of distilled water was added. A red ring formed at the interface of the two layers indicated the presence of glycosides[32](#16157d3833c9e1fd37d0f726e4e4fcd8).

### Detection of Anthraquinones

To detect the presence of anthraquinones, Borntrager’s test was employed. 2 ml of the extract was shaken with 5 ml of 10% aqueous ammonia. A pink color indicated the presence of anthraquinones[36](#80ab07f963222ed2a00a33921c7138d8).

### Total Phenolic Content Quantitative Analysis

The total phenolic content of the *Carpobrotus edulis* extract was quantified using the Folin-Ciocalteu reagent assay, following established protocols with slight modifications[32](#16157d3833c9e1fd37d0f726e4e4fcd8). A gallic acid stock solution was prepared at a concentration of 5 mg/ml by dissolving 5 mg of gallic acid in 1 mL of distilled water. Serial dilutions of this stock solution were performed to create five different concentrations of gallic acid standard solutions. The lyophilized leaf extract of *Carpobrotus edulis* was reconstituted in distilled water to achieve a concentration of 10 mg/ml. Immediately before use, the Folin-Ciocalteu reagent was diluted 10-fold with deionized water. Aliquots of 0.25 mL of each gallic acid standard solution and 0.25 mL of the *C. edulis* extract solution were transferred into separate test tubes. To each test tube, 1.25 mL of the 10-fold diluted Folin-Ciocalteu reagent was added, and the mixture was allowed to react for 5 minutes at room temperature. Subsequently, 2 mL of 7.5% sodium carbonate combined with folin solution was added to each test tube, and the final volume in each test tube was adjusted to 5 mL with distilled water. The test tubes were then incubated in the dark at room temperature for 60 minutes to facilitate color development. After incubation, the absorbance at 760 nm was measured spectrophotometrically, using the Folin-Ciocalteu reagent combined with sodium carbonate solution as a blank. All experiments were performed in triplicate. A calibration curve was generated by plotting the absorbance values of the gallic acid standard solutions against their corresponding concentrations. This calibration curve was used to determine the total phenolic content in *C. edulis* leaf extracts, by comparing the absorbance of the extract solution to the gallic acid calibration curve. The results were expressed as milligrams of gallic acid equivalents per gram of the sample. The total phenolic content was calculated using the formula derived from the gallic acid calibration curve: Total Phenolic Content (mg GAE/g) = (C × V) / m, where C is the concentration of gallic acid determined from the calibration curve (mg/mL), V is the volume of the extract in mL, and m is the weight of the dry plant extract in grams.

## Anti-inflammatory effect of lyophilized leaf extract of *C. edulis*

The anti-inflammatory activity of the lyophilized leaf extract of *C. edulis* was evaluated using the egg albumin denaturation test with minor modifications[32](#16157d3833c9e1fd37d0f726e4e4fcd8). The methodology involved assessing the extract's capacity to prevent protein denaturation, a mechanism linked to inflammation[32](#16157d3833c9e1fd37d0f726e4e4fcd8). The assay monitored the extract's ability to inhibit egg albumin denaturation in a phosphate-buffered saline solution. This method is based on the principle that inflammatory Tlesponses often lead to protein denaturation, and substances with anti-inflammatory properties can inhibit this denaturation process[33](#d5c2466646bce2022c8e3f4056d0084f).

### Reagents and Materials

Fresh chicken egg albumin was used, and phosphate-buffered saline with a pH of 7.2 served as the buffer solution. Lyophilized *Carpobrotus edulis* leaf extracts were prepared in 0.4% dimethyl sulfoxide, while diclofenac sodium, the positive control, was also dissolved in 0.4% DMSO. The vehicle control consisted of 0.4% DMSO.

### Preparation of Stock Solutions and Test Concentrations

A stock solution of lyophilized *C. edulis* extract was prepared at a concentration of 10 mg/mL in 0.4% DMSO by dissolving 100 mg of each lyophilized extract in 10 mL of 0.4% DMSO. Serial dilutions were then made from the stock solution to achieve the following final concentrations in the total reaction mixture: 50, 100, 250, 500, 750 and 1000 µg/ml.

### Reaction Mixture Preparation

The protocol involved using egg albumin from free-range domesticated hens, phosphate-buffered saline at pH 7.2, and solutions of varying concentrations of the lyophilized leaf extracts in 0.4% DMSO. The concentrations of the lyophilized extracts in the total reaction solution ranged from 50 to 1000 µg/mL[32](#16157d3833c9e1fd37d0f726e4e4fcd8). The reaction mixture comprised 0.4 mL of egg albumin, 10 mL of phosphate-buffered saline, and 5 mL of the *C. edulis* extract solutions. The positive control (Diclofenac) group consisted of a similar mixture without the extract. Negative controls consisting of 0.4 mL of fresh egg albumin, 0.5 mL of 0.4% DMSO, and 3 mL of PBS. Diclofenac sodium was used as a positive control for the study at similar concentrations[32](#16157d3833c9e1fd37d0f726e4e4fcd8).

### Incubation and Absorbance Measurement

The samples were incubated for 20 minutes at 37°C; by heating at 65°C in a water bath for an additional 30 minutes to induce denaturation of the egg albumin. After cooling the mixture, the absorbance was measured at 660 nm using the vehicle as blank.

### Determination of Percentage Inhibition

The percentage of inhibition, which translates to the anti-inflammatory activity of the extracts and standards, was calculated by the following equation:

*Equation 1*

*% Inhibition = 100 × (Abs control – Abs sample)/Abs control*

Where: Abs test is the absorbance of the test sample (containing the extract or standard), and Abs control is the absorbance of the control sample (without the extract or standard).

## Antibacterial activity of lyophilized *C. edulis* against *Escherichia coli* using well diffusion method.

The antibacterial activity of *Carpobrotus edulis* extracts was assessed through a qualitative screening process, specifically by measuring the zones of inhibition against *Escherichia coli* using the well diffusion method. The well diffusion method is widely recognized for its simplicity and reliability in determining the antibacterial potential of various substances, making it an ideal choice for this study[37](#84eeb59ec734e0aedeab6908bd06b043). This method allows for direct observation of the inhibitory effects of the extracts on bacterial growth, providing valuable insights into their antibacterial properties.

### Procedure:

Tryptone soya agar was sterilized by autoclaving at 121°C for 15 minutes[35](#4c57850881afa4e90555f6c333226247). The sterilized agar was allowed to cool to approximately 45-50°C before pouring into sterile Petri dishes under aseptic conditions. A uniform depth of approximately 4 mm was ensured. The agar was allowed to solidify completely. An *E. coli* suspension was prepared and adjusted to a 0.5 McFarland turbidity standard. The bacterial suspension was then evenly swabbed onto the surface of the solidified Mueller-Hinton agar plates using a sterile swab[35](#4c57850881afa4e90555f6c333226247). Each Petri dish was divided into four sections by marking on the lid. Using a sterile cork borer, four wells of 6 mm diameter were created in the agar within each section of the plate. The different concentrations of each plant extract (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml), ciprofloxacin solutions (positive control at the same concentrations), and sterile distilled water (negative control) were prepared. Using a sterile pipette, one drop (approximately 50 μl) of each concentration was carefully added into the appropriately labeled well. Each concentration corresponded to a specific section of the Petri dish, and each Petri dish had four wells. The Petri dishes were incubated in an incubator at 37°C for 24 hours[38](#718026e4ea4f884f868c88800e98e529). After incubation, the diameter of the zones of inhibition around each well was measured using a ruler. The measurement was taken from the back of the petri dish to ensure accuracy. The experiment was performed in triplicate to ensure the reproducibility of the results. Each Petri dish was labeled appropriately to indicate the plant extract and concentrations tested. The diameter of the inhibition zones was recorded for each concentration of the plant extracts, positive control, and negative control. The mean and standard deviation of the inhibition zone diameters were calculated for each treatment group. The antibacterial activity of the plant extracts was determined by comparing the size of the inhibition zones with those of the positive and negative controls[39](#d5f0284449732ad8585b082239644490). The extent of antibacterial activity was quantified by measuring the diameter of the clear zone surrounding each well, representing the inhibition of bacterial growth; these measurements were then used to calculate the area of the inhibition zone using the formula:

*Equation 2*

*Net Zone of inhibition= (Observed Zone of Inhibition diameter - Well diameter)*

% Inhibition = Net ZOI of extract at conc X/ Net ZOI of ciprofloxacin at conc X×100; Where Net ZOI of test compound = ZOI of test compound - ZOI of solvent control.

### Acute toxicity studies

To ascertain the potential hazards associated with the administration of lyophilized *C. edulis* extracts, a comprehensive acute toxicity study was undertaken, following the Organization for Economic Cooperation and Development guidelines 425 for the assessment of chemical. The study utilized 12 healthy, nulliparous, and non-pregnant female Sprague-Dawley rats, aged 8-12 weeks and weighing between 200-250g. The rats were individually housed and acclimatized to the laboratory conditions for a minimum of 5 days, with free access to commercial rodent feed and water. A stock solution of the extract was prepared by dissolving the lyophilized extract in distilled water to achieve a concentration of 5000mg/m. The rats were randomly divided into three treatment groups and a control group. Following an overnight fast, the treatment groups received a single oral dose of the plant extract at 500mg/kg, 2500mg/kg, and 5000 mg/kg body weight, respectively, while the control group received an equivalent volume of distilled water. The extracts were administered via oral gavage. The animals were closely observed for the first 4 hours after dosing, with attention to any signs of tremors, convulsions, salivation, diarrhea, or other abnormal behavior. Thereafter, observations were made daily for 14 days, recording various parameters such as mortality, changes in skin and fur, changes in eyes and mucous membranes, respiratory rate and pattern, activity level, gait abnormalities, changes in food and water consumption, and presence of diarrhea or changes in urine output. The body weight of each animal was recorded on days 0, 7, and 14 post-dosing, and food and water intake were measured daily. Throughout the study, a veterinary specialist monitored the animals daily for any signs of distress or toxicity, and any adverse clinical signs or unexpected deaths were recorded immediately. At the end of the 14-day observation period, all surviving animals were humanely euthanized via cervical dislocation, a method aligned with AVMA guidelines for the humane euthanasia of animals.

### Ethical Considerations

This study was conducted in accordance with the ethical guidelines for the care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering. Animals exhibiting signs of severe distress or moribund condition were humanely euthanized according to approved protocols.

## Subacute Oral Toxicity Study of *Carpobrotus edulis* Extract in Sprague Dawley Rats

This study aimed to evaluate the subacute oral toxicity of lyophilized extracts of *Carpobrotus edulis* in Sprague Dawley rats. Subacute toxicity studies are crucial for assessing the potential health risks associated with repeated exposure to a substance over a defined period, typically ranging from 14 to 28 days[40](#cde51eadea3f697ca26f432978068565). These studies provide valuable insights into the target organs affected, the nature of toxic effects, and the dose-response relationship, thereby aiding in the determination of safe exposure levels for further investigations The study was conducted in accordance with OECD Test Guideline 407, with slight modification, specifically on the number of study groups and dosages: Repeated Dose 28-day Oral Toxicity Study in Rodents[41](#a0fb1eb1cd426dc287511545c9347fb3).

### Animals and Housing

A total of 18 healthy, nulliparous and non-pregnant female Wistar rats, aged 8-12 weeks and weighing between 200-250g, were used. The groups of rats were divided into control, low-dose, and high-dose groups, each group having six rats (n=6). The rats were obtained from a certified animal breeding facility, University of Zimbabwe Animal House and acclimatized to the laboratory conditions for a minimum of 7 days prior to the experiment. This acclimatization period allows the animals to adjust to the new environment, minimizing stress[32](#16157d3833c9e1fd37d0f726e4e4fcd8). Animals were housed individually in standard rat cages with stainless steel wire mesh floors. The animal room was maintained at a temperature of 22 ± 3°C, with a relative humidity of 50-60% and a 12-hour light/dark cycle. Rats were provided with free access to commercial rodent feed and water *ad libitum* throughout the acclimatization and experimental periods. The dose levels for the subacute toxicity study were selected based on the results of the acute toxicity study.

### Clinical Observations

The animals were observed twice daily for any clinical signs of toxicity. Detailed observations, including the following parameters, were recorded daily: Mortality, changes in skin and fur, changes in eyes and mucous membranes, respiratory rate and pattern, activity level (drowsiness, excitability), gait abnormalities, changes in food and water consumption, presence of diarrhea or changes in urine output and erection of fur.

### Body Weight and Food Consumption

The body weights of the animals were recorded daily. Additionally, the food and water consumption of each group was measured and documented on a daily basis.

## Hematology, Clinical Biochemistry and Histopathology

Hematology, clinical biochemistry, and histopathology analyses were performed according to established protocols[4](#38a24be4dca31cd3230279d3cfe819f5). Following the 28-day treatment period, the rats were fasted overnight to minimize the influence of recent food intake on biochemical parameters. 15 Sprague Dawley rats of both sexes were used in these studies.

### Hematology

#### Blood Collection

The rats were anesthetized using chloroform. Blood was collected via cardiac puncture and transferred into blood collection tubes containing Ethylenediaminetetraacetic acid as an anticoagulant. It is worth noting that anesthesia is recommended to minimize discomfort during blood collection[42](#bf7f4f4d63068aaf9f62cd197c2a5a4a).

#### Hematological Analysis

The blood samples in EDTA tubes were analyzed using a Mindray BC-2800Vet hematology analyzer. The following parameters were determined: red blood cell count, white blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume and platelet count.

### Biochemistry

Serum Preparation: Blood was collected from all animals through cardiac puncture under chloroform anesthesia and stored in plain blood collection tubes. The blood was then centrifuged using a Hermle Centrifuge at 3000 revolutions per minute to obtain serum. The separated serum was collected and stored in Eppendorf tubes at -20°C until analysis.

#### Biochemical Analysis

Serum samples were analyzed using a Mindray BS-120 chemistry analyzer. The following biochemical parameters were assessed: urea, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, total protein and albumin.

## Histopathology

### Organ Removal and Fixation

After the treatment period, the rats were euthanized using hexane and subjected to post-mortem examination. Major organs, including the liver, heart, and kidney, were carefully removed. The organ weights of the heart, liver, and kidney were recorded. These organs were then fixed in 10% buffered formalin to preserve tissue structure.

### Tissue Processing

The fixed organs were processed for histopathology following standard protocols. This involved trimming the tissues and embedding them in paraffin wax.

### Sectioning and Staining

The paraffin-embedded tissues were sectioned at a thickness of 5 μm using a microtome. The sections were then stained with hematoxylin and eosin to visualize cellular structures.

### Microscopic Examination

The stained slides were examined under a light microscope at 10x and 40x objective magnification by Dr. Mudimba, who analyzed the slides for any pathological changes.

### Statistical Analysis

Data was analyzed using the Statistical Package for Social Sciences. Hematological and biochemical parameters were expressed as means ± standard error of the mean for all groups. One-way analysis of variance was used to compare the variation of hematological profiles and biochemical differences within groups. Where ANOVA indicated significant differences, a post-hoc test (e.g., Dunnett's test) was applied to determine the specific pairs of groups that differed significantly[43](#f3db3fd0449dfe59664264467054ef58). A 95% level of significance (p ≤ 0.05) was used to determine statistically significant differences. For histopathology, descriptive statistical analysis was conducted.

# Results and Discussion

## Evaluation of phytochemical studies

The phytochemical screening of the lyophilized *C. edulis* leaf extract reveals a diverse array of secondary metabolites, including phenolics, alkaloids, flavonoids, tannins, saponins, terpenoids, anthraquinones, glycosides, and sterols (Table 1). This rich phytochemical profile supports the plant's potential therapeutic effects, particularly in the context of pancreatic insufficiency, where antibacterial, antioxidant, and anti-inflammatory properties are highly relevant. Phenolics and flavonoids, which are highly present in the extract, are well-known for their antioxidant and anti-inflammatory activities[44](#ca1726c4820f04e87d8ed90850be1ae7), [3](#53fca0c717deb18ac139628c1fcdd05a). Their ability to scavenge free radicals and modulate inflammatory pathways can help mitigate oxidative stress and inflammation, both of which can exacerbate pancreatic damage and dysfunction. These compounds are effective in the treatments against several debilitating diseases such as cancer, diabetes, inflammation, pathogenic infections and aging[3](#53fca0c717deb18ac139628c1fcdd05a). Tannins, also abundant in the extract, possess antibacterial properties that can combat bacterial infections, which are a common complication in pancreatic insufficiency[45](#08891f3f1d93a16d3c9b6ed04f8b3c8f). Saponins, moderately present, have been shown to exhibit both anti-inflammatory and immunomodulatory effects, further contributing to the plant's therapeutic potential[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Given this diverse range of bioactive compounds, further research is warranted to isolate and identify the specific compounds responsible for the observed therapeutic effects, determine their mechanisms of action, and assess their safety and efficacy in preclinical and clinical studies. By elucidating the intricate interplay between these phytochemicals and their therapeutic activities, we can unlock the full potential of *C. edulis* as a valuable natural remedy for pancreatic insufficiency[6](#d73ec6c53d6bcec7882fec6ac0484899).

## *Table 1: Secondary Metabolomics screening tests done on Carpobrotus edulis lyophilized leaf extract*

|  |  |
| --- | --- |
| **Secondary Metabolites** | **Presence in hydro-ethanolic extract** |
| Phenolics | +++ |
| Alkaloids | + |
| Flavonoids | ++ |
| Tannins | +++ |
| Saponins | ++ |
| Terpenoids | + |
| Anthraquinones | ++ |
| Glycosides | + |
| Sterols | + |

*Highly present+++, Moderately present++, lowly present+*

## Evaluation of total phenolic content

The total phenolic content of 279.7 mg GAE/g in the lyophilized leaves of *Carpobrotus edulis* suggests a notably high antioxidant potential (Fig 2). This is significant because oxidative stress plays a crucial role in the pathogenesis of pancreatic insufficiency, exacerbating damage to pancreatic tissue and impairing its function. Phenolic compounds are known for their free radical scavenging abilities, which can mitigate oxidative damage[39](#d5f0284449732ad8585b082239644490). This value suggests that the *C. edulis* leaves from Zvimba, Zimbabwe, could be a potent source of antioxidants. While a direct comparison to other studies on *C. edulis* is necessary for validation, several factors could influence this high value. The altitude and specific environmental conditions in the Zvimba area could contribute to increased phenolic production as a stress response in the plant[32](#16157d3833c9e1fd37d0f726e4e4fcd8). Further investigation, including comparisons with *C. edulis* from different regions and detailed phenolic profiling, would be valuable to confirm this result and fully understand its implications[46](#b49a0b3e126be4511acc2fd858989a16). Overall, this high phenolic content supports the potential of *C. edulis* as a complementary treatment for pancreatic insufficiency by combating oxidative stress[5,47](#3f583438bd56505262bed78fb846dd79).

Figure 2 Total phenolic content assay of C edulis

## Evaluation of Anti-Inflammatory Activity of lyophilized leaves of *c edulis* extracts

The observed anti-inflammatory activity of the lyophilized hydroethanolic leaf extract of *C. edulis*, with a peak of 69.37% inhibition at 1000 µg/mL relative to diclofenac (Table 2 and Fig 3), strongly suggests its potential therapeutic application in pancreatic insufficiency. This activity is consistent with the traditional use of *C. edulis* for treating inflammatory conditions and warrants further investigation into its use as a natural anti-inflammatory agent[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e), [5](#a31d280e1cc73610cd1bfa01f1a896cf). The greater inhibition percentage observed at the highest concentration tested, compared to lower concentrations, indicates a dose-dependent response up to a certain point. It is also possible that synergistic effects occur at higher concentrations between certain compounds within the extract, leading to enhanced anti-inflammatory activity[32](#16157d3833c9e1fd37d0f726e4e4fcd8). It is also plausible that the 69.37% inhibition is attributable to other phytochemical constituents with significant anti-inflammatory potential[32](#16157d3833c9e1fd37d0f726e4e4fcd8). Sabiu et al[3](#53fca0c717deb18ac139628c1fcdd05a) identified a number of phenolics. The observed anti-inflammatory activity might also be linked to compounds that interfere with inflammatory pathways. Given the potential of *C. edulis* as an anti-inflammatory agent, further research is justified to fully elucidate its mechanisms of action, optimize extraction methods, and determine safe and effective dosages. Future studies should explore the effects of *C. edulis* extracts on specific inflammatory mediators and signaling pathways relevant to pancreatic insufficiency. Furthermore, in vivo studies are crucial to validate the anti-inflammatory efficacy of *C. edulis* in a physiological context.

## *Table 2. Anti-inflammatory test of extracts of C. edulis*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample** | **Concentration(ug/ml)** |  | **Absorbance** | **% Inhibition** |
| **Negative control** | 0.4% DMSO |  | 0.924 | 0.00 |
| **Diclofenac** | 50 |  | 0.781 | 15.48 |
|  | 100 |  | 0.653 | 29.33 |
|  | 250 |  | 0.482 | 47.84 |
|  | 500 |  | 0.317 | 65.69 |
|  | 750 |  | 0.209 | 77.38 |
|  | 1000 |  | 0.152 | 83.55 |
| ***C. edulis*** | 50 |  | 0.847 | 8.33 |
|  | 100 |  | 0.723 | 21.75 |
|  | 250 |  | 0.598 | 35.28 |
|  | 500 |  | 0.452 | 51.08 |
|  | 750 |  | 0.347 | 62.45 |
|  | 1000 |  | 0.283 | 69.37 |

Figure 3:Inflammation-inhibiting effects of C. edulis in comparison to diclofenac sodium

## Evaluation of Antibacterial activity of lyophilized leaf extract of *C. edulis*

*Carpobrotus edulis* has demonstrated antibacterial activity, making it potentially useful in managing bacterial imbalances associated with pancreatic insufficiency. The results show that at a concentration of 100 mg/ml, *C. edulis* inhibited bacterial growth with a mean zone of inhibition of 18.7±1.5 mm, while the positive control, Ciprofloxacin, had a ZOI of 38.3±1.5 mm (Table 3,4, Fig 4). The net ZOI for *C. edulis* and Ciprofloxacin were 12.7±1.5 mm and 32.3±1.5 mm, respectively. The results are shown in table 3 and 4. The likely minimum inhibitory concentration value lies between 12.5 mg/ml and 25 mg/ml, as at 12.5mg/ml the net ZOI of *C. edulis* is 4.00±1.0mm. The highest ZOI observed for *C. edulis* in this study was 18.7±1.5 mm at 100 mg/ml concentration. The antibacterial activity of *C. edulis* extracts has significant implications for managing pancreatic insufficiency. The antibacterial effects may also help mitigate the complications associated with bacterial overgrowth in the gut, further improving digestive health and nutrient absorption[48](#09d796d816f61328342f6c9a44f1e7c0). The seasonal variation in the antimicrobial activity of *C. edulis* against *Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli*, and *Staphylococcus aureus* was also evaluated[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Another study found that *C. edulis* from the Tunisian coast displayed notable antibacterial activity against Pseudomonas aeruginosa, *Escherichia coli and* Staphylococcus aureus, with MIC values between 6.25 and 50 mg/ml. When compared to other studies, the antibacterial activity of *C. edulis* observed in this study is consistent with previous reports. For instance, ethanolic extracts of *C. edulis* have shown significant activity against *Staphylococcus aureus, Bacillus cereus*, and *Mycobacterium aurum*[4](#38a24be4dca31cd3230279d3cfe819f5). However, the variations in the antibacterial activity of *C. edulis* across different studies can be attributed to several factors. The extraction method used to obtain the plant extracts can significantly influence their antibacterial activity. Different solvents and techniques may selectively extract different antibacterial compounds, leading to variations in the composition and potency of the extracts[49](#6c48722e3f4d36a8122d7c028fbbef04). Geographical location and environmental factors can also affect the phytochemical profile of *C. edulis* plants. Plants collected from different regions may possess unique compositions due to variations in soil composition, altitude, and climate, affecting the production of antibacterial metabolites[32](#16157d3833c9e1fd37d0f726e4e4fcd8). Additionally, different bacterial strains may exhibit varying degrees of sensitivity to the antibacterial compounds present in *C. edulis* extracts. The antibacterial properties of *C. edulis* can be attributed to its rich content of bioactive compounds. *C. edulis* found on the Tunisian coast also displayed notable antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*[*2*](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Specific phenolic compounds within *C. edulis* contribute to its antibacterial prowess; for example, hyperoside and the flavonone glycoside neohesperidin have both demonstrated antibacterial activity against *Pseudomonas aeruginosa*[*2*](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Further, rutin, cactichin, and ferulic acid have demonstrated antibacterial activity[50](#a0461330dccb32d971a4627f2ee91ed6). Furthermore, previous studies have shown that rutin, cactichin, and ferulic acid, compounds found in *C. edulis*, possess antibacterial activity[50](#a0461330dccb32d971a4627f2ee91ed6). Compared to other studies, the antibacterial activity of *C. edulis* holds promises for managing bacterial imbalances in pancreatic insufficiency. Future research is needed to explore the specific mechanisms of action of *C. edulis* against different bacterial strains and to optimize extraction and formulation methods for enhanced antibacterial efficacy. Additionally, clinical trials are necessary to evaluate the effectiveness of *C. edulis* in managing bacterial-related complications in patients with pancreatic insufficiency.

*Table 3: Observed Zone of Inhibition diameter(mm) with respect to concentration of Lyophilized C. edulis and Ciprofloxacin*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration** | **Observed Zone of Inhibition** | | | | **Observed Zone of Inhibition** | | | |
| ***C. edulis(mm)*** | | | | ***Ciprofloxacin(mm)*** | | | |
| mg/ml | Trial  1 | Trial 2 | Trial 3 | Mean ± SD | Trial 1 | Trial 2 | Trial 3 | Mean ± SD |
|  |  |  |  |  |  |  |  |  |
| 100 | 19 | 17 | 20 | 18.7±1.5 | 38 | 40 | 37 | 38.3±1.5 |
| 50 | 16 | 15 | 17 | 16.0±1.0 | 35 | 36 | 33 | 34.7±1.5 |
| 25 | 11 | 13 | 14 | 12.7±1.5 | 28 | 30 | 26 | 28.0±2.0 |
| 12.5 | 10 | 9 | 11 | 10.0±1.0 | 22 | 24 | 21 | 22.3±1.5 |

*Table 4: Net Zone of Inhibition diameter (mm) with respect to concentration of Lyophilized C. edulis and Ciprofloxacin*

|  |  |  |
| --- | --- | --- |
| Concentration | Net Zone of Inhibition(mm) | Net Zone of Inhibition(mm) |
| mg/ml | *C. edulis* | *Ciprofloxacin* |
| 100 | 12.7±1.5 | 32.3±1.5 |
| 50 | 10.0±1.0 | 28.7±1.5 |
| 25 | 6.70±1.5 | 22.0±2.0 |
| 12.5 | 4.00±1.0 | 16.3±1.5 |

Figure 4 Zone of Inhibition diameter(mm) with respect to concentration of Lyophilized C. edulis and Ciprofloxacin

## Evaluation of toxicity studies of Lyophilized extract of *C. edulis*

Acute and sub-acute toxicity studies are essential for evaluating the safety profile of *Carpobrotus edulis* extracts, ensuring their suitability for therapeutic applications in pancreatic insufficiency.

### Acute toxicity studies and validation of oral toxicity profile of *C. edulis*

The acute toxicity studies revealed no mortality or significant signs of toxicity in rats administered *C. edulis* extracts at doses up to 5000 mg/kg body weight as shown in table 5, suggesting a high margin of safety. These findings align with previous research demonstrating the non-toxic nature of aqueous *C. edulis* extracts at a dose of 2000 mg/kg[4](#38a24be4dca31cd3230279d3cfe819f5). According to the Globally Harmonized System of Classification and Labelling of Chemicals, substances with an LD50 greater than 5000 mg/kg are considered non-toxic[32](#16157d3833c9e1fd37d0f726e4e4fcd8). The absence of mortality and visible signs of toxicity at these high doses indicates that *C. edulis* extracts have a wide therapeutic window, minimizing the risk of adverse effects even at elevated concentrations. These results provide initial reassurance regarding the safety of *C. edulis* extracts for potential use in treating pancreatic insufficiency. Furthermore, the detailed observations during the acute toxicity studies, including monitoring of behavioral changes, clinical signs, and body weight (table 6), offer valuable insights into the immediate effects of *C. edulis* extracts on the physiological and behavioral parameters of the tested animals. The fact that no significant changes were observed in these parameters further supports the safety profile of *C. edulis* extracts during acute exposure.

*Table 5: Weekly mean body weight of Sprague Dawley rat groups used in acute toxicity study of C. edulis hydro-ethanolic extract*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Group (n=3)** | **Dose Levels (mg/kg)** | **Weight in grams prior to *C. edulis* extract exposure** | **Weight in grams, one week after exposure** | **Weight in grams, two weeks after exposure** |
| A | 0 | 120.00 ± 8.50 | 123.00 ± 7.90 | 127.00 ± 9.10 |
| B | 500 | 115.50 ± 9.20 | 118.00 ± 8.80 | 122.50 ± 9.50 |
| C | 2500 | 118.00 ± 7.80 | 121.50 ± 8.10 | 125.00 ± 8.70 |
| D | 5000 | 117.00 ± 8.10 | 120.00 ± 7.50 | 124.50 ± 8.00 |

Values are expressed as mean ± SEM. \*p value less than 0.05, (p<0.05) significant value.

## Evaluation of Subacute toxicity studies

### Observations from subacute toxicity profiling of *C. edulis*

The sub-acute toxicity study, conducted over 28 days, supported the safety of *C. edulis* extracts at doses up to 1000 mg/kg body weight as shown in table 6 and Figure 5. The treated rats exhibited normal food and water intake, similar to the control group, suggesting that the extract did not significantly affect their appetite or general physiology. Importantly, no deaths occurred in any of the groups, indicating a lack of acute toxicity at the tested doses. The treatment did not cause significant change in weights of rats (fig 5). Furthermore, the absence of clinical signs, such as drowsiness or erection of fur, reinforced the non-toxic nature of the extract. However, increased urination was noted in the 1000 mg/kg group, prompting further toxicity studies, including hematology, biochemistry, and histopathology[4](#38a24be4dca31cd3230279d3cfe819f5). These findings align with previous studies[4](#38a24be4dca31cd3230279d3cfe819f5), which also found no adverse effects on body weight, feed/water intake, or tested biochemical parameters in Sprague Dawley rats, reinforcing the safety profile of *C. edulis*. Considering its favorable side effect profile, the potential use of *C. edulis* in pancreatic insufficiency could be validated, as the plant appears to be relatively safe at the tested doses, with no significant adverse effects observed on the general health and behavior of the treated animals. The increased urination observed at the highest dose (1000 mg/kg) may indicate a potential diuretic effect of the extract, which could be further investigated to determine its underlying mechanism and potential implications for fluid balance.

*Table 6. Observations from Acute and subacute toxicity profiling of C. edulis*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Observed Parameter** | **Dose of *C. edulis in mg/kg body weight*** | | | |
|  | **Control** | **2500mg** | **5000mg** |  |
| **Food intake** | Normal | Normal | Normal |  |
| **Water intake** | Normal | Normal | Normal |  |
| **Death** | Alive | Alive | Alive |  |
| **Breathing** | Normal | Normal | Normal |  |
| **Defecation** | Normal | Normal | Normal |  |
| **Skin color** | Normal | Normal | Normal |  |
| **Drowsiness** | Not observed | Not observed | Not observed |  |
| **Erection of Fur** | Not observed | Not observed | Not observed |  |
| **Urination** | Normal | Normal | Increased | 1 |

Figure 5 Sprague-Dawley rats’ weight over the subacute toxicity test investigation period (g)

### Food and water consumption

The average daily water intake feed intake is shown in Table 7 and 8. There were no ssignificantdifferences (p>0.05) in water and food consumption at any time point. In water intake the 1000mg/kg group showed a mild, no significant increase in water intake over time, which could reflect natural variability or a weak biological effect. For feed intake, all groups maintained stable intake across weeks with no dose-dependent effects. Variability (SEM) was consistent across groups and no interaction between dose and time reached significance, suggesting the treatment had no measurable impact on consumption behaviors under the tested conditions.

*Table 7: Effects of Carpobrotus edulis on average daily water intake (ml) of Sprague Dawley Rats over 28 days.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Dosage | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 0 | 97.50±2.10 | 99.83±4.80 | 113.20±8.50 | 96.67±4.10 |
| 1 | 500 | 95.33±3.50 | 98.67±5.20 | 108.17±9.20 | 105.50±3.90 |
| 2 | 1000 | 105.83±5.40 | 104.17±6.80 | 122.33±10.20 | 112.83±5.50 |

Values are presented as mean ± SEM. \*p < 0.05 indicates statistical significance.

*Table 8: Effects of Carpobrotus edulis on average feed intake(g) of Sprague Dawley Rats over 28 days.*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Dose (mg/kg) | Week 1 | Week 2 | Week 3 | Week 4 |
| Control | 0 | 65.83±5.50 | 72.33±2.70 | 78.17±3.20 | 72.50±3.00 |
| 1 | 500 | 63.17±5.20 | 68.83±2.60 | 77.67±3.10 | 71.83±2.90 |
| 2 | 1000 | 66.50±5.80 | 73.17±2.40 | 78.83±4.30 | 74.33±3.20 |

*Values are presented as mean ± SEM. \*p < 0.05 indicates statistical significance*.

### Organ weight

The average weight changes in organs weights are shown in Table 9. No statistically significant differences (p<0.05) were observed in liver, heart, or kidney weights between the control and treatment groups (500mg/kg and 1000mg/kg).

*Table 9: Average Organ weights of Sprague Dawley rats following 28 days of repeated exposure to hydro-ethanolic extract of Carpobrotus edulis.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group  n=6 | Dose (mg/kg | Liver | Heart | Kidney |
| Control | 0 | 6.24±0.35 | 0.76±0.07 | 1.44±0.16 |
| 1 | 500 | 6.00±0.71 | 0.68±0.06 | 1.27±0.16 |
| 2 | 1000 | 6.59±0.54 | 0.71±0.07 | 1.41±0.10 |

*Values are presented as mean ± SEM. \*p < 0.05 indicates statistical significance.*

## Hematology

The hydro-ethanolic extract of *C. edulis* showed statistically significant alterations in several hematological parameters in the treated rats compared to the control group. Specifically, RBC counts were significantly lower in both the 500mg/kg and 1000mg/kg groups, alongside decreased PLT counts, and altered red cell indices in the treatment groups as shown in Table 10. These findings suggest that the hydro-ethanolic extract of *C. edulis* may have some level of hematological toxicity. The reduction in RBC count could indicate potential bone marrow suppression or increased RBC destruction. The decrease in platelet count could indicate an increased risk of bleeding[31](#9bab0014f2164b59f8d56aedb072cf0f). The changes in MCV, MCH, and MCHC suggest alterations in red blood cell size and hemoglobin concentration, potentially affecting oxygen-carrying capacity. When comparing these results to the study by Mudimba et al.[4](#38a24be4dca31cd3230279d3cfe819f5), differences in the extraction method are crucial. Mudimba et al.[4](#38a24be4dca31cd3230279d3cfe819f5) used an aqueous extract and found no significant differences in hematological parameters. The use of a hydro-ethanolic extract likely extracted different bioactive compounds, some of which may be responsible for the observed hematological changes. Studies done by Mudimba et al[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e) shows that the solvent system affects the types of compounds that end up in the final extract. Akinyede et al[6](#d73ec6c53d6bcec7882fec6ac0484899),notes that the traditional use of the plant, disease prevalence, availability and sustainability of the plant should be considered during research and drug development. It's possible that the compounds extracted by the hydro-ethanolic solvent exhibit some toxicity that was not present in the aqueous extract[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e). Given these hematological changes, there is a need for further dosage evaluation and validation when considering *C. edulis* for the treatment of pancreatic insufficiency. While the plant has demonstrated potential benefits, such as antibacterial properties, anti-oxidant, anti-inflammatory and anti-diabetic effects, the potential for hematological toxicity needs to be carefully evaluated. If *C. edulis* is to be considered, further studies should focus on identifying the specific compounds responsible for the observed hematological changes and determining safe dosage levels.

*Table 10: Hematology values of rats treated with hydro-ethanolic extract of C. edulis in sub-acute toxicity (n=15), n=5 per group*

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | Control  0mg/kg | Group 1  500mg/kg | Group 2  1000mg/kg |
| WBC | 6.0 ± 0.2 | 4.2 ± 0.60 | 4.3 ± 1.2 |
| RBC | 9.3 ± 0.8 | 7.8 ± 1.00\* | 8.3 ± 0.4\* |
| Hb | 18.1 ± 0.1 | 16.3 ± 0.4 | 17.3 ± 0.9 |
| HCT | 52.3 ± 0.7 | 48.5 ± 0.5 | 51.5 ± 2.3 |
| MCV | 57.2 ± 0.2 | 61.1 ± 0.8\* | 60.8 ± 0.4\* |
| MCH | 19.2 ± 0.3 | 20.4 ± 0.2\* | 20.3 ± 0.2\* |
| MCHC | 34.2 ± 0.3 | 33.0 ± 0.2\* | 33.2 ± 0.3\* |
| RDW | 14.1 ± 0.2 | 12.0 ± 0.3\* | 12.7 ± 0.6 |
| PLT | 1220.2 ± 6.1 | 1045.2 ± 28.3\* | 1175.8 ± 47.1\* |
| MPV | 5.8 ± 0.2 | 5.4 ± 0.1 | 6.0 ± 0.5 |

*Values are expressed as mean ± SEM, n=15, \*p values less than 0.05 (p<0.05) significant value*

## Biochemistry

Evaluating the biochemical data in light of *Carpobrotus epulis*’s potential use in pancreatic insufficiency requires a nuanced approach, focusing on the statistical significance of the observed changes and their potential implications for organ function. The study found that rats treated with a hydro-ethanolic extract of *C. edulis* exhibited statistically significant alterations in several biochemical parameters. Specifically, albumin, ALP, urea, creatine, and total protein levels were significantly elevated in the treatment groups compared to the control as shown in Table 11. The elevated albumin and total protein levels could suggest a response to inflammation or altered protein metabolism. The significant increase in ALP at both doses may indicate potential hepatobiliary effects, as ALP is often used as an indicator of liver function. Similarly, the elevated urea and creatinine levels point towards possible alterations in renal function, since those are markers of kidney health[51](#d0e7837b8990e486bde5fe13a2261714). Connecting these findings to the work of Mudimba et al. [4](#38a24be4dca31cd3230279d3cfe819f5) is crucial, but with careful consideration. Mudimba et al.[4](#38a24be4dca31cd3230279d3cfe819f5) employed an aqueous extract of *C. edulis* and reported no significant changes in biochemical parameters. The contrasting results likely stem from the differences in extraction methods. The hydro-ethanolic solvent likely extracted different compounds than the aqueous solvent used by Mudimba et al. [4](#38a24be4dca31cd3230279d3cfe819f5), and some of these compounds may be responsible for the observed biochemical changes[2](#6b7ce6dfdc070894b5fad73ffb9d4e7e). states the importance of the different solvent systems and how they may affect the compounds. Considering these biochemical alterations, a cautious approach is necessary when evaluating *C. edulis* for pancreatic insufficiency. The plant has demonstrated potential benefits, such as antibacterial, antioxidant, anti-inflammatory and antidiabetic effects [5](#a31d280e1cc73610cd1bfa01f1a896cf). However, the potential for hepatobiliary and renal effects, as suggested by the study, needs careful evaluation. If *C. edulis* is to be considered for pancreatic insufficiency, further investigations should prioritize identifying the specific compounds responsible for the observed biochemical changes and establishing safe dosage levels. Furthermore, it would be beneficial to understand if the hydro-ethanolic extract's effects are dose-dependent.

*Table 11: Biochemical values of rats treated with hydro-ethanolic extract of C. edulis in sub-acute toxicity studies (n=15), n=5 per group*

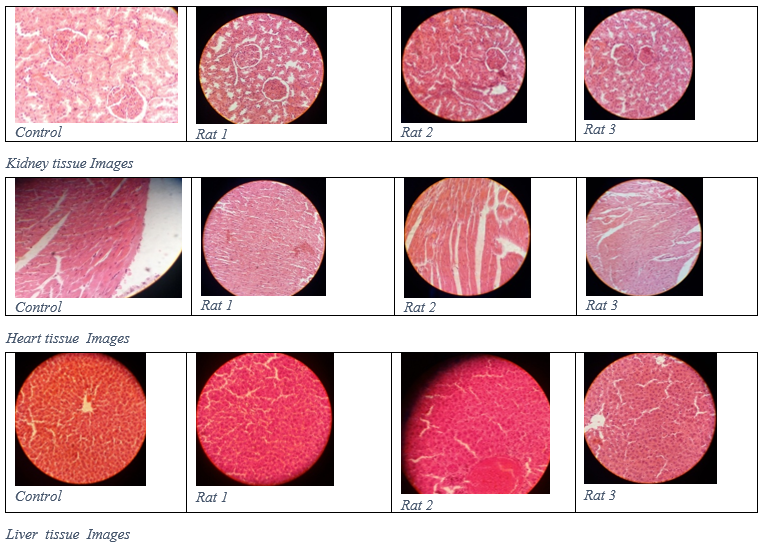
|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | Control  0mg/kg | Group 1  500mg/kg | Group 2  1000mg/kg |
| ALT | 87.6 ± 0.7 | 75.3 ± 1.5 | 88.9 ± 6.6 |
| ALB | 34.3 ± 0.4 | 35.4 ± 0.3 | 38.0 ± 0.7\* |
| ALP | 438.7 ± 0.4 | 267.5 ± 61.6\* | 472.7 ± 20.7\* |
| AST | 130.2 ± 0.3 | 115.5 ± 25.9 | 167.8 ± 2.6 |
| UREA | 4.8 ± 0.2 | 5.7 ± 0.2\* | 6.1 ± 0.1\* |
| CREATINE | 45.2 ± 0.2 | 51.6 ± 1.4\* | 55.3 ± 2.1\* |
| Total Protein | 4.5 ± 0.2 | 6.2 ± 0.3\* | 6.8 ± 0.5\* |

*Values are expressed as mean ± SEM, n=15, \*p values less than 0.05 (p<0.05) significant value*

## Histopathology

No histopathological changes were observed in major organs (heart, liver and kidney) in all treatment groups compared as well as the control. No abnormalities were found (Table 12). This corresponds well with the biochemical and hematology results observed above.

*Table 12: Slide images of histopathology investigations*

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Sub-acute toxicity evaluation is based on observing and identifying any adverse effects that may occur after repeated exposure of laboratory animals to a substance over a prolonged duration. This type of testing is important for nutraceutical remedies that are used for long periods for multiple uses. It was therefore prudent to carry out this assay on *C. edulis* due to its cardinal prevalence in traditional medicinal practices and multiple uses in various ailments.

# Conclusion

Phytochemical analysis revealed various bioactive compounds, which explain the plant's antibacterial, antioxidant, and anti-inflammatory effects. In vitro tests showed anti-inflammatory promise, and initial toxicity tests suggested general safety at the tested doses. However, longer-term toxicity studies indicated statistically significant changes in blood and biochemical markers, specifically affecting red blood cell and platelet counts, as well as albumin, ALP, urea, and creatinine levels. These results emphasize the need for further research to identify the specific compounds causing these changes and to determine safe dosages for therapeutic use. Comparative studies using different extraction methods, such as aqueous versus hydro-ethanolic, are also essential to understand how solvent systems affect the plant's chemical composition and biological activity, given the discrepancies between this study and previous research using aqueous extracts. Overall, *C. edulis* shows a complex profile, with both therapeutic potential and potential risks, requiring careful evaluation and further investigation to maximize its benefits while minimizing potential harm.

# Competing interests

The authors declare that there are no conflicts of interest. The content of this research and the preparation of this report are the sole responsibility of the authors.

# Disclaimer (Artificial intelligence)

The Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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