***Original Research Article***

***Therapeutic Potential and Toxicological Profile of Carpobrotus edulis Extract***

**Abstract**

*Carpobrotus edulis* (*C. edulis*) commonly known as sour fig, from the *Aizoaceae* family is a widespread plant species prevalent in tropical and subtropical regions of Africa. The plant is extensively utilized in traditional medicine for various ailments, including sore throat, bad breath, mouth ulcers, and tonsillitis. The endpoint biomarkers of the tonsillitis diseased state include inflammation, pain and degeneration of cells and tissues. The current therapeutic approaches for managing tonsillitis are characterized by several limitations such as adverse side effects and prohibitive costs. This, therefore, necessitates the need to explore safer, effective, and affordable alternatives that can inhibit, ameliorate, and/or reverse the biological endpoints of tonsillitis. Our current study qualitatively and quantitatively determined the phytoconstituents of *Carpobrotus edulis*, as well as its activity as an antimicrobial, antioxidant, and anti-inflammatory agent. The phytochemical screening confirmed the presence of numerous biomedically relevant secondary metabolites with high polyphenolic and flavonoid yields. The biosafety study using Sprague Dawley rat models confirmed that *Carpobrotus edulis* is non-toxic with an LD50 above 2000mg/kg. The hydroethanolic extract demonstrated both high antioxidant and anti-inflammatory activities, which were comparable to the standards, ascorbic acid and diclofenac, respectively. It was therefore concluded that the presence of bioactive secondary metabolites in lyophilized extracts of the leaves of *Carpobrotus edulis* possess satisfactory anti-bacterial, anti-inflammatory, and antioxidant profiles. These results thereby support the use of *Carpobrotus edulis* as an adjunct therapy for known biological endpoints of tonsillitis in traditional medical practices in Southern Africa.

**Keywords**: C*arpobrotus edulis*, anti-inflammatory, antibacterial, tonsilitis, polyphenols, secondary metabolites, phytochemicals.

# Introduction

## *Carpobrotus edulis*

*Carpobrotus edulis*, commonly known as *Mukuyukono* by the Shona communities of Zimbabwe is a succulent, ground-cover plant that is used widely in Southern Africa. This plant belongs to the Aizoaceae family and is characterized by its mat-forming growth habit, vibrant foliage, and ability to thrive in saline environment. It is characterized by fleshy green leaves that can store water, making it well-suited for arid environments. The plant has mat-forming leaves and low-lying stems that may reach up to 2m in length. The leaves have margins serrated along the keel and apex sharply pointed. *Carpobrotus* usually flowers in August up to 50mm in diameter of the flowers, with numerous pale yellow petals, turning pinkish as the flowers age with edible fleshy fruits. The traditional use of the leaves for wound, sore, or ulcer healing, makes the extract a promising natural and sustainable option for the management of mouth ulcerations, sore throats, scurvy, and also bacterial tonsillitis, particularly in resource-limited settings. Traditional medicine in various regions has long utilized the *Carpobrotus edulis* plant for its ability to promote ulcer recovery and antibacterial activity. The crude extract is believed to contain many bioactive compounds such as flavonoids, tannins, and phenolic acid which have demonstrated antimicrobial, anti-inflammatory, and wound-healing properties in previous studies [1]

 

Figure 1: Images of C edulis plant, aerial parts, foliage, and fruit

## Tonsillitis

Tonsillitis is an inflammation of the tonsils, which are two lymph nodes located on each side of the back of the throat. It can be a result of viral or bacterial infections, with the most common bacterial cause being *Streptococcus pyogenes*. Tonsillitis typically begins when bacteria enter the body through the mouth or nose. The tonsils act as a first line of defense via a mechanism of trapping these pathogens. The bacteria are agglomerated by the proliferation of lymphocytes, a type of white blood cell, and the release of cytokines, which are signaling proteins that help coordinate the immune response. This activation leads to inflammation, causing the tonsils to swell and become painful. In the progression of the process, symptoms of tonsillitis develop and the most common include sore throat, difficulty swallowing, fever, swollen lymph nodes, and sometimes white or yellow patches on the tonsils. In some cases, the immune system clears the infection, and symptoms subside though it takes some days to recover. However, in most cases, particularly strep throat, antibiotic treatment is necessary to prevent complications such as rheumatic fever or kidney inflammation. Some individuals may experience recurrent episodes of tonsillitis, leading to chronic tonsillitis which results in persistent inflammation and may require surgical intervention, such as a tonsillectomy, to remove the tonsils.

## Antibacterial herbal remedies' role in tonsilitis management

Tonsillitis is an inflammation of the tonsils, commonly caused by viral or bacterial infections. In bacterial tonsillitis, Streptococcus pyogenes adheres to the surface of the tonsils, evading the immune system with virulence factors such as the M protein and various exotoxins, which contribute to tissue damage and inflammation [2]. The symptoms, such as sore throat, fever, and swollen tonsils, are primarily the result of local tissue inflammation and the body's immune response to the infection [3]. Tonsils act as filters, trapping bacteria and viruses that enter the body through the mouth and nose.

In Zimbabwe and much of Africa, tonsillitis is commonly treated with a combination of medical interventions and home remedies. For bacterial tonsillitis, antibiotics are the standard medical treatment. Penicillin or amoxicillin is commonly prescribed, as Streptococcus pyogenes is typically sensitive to these medications [3]. However, the overuse and misuse of antibiotics in many parts of Africa, due to self-medication or inadequate medical supervision, have led to concerns about antimicrobial resistance. In addition to antibiotics, pain relievers such as paracetamol and anti-inflammatory drugs are used to manage symptoms such as fever and throat pain. Relying on oral medications can present challenges for individuals with sore throats, where swallowing is painful [4]. The use of indigenous plants in the treatment of tonsillitis is quite prevalent in Southern Africa and has been for years. Many home remedies including ginger, honey aloe vera, and saltwater gaggling are employed. Although *Carpobrotus edulis* is widely used traditionally to cure many ailments, its bioactivity and safety profiles are not well characterized. The activity of anti-bacterial plants is a consequence of their richness in secondary metabolites including polyphenols. Secondary metabolites are organic compounds produced by plants that are not directly involved in growth, development, or reproduction, but are crucial for environmental adaptation. Compounds such as alkaloids, flavonoids, terpenoids, phenolic acids, and saponins have demonstrated significant anti-bacterial effects through a variety of mechanisms. These include antioxidant activity and inhibition of bacterial growth. Flavonoids are known for their antioxidant properties and have been shown to possess antibacterial activity. Research indicates that flavonoids can disrupt microbial membranes and inhibit enzymes essential for bacterial growth [5]. Phenolic compounds are significant in plant defense and have been associated with antimicrobial properties. They can act by interfering with bacterial cell wall synthesis or by inhibiting crucial metabolic pathways [6]. Tannins are polyphenolic compounds that exhibit astringency and have been documented for their ability to inhibit microbial growth[7]. The antibacterial action of tannins is believed to be due to their ability to precipitate proteins and disrupt microbial cell membrane integrity [8]. Terpenoids, present in significant amounts, have demonstrated antibacterial effects by interacting with bacterial cell membranes, causing leakage of intracellular components and subsequent cell death [9]. The antibacterial effects of *Carpobrotus edulis* leaf extracts can be attributed to the synergistic action of these phytochemicals. For example, flavonoids and phenolic compounds may work together to enhance the permeability of bacterial membranes, making them more susceptible to damage from other components. Furthermore, the high antioxidant capacity of these phytochemicals could limit oxidative stress in host cells while targeting bacterial pathogens. The antibacterial properties of secondary metabolites make them promising candidates for the management of tonsillitis. The present study seeks to investigate the biosafety and bioactivity of the hydroethanolic extract of the aerial parts of *Carpobrotus edulis*, to validate its use in the management of tonsillitis in traditional medicine in Zimbabwe.

## Anti-inflammatory metabolites role in tonsillitis management

Tonsillitis, the inflammation of the tonsils, is primarily caused by viral or bacterial infections, with *Streptococcus pyogenes* being the most common bacterial agent. One of the key features of the disease is inflammation, which plays a central role in both the pathogenesis and symptom development of the condition. The clinical manifestations—pain, swelling, redness, and fever—are direct outcomes of inflammatory responses initiated by the immune system in reaction to the invading pathogens. The inflammatory cascade in tonsillitis begins when pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) on epithelial and immune cells of the tonsils. This recognition activates intracellular signaling pathways, most notably the nuclear factor kappa B (NF-κB) pathway, which regulates the expression of pro-inflammatory genes. As a result, immune cells such as neutrophils, macrophages, and T-cells are recruited to the site, amplifying the inflammatory response. Several pro-inflammatory cytokines have been implicated in the pathology of tonsillitis, including interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). These cytokines not only mediate local inflammation and tissue swelling but also contribute to systemic symptoms such as fever and malaise. Elevated levels of these cytokines in tonsillar tissues and serum have been documented in patients with acute tonsillitis, affirming their role in disease progression. At the molecular level, the inflammation in tonsillitis involves the upregulation of adhesion molecules, chemokines, and matrix metalloproteinases (MMPs), which facilitate leukocyte infiltration and tissue remodeling. Prolonged inflammation can result in fibrosis, crypt hyperplasia, and lymphoid tissue hypertrophy, which in turn may impair normal immune function in the tonsils. Environmental factors, such as exposure to allergens, air pollutants, or cold air, can exacerbate inflammatory responses by priming the immune system or disrupting mucosal barriers. These factors can lead to repeated infections and chronic inflammation, thereby contributing to recurrent tonsillitis [9]. Moreover, certain genetic predispositions and epigenetic modifications have been shown to influence how the body reacts to these environmental triggers, adding complexity to the inflammatory mechanisms. Given these findings, there is growing research interest in targeting inflammation as a therapeutic strategy for managing tonsillitis. Anti-inflammatory drugs, both steroidal and non-steroidal, are commonly prescribed to manage symptoms, yet they may come with adverse effects or contraindications. This has spurred interest in exploring plant-based anti-inflammatory agents, especially in regions with rich traditional medicine systems such as Southern Africa. The growing understanding of the link between inflammation and tonsillitis has spurred interest in anti-inflammatory therapies to manage and prevent the condition, as well as in using inflammatory biomarkers to assess risk for chronic tonsillitis. The identification of potential pathways connecting inflammation to tonsillitis has produced growing interest in targeting inflammation to help manage and control tonsillitis. Aiming to broaden the scope of orally administered anti-tonsillitis agents, the present study was carried out to confirm the bioactivity of lyophilized hydroethanolic aerial extracts of *Carpobrotus edulis* against known biological endpoints associated with tonsillitis conditions including inflammation and oxidative cell damage. The study also serves to evaluate its safety in animal models and to complement earlier studies in different geographical setups on this widespread medicinal plant.

# Materials and methods

## Materials, equipment, and facilities

All chemicals, associated reagents, equipment, and facilities for the *in-vivo* laboratory animal toxicity investigations and the bioactivity assays were obtained from the University of Zimbabwe, Faculty of Medicine and Health Sciences laboratories.

### Animal use approval

Prior to the investigations, animal use and research ethics approvals were obtained from the Joint Parirenyatwa Research Ethics Committee (JREC) which is the local research Institutional Review board for the University of Zimbabwe.

### Plant material collection and preparation

Plant material was collected from the Marondera town of Zimbabwe, 74 km Eastwards from the City of Harare (18.2493° S, 30.8556° E). In observing the rules for Zimbabwe's Sustainable Harvesting of Traditional Medicinal Plants, the material was collected from 5 different plants. The plant material was authenticated as *Carpobrotus edulis* by the National Herbarium and Botanical Garden in Harare, Zimbabwe. The leaves were thoroughly washed using distilled water to remove debris and other contaminants, oven-dried at 40°C to constant weight for 24 hours, and then pulverized using mortar and pestle. The pulverized material was ground into a fine powder using a coffee grinder (Hamilton Beach Coffee Grinder Model- 80410).

The phytoextraction was done by adding 500g plant powder into 1200 ml of 70% (v/v) hydro-ethanolic mixture in a 2-liter sterile amber bottle and macerated for 3 days, with 3 minutes physical shaking twice a day. A muslin cloth was used to obtain a filtrate from the solution, which was further clarified by filtration using Whatman filter paper number 1. The filtrate was then evaporated under vacuum and low pressure (Rotavapor® R-300, Buchi, Switzerland), followed by lyophilization (Lyovapor l-200, Buchi, Switzerland) under 140Pa pressure and -50 °C. The lyophilized extract was stored in an airtight sample bottle, at 4oC in a refrigerator until required.

## Phytochemical Screening of secondary metabolites

In a 200ml round-bottomed flask, 10g of the lyophilized hydro-ethanolic extracts of *Carpobrotus edulis* were dissolved in 100ml of distilled water and subjected to various phyto-screening techniques to confirm the presence or absence of relevant phytoconstituents of pharmacological interest. The following qualitative tests were conducted on the extract liquor.

### Detection for alkaloids by the Iodine test

The Iodine test was used to determine the presence of alkaloids. In this assay, to 3ml of the lyophilized extract solution, a few drops of iodine solution were slowly added along the sides of the test tube. The presence of alkaloids was then identified by the appearance of a blue colour, which disappears on boiling and reappears on cooling [11].

### Detection of tannins by the Braymer’s test

The simplified Braymer's test was used to detect the presence of tannins. To 1ml lyophilized extract solution, 3 drops of a 10% Ferric chloride solution were added. The presence of tannins was confirmed by conversion of the solution to a blue-green colour [12].

### Detection of flavonoids by the Ammonia test

Flavonoids were detected by means of the Ammonia test where 5ml dilute ammonia solution was added to 5ml of the lyophilized solution followed by a few drops of concentrated H2SO4. The emergence of a yellow colour indicates the presence of flavonoids [1].

### Detection of Glycosides by the Keller-Killani test

The presence of glycosides was done by the Keller-Killani test [1]. To 1mL of the lyophilized solution, 1.5mL glacial acetic acid was added and a few drops of 5% ferric chloride were added as well as cons. H2SO4 (along the side of the test tube). The presence of glycosides was confirmed by the emergence of a blue-coloured solution in the mixture acetic acid layer.

### Detection of Phenolic compounds by the Gelatin test

Phenolic compounds were detected using the Gelatin test. In this assay, 2ml of the lyophilized extract solution was added to 5ml of a 1% gelatin solution and 5 drops of 10% NaCl were further added. Phenolics were identified by the appearance of a white precipitate [13].

### Detection of saponins by the simplified foam test

The simplified foam test was used to determine the presence of saponins. In this assay, 2 ml of the extract was added to 20 ml distilled water. The mixture was shaken in a graduated cylinder for 15 minutes. The presence of saponins would be confirmed by the formation of a form with a head height of at least 1 cm [14].

### Detection of anthraquinones by the ammonium hydroxide test

The ammonium hydroxide test was used to determine the presence of anthraquinones. One drop of concentrated ammonium hydroxide was added to 10mg of the extract, previously dissolved in isopropyl alcohol. After two minutes, the formation of a red colour indicated the presence of anthraquinones.

### Detection of terpenoids by the Salkowski test

The Salkowski test was used to determine the presence of terpenoids. To 2ml of the lyophilized extract solution, a few drops of concentrated sulfuric acid was added down the side of the test tube without agitation. Observations were made at the interface between the two layers. The presence of terpenoids was confirmed by the appearance of a reddish-brown color at the interface [15].

### Detection of proanthocyanidins by the ferric chloride test

The ferric chloride test was used to determine the presence of proanthocyanidins. To 1ml of the lyophilized plant extract solution, a few drops of 1% ferric chloride solution were added . The presence of proanthocyanidins was confirmed by the appearance of a dark green color [16].

## Antibacterial Activity Test of *Carpobrotus edulis*

### Test microorganisms

The bacterial species: multidrug-resistant strains ® of gram-positive *Streptococcus pyogenes* and meticillin-sensitive *Staphylococcus Aureus* as well as gram-negative *Escherichia coli* were obtained from the University of Zimbabwe Department of Medical Microbiology in Harare, Zimbabwe. The antibacterial, laboratory evaluations were performed according to CLSI guidelines.

### Determination of the zone of inhibition

A modified, simplified Kirby-Bauer test [17] was configured and used in this study to determine and compare the susceptibility or resistance of the chosen pathogenic bacteria to the crude *Carpobrotus edulis* through the zone of inhibition observations and MIC determination through serial dilution techniques.

### Zone of inhibition MIC test requirements

* Mueller-Hinton agar plates
* Sterile swabs and forceps
* Pure bacterial cultures
* Samples of *Carpobrotus edulis* extract liquor

### Zone of inhibition measurement and MIC determination test protocol

Bacterial inoculum suspensions were spread uniformly on solidified Muller−Hinton Agar (MHA) using a sterile swab [18]. The bacterial strains to confirm the broad-spectrum antibacterial effects of *Carpobrotus edulis* were multi-drug-resistant strains ® of gram-positive *Streptococcus pyogenes* and meticillin-sensitive *Staphylococcus aureus* as well as gram-negative *Escherichia coli*.

In the procedure: To the Mueller-Hinton agar plates, swabs of the pure bacterial cultures were evenly spread over and 2 -3 drops of the test samples were placed in the media plate using sterile forceps.  The petri plates were incubated for 24 hours at 36oC with controlled humidity. After the incubation period and diffusion of the test samples, the clear area (zone of inhibition) around the point of introduction of the samples was observed and measured. The size of the zone of inhibition is directly proportional to the antibacterial activity.

For the determination of MICs, the same experimental setup was used. Serial dilutions of the test materials with distilled water were done and the minimum concentrations of the test materials needed to inhibit the ability of the microorganism's ability to produce any visible growth in the agar plates was noted. In this simplified modified method, the lowest concentration of the antimicrobial agents (in mg/ml) which prevented the appearance of visible growth of the microorganisms within 24 hours was determined as the MIC.

## Anti-inflammatory activity of *Carpobrotus edulis* using the egg albumin denaturation test

The anti-inflammatory activity of the lyophilized leaf extract of *Carpobrotus edulis* was determined using the egg albumin protein denaturation assay, with slight modifications as described by Chifamba *et al* (2024) [19]. The samples and reagents used for this assay include 0.4 mL of egg albumin (fresh) from a free-range domesticated hen (*Gallus domesticus*), 10 mL of phosphate-buffered saline (PBS) at pH 7.2, and 5ml 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. The samples were incubated (Shel lab SRI3 Low Temperature BOD Incubator) for 20 minutes at 37°C followed 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 (UV spectrophotometer, Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments) using the vehicle as blank. Negative controls consisting of 0.4 mL of fresh egg albumin, 0.5 mL of 0.4% DMSO, and 3 mL of PBS were included in the experiment. Diclofenac sodium was used as a positive control for the study at similar concentrations. The percentage of inhibition, which translates to the anti-inflammatory activity of the extracts and standards, was calculated by the following equation.

***Equation 1***

where,

Abs sample = absorbance of sample, Abs control = absorbance of control.

## Acute oral toxicity evaluation

The acute oral toxicity evaluation of *Carpobrotus edulis* lyophilized extract was done using a modified OECD technical guideline 425 (The up and down test) [20]. Female Sprague Dawley rats (24) were used, which were acclimatized to the test environment for 10 days before the commencement of the test protocols. The participating animals were fed with a commercial standardized rodent pellet from Agrofeeds® and were given water *ad libitum*. The animal habitat was kept at an average ambient temperature of 25°C throughout the study with a relative humidity level of 40% and an artificially controlled photoperiod of 12:12-hour light/dark cycle. The animal welfare, observations, and care were supervised by a practicing veterinary officer.

In our investigation, sequentially ordered progressions of doses were orally administered to the animals at 48-hour intervals. The animals were divided into 2 groups of 12 female rats each; the first group (group 1) received distilled water and served as the control group. The second (group 2) received incremental doses of the *Carpobrotus edulis* solution. The selected animals were marked to facilitate individual identification. The experimental animals were fasted for 18 hours with water before dosing. Initial starting doses were chosen based on related toxicological studies. The first animal received a dose of 250mg/kg body weight, which was below a randomly selected estimated LD50. When animals survived the dose, the next dose was doubled, subject to our observations of the test animals over 48 hours. The *Carpobrotus edulis* was orally gavaged in a water solution in 4 different sets of doses of: 250, 500, 1000 and 2000 mg/kg body weight. The female rats were observed by a veterinary specialist for morbidity and mortality twice daily. In the absence of mortality, the rats were observed for any visible changes and clinical signs and symptoms of toxicity every 1 hour, up to 12 hours on day 1, and thereafter, once daily up to a maximum of 14 days. The animals were also weighed daily up to a maximum of 21 days.

# Results and discussion

## Phytochemical screening

Table 1: Qualitative screening of Carpobrotus edulis secondary metabolites

|  |  |  |
| --- | --- | --- |
| Test | Presence in hydro-ethanolic extract | Presence in distilled water extract |
| Phenols | ++ | ++ |
| Flavonoids | +++ | +++ |
| Anthraquinones | +++ | ++ |
| Alkaloids | ++ | + |
| Terpenoids | +++ | + |
| Saponins | + | ++ |
| Glycosides | ++ | ++ |
| Tannins | ++ | +++ |
| Proanthocyanidins |  |  |

*(-): Indicates the absence of the phytochemical*

*(+): Indicates the presence of the phytochemical*

*(++): Indicates moderate presence of the phytochemical*

*(+++): Indicates strong presence of the phytochemical*

From the phytochemical screening protocols, we confirmed the abundance of primary and secondary metabolites of biomedical relevance to tonsillitis (Table 1). In the ethanolic extract, flavonoids, anthraquinones and terpenoids were found to be especially abundant, giving this extract stronger antimicrobial and anti-inflammatory potential. The aqueous extract is particularly rich in flavonoids and tannins. These results correlate with other published studies which identified tannins, flavonoids, glycosides, phenols, and saponins among other compounds in *Carpobrotus edulis* [17]. This proliferation of medically relevant phytoconstituents validates the numerous uses of the plant in traditional medical practice in general, and the management of tonsillitis in particular.

## Anti-inflammatory tests

Table 2: Anti-inflammatory activity of lyophilized Carpobrotus edulis extracts

|  |  |  |
| --- | --- | --- |
| Concentration | % Inhibition | % Inhibition |
| µg/ml | ***Carpobrotus edulis* hydroethanolic extract** | **Diclofenac (standard)** |
| 250 | 15± 0.11 | 39±0.84 |
| 500 | 27.3±0.18 | 65.1±1.78 |
| 1000 | 41.8±0.29 | 82.6±2.44 |
| 2000 | 62.5±1.42 | 93.0.± 2.92 |
| 4000 | 77.9±1.65 | Not Tested |
| 6000 | 86.7±1.98 | Not Tested |
| 8000 | 91.3±2.11 | Not Tested |

At related concentrations, the lyophilized hydroethanolic leaf extracts of *Carpobrotus edulis* exhibited anti-inflammatory effects that were consistently lower than those of the standard Diclofenac, though still significant in a dose-dependent manner (Table 2). The anti-inflammatory activity of *Carpobrotus edulis* became comparable to the lowest dose of the positive control (Diclofenac at 250 µg/mL) at approximately 900–1000 µg/mL. At 8000 µg/mL, *Carpobrotus edulis* showed 91.3 ± 2.11% inhibition of protein denaturation, while 2000 µg/mL of Diclofenac produced 93.0 ± 2.92% inhibition, indicating that the crude extract has near-comparable activity to Diclofenac at higher concentrations. The high activity of *Carpobrotus edulis* at 8000 µg/mL, despite being in crude form, suggests that the plant contains active anti-inflammatory constituents. This activity is expected to increase with further fractionation and isolation of bioactive compounds. The findings from this study suggest that *Carpobrotus edulis* has the potential to play a supportive role in tonsillitis. Tonsillitis involves acute inflammation of the tonsils, primarily triggered by bacterial or viral infections, where excessive production of pro-inflammatory cytokines contributes to symptoms such as pain, swelling, and fever. By targeting inflammatory pathways, *Carpobrotus edulis* may help reduce these symptoms and contribute to the resolution of infection-associated inflammation. The development of standardized, dosage-controlled formulations based on this extract could therefore provide a safe and accessible alternative or adjunct to conventional therapy for tonsillitis.

## Acute oral toxicity evaluation

Table 3: Acute Oral Toxicity Study of C edulis Behavioural Observations

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observed parameter | Dose of *Carpobrotus edulis* in mg/kg body weight | | | | |
|  | **250mg** | **500mg** | **1000mg** | **2000mg** | **Control** |
| Food intake | Normal | Normal | Normal | Normal | Normal |
| Water intake | Normal | Normal | Normal | Normal | Normal |
| Death | Alive | Alive | Alive | Alive | Alive |
| Breathing | Normal | Normal | Normal | Normal | Normal |
| Diarrhea | Not observed | Not observed | Not observed | Not observed | Not observed |
| Urination | Normal | Normal | Normal | Normal | Normal |
| Skin colour | Normal | Normal | Normal | Normal | Normal |
| Drowsiness | Not observed | Not observed | Not observed | Not observed | Not observed |
| Erection of Fur | Not observed | Not observed | Not observed | Not observed | Not observed |

The acute toxicity study was carried out as per OECD technical guideline 425. The observations, results, and interpretation were done by a qualified veterinary expert. Our findings indicated that the extract at doses up to 2000 mg/kg body weight imparted neither visible signs of toxicity nor mortality in rats, suggesting its safety. No animals were withdrawn from the study for any reason during the observation period. Our findings imply that the use of *Carpobrotus edulis* extract in high concentrations to achieve the desired bioactivity effects is relatively safe.

## Rat weights observations

Figure 2: Observed rat weights during acute oral toxicity studies of C edulis

In toxicity evaluations, generally, unexpected fluctuations in body weight are a simple and sensitive reflection of toxicity after exposure of study animals to materials. Progressive Weight loss or gain in animals is usually indicative of stress, failure to feed, or a response to observed or underlying adverse health conditions. In the present study, the lyophilized extracts did not signiﬁcantly affect normal body weight growth during the study period suggesting that the extract did not alter rat growth at the concentrations investigated.

## Anti-bacterial assays

In the antibacterial tests against susceptible common microbes, the lyophilized *Carpobrotus edulis* exhibited considerable antibacterial effects. The lyophilized plant extract was slightly lower in activity against meticillin sensitive and multi drug resistant strains of Staphylococcus aureus when comparable to the commercial drug used as standard. The commercial drug was ineffective against multidrug-resistant strains of Streptococcus pyogenes (Table 4).

Table 4: Antibacterial activity zones of inhibition for Carpobrotus edulis as well as Doxycycline on bacterial strains.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Test Sample | mg/ml |  |  | Inhibition zone in diameter (mm) | | |
|  |  | **Gram-positive bacteria** | | | | **Gram-negative Bacteria** |
|  |  | ***MRSA®*** | ***MSSA*** | | ***S.Pyogenes®*** | ***E.coli®*** |
| *Carpobrotus edulis* | 10 | 18.03±0.7 | 19.24±0.3 | | 12.00±0.5 | 14.20±0.9 |
| Carpobrotus edulis | 20 | 18.24±0.5 | 18.45±0.8 | | 14.50±0.4 | 20.00±0.5 |
| Doxycycline | - | 19.50± 0.8 | 19.26±0.8 | | - | - |
| Control | - | - | - | | - | - |

*All values are mean ± standard deviation of 3 determinations, ® =the multidrug-resistant strain, R =Reference strain (susceptible), S. pyogenes= Streptococcus pyogenes, MSSAR =meticillin-sensitive Staphylococcus aureus, MRSA =meticillin resistant Staphylococcus aureus*

*E.coli =Escherichia coli.*

Table 5: Minimum inhibition concentrations for Carpobrotus edulis as well as doxycycline on multidrug resistant strains

|  |  |  |  |
| --- | --- | --- | --- |
| Bacterial strain | | Minimum inhibitory concentrations mg/ml | |
|  |  | ***Carpobrotus edulis extract*** | **Doxycycline** |
| MRSA® |  | 20 | 6.25 |
| *S.Pyogen®* |  | 10 | - |
| E Coli® |  | 18 | - |

The antibacterial activity of *Carpobrotus edulis* extract was assessed against both Gram-positive and Gram-negative bacteria using the agar well diffusion method. At different concentrations, the extract exhibited measurable zones of inhibition across all tested bacterial strains, indicating a broad-spectrum antibacterial effect. The antibacterial activity of the lyophilized hydro-ethanolic extract obtained at 20mg/ml is much better than those reported in many studies. There were not many differences in antibacterial activities of *Carpobrotus edulis* between the bacterial strains, despite the structural differences in bacterial morphologies between gram-positive and gram-negative types. The increase in zone diameters with rising concentration confirms a dose-dependent response, and the extract’s efficacy, especially against a MSSA and E. coli, suggests the presence of potent antibacterial phytochemicals such as tannins, flavonoids and alkaloids. These compounds are known to disrupt bacterial cell walls, interfere with protein synthesis and inhibit nucleic acid replication.

# Conclusions

The lyophilized hydro-ethanolic extracts of *Carpobrotus edulis* have shown topossess considerable antibacterial and anti-inflammatory activities. The observed activities were attributable to the presence of secondary metabolites including tannins, phenolic compounds, flavonoids, and alkaloids. These contribute to the underlying mechanisms behind the plant’s proven antibacterial effects and therapeutic activities in tonsillitis*. C.edulis* was nontoxic at 2000mg/kg. Our biosafety and bioactivity studies therefore authenticate the use of *Carpobrotus edulis* as a potential antibacterial remedy in traditional medicine.

# Disclaimer (Artificial intelligence)

Author(s) 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.

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