***Original Research Article***

***Validation of biosafety and activity of Albizia antunesiana lyophilised leaf extracts***

**Abstract**

*Albizia antunesiana,* from the *Fabaceae* 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, particularly in the management of acute and chronic wounds. The use of *Albizia antunesiana* in traditional medicine has never been scientifically validated for its biosafety and activity. Our current study aimed to validate its biosafety and the pharmacological activity of the plant to ensure therapeutic efficacy while minimizing potential risks. Our investigations qualitatively and quantitatively determined the secondary metabolites of *Albizia antunesiana*, its activity as an anti-bacterial and anti-inflammatory agent as well as assessing the toxicity profile of the lyophilised leaf extract. The phytoscreening was done using various classical wet chemistry techniques. The biosafety study was based on an amended OECD guideline 425, using Sprague Dawley rat models. The anti-bacterial evaluations were done using a modified Kirby-Bauer test (disk diffusion test) on *Pseudomonas aeruginosa*, methicillin-sensitive *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae* with doxycycline as a standard*.* The egg albumin test was used to determine the anti-inflammatory potential of the plant. The phytoscreening assays confirmed the presence of numerous biomedically relevant secondary metabolites with high polyphenolic and flavonoid yields. The antibacterial tests demonstrated high antibacterial activity with an MIC of 300mg comparable to 10mg doxycycline and the anti-inflammatory activity at 8000mg/ was comparable to the standard diclofenac at 2000mg. The toxicity tests showed an LD50 above 2500mg/kg body weight. It was therefore concluded that the presence of bioactive secondary metabolites in lyophilized extracts of the aerial parts of *Albizia antunesiana* possess significant anti-inflammatory and antioxidant profiles. The extracts were also toxicologically safe according to the Hodge and Sterner toxicity scale, thereby supporting their continued use as an adjunct therapy in traditional medical practices in Southern Africa.

**Key words**: *Albizia antunesiana*, ~~anti-inflammatory, wounds, polyphenols,~~ secondary metabolites

# Introduction

## Albizia antunesiana

*Albizia antunesiana* (*A. antunesiana*) is a medium to large deciduous tree with a spreading crown, growing up to a height of 18m which belongs to the *Fabaceae* family. Known locally as *muriranyenze* in Zimbabwe due to its popularity with cicada insects, the tree bark varies, it may either be rough or smooth with grey to brown colours. The leaf colours vary as well from purple to green usually occurring as 1 to 3 pairs of pinnae, each bearing 3 to 8 large, ovate leaflets (up to 25 mm long) with a pale undersurface. It has been observed that it is mostly the young trees that sometimes display purple leaves[1]. In early spring to early summer, the tree bears large, showy, half‑spherical, fluffy inflorescences composed of 1 to 2 bisexual central flowers that are creamy white [2].The plant fruits are flat, light brown pods, reaching up to 230mm long, which ripen from autumn through spring (April to September) [1]. *A. antunesiana* grows in deciduous woodland and wooded grassland at elevations ranging from 900 to 1700m, preferring sunny positions on loamy soils. In Zimbabwe, the species is more densely distributed in the eastern highlands compared to other regions [1][2].

In traditional Zimbabwean medicinal practices, *A. antunesiana* plays important roles in various treatment protocols. The bark extracts are used as a remedy for constipation, the leaf extract serves as a purgative, and a preparation from the root extract is taken to alleviate diarrhoea and gonorrhoea [2]. Moreover, the roots, bark, and leaves are widely employed in treating skin disorders, wounds, colds, and certain sexually transmitted diseases, while the plant also serves as an aphrodisiac for both men and women[2]. Externally, the roots are applied to swollen legs, cuts, sore eyes, ulcers, and pneumonia, and they have shown significant *invitro* anthelminthic activity against the rat tapeworm (the agent of hymenolepiasis) as well as against trematodes responsible for schistosomiasis. Additionally, the plant is used for culinary purposes with young leaves being cooked with groundnuts, soda, and gravy and eaten as a vegetable [2].



Figure 1: Images of *A. antunesiana plant*, aerial parts, foliage and fruit

## Secondary metabolites, antibacterial effects

The management of burn wounds presents a significant challenge, particularly in resource-limited settings where access to advanced medical care is often restricted. Complications such as bacterial infections, delayed healing, and scarring are common, underscoring the need for effective and affordable treatment options. Traditional medicine, with its rich history of utilizing indigenous plants, offers a promising avenue for developing novel therapeutic agents [1]. The *Fabaceae* plant genus has numerous sub species members widely used in traditional healing practices around the world. Most investigated sub species members used in medicinal practices possess a diverse array of secondary metabolites that contribute to their potential efficacy in wound treatment. The bioactivity of plant extracts in general is primarily attributed to the abundance of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and terpenoids [3]. These compounds, while not directly involved in the plant's growth and development, play crucial roles in its defense and adaptation to the environment. Flavonoids, for instance, are known for their potent antioxidant and anti-inflammatory properties, which can accelerate wound healing by reducing oxidative stress and inflammation [4]. They also exhibit antimicrobial activity by disrupting bacterial cell membranes and inhibiting essential enzymes such as DNA gyrase [5]. Tannins, another group of phenolic compounds, exert antibacterial effects by precipitating bacterial proteins and disrupting cell membrane integrity [6]. This protein-binding capacity can also aid in wound healing by forming a protective layer over the damaged tissue. Saponins, which are glycosidic compounds, demonstrate antimicrobial properties by interacting with bacterial cell membranes, causing leakage of cellular contents and ultimately leading to cell death [7]. Additionally, saponins can enhance the permeability of other antimicrobial compounds, thereby increasing their efficacy. Alkaloids, known for their diverse biological activities, can inhibit bacterial DNA replication and protein synthesis [8]. Terpenoids, including triterpenoids and steroids, exhibit antibacterial activity by disrupting bacterial cell membrane function and inhibiting essential bacterial enzymes [9]. In various structured studies, secondary metabolites in plantshave demonstrated potent inhibitory effects against common wound pathogens like *S. aureus* and *P. aeruginosa*, as well as *S. aureus*, another common cause of skin and soft tissue infections due to the membrane-disrupting effects of flavonoids, saponins, and terpenoids [10]. *P. aeruginosa*, known for its resistance to many antibiotics, is also vulnerable to the combined effects of these metabolites, particularly tannins and alkaloids, which can interfere with its quorum sensing and biofilm formation [11]. The synergistic action of these secondary metabolites in plants provides a multi-targeted approach to combating bacterial infections, making it a promising candidate for wound healing applications. By targeting multiple bacterial pathways, these compounds can reduce the likelihood of resistance development, a critical advantage over single-target antibiotics. Furthermore, the anti-inflammatory and antioxidant properties of these metabolites can promote tissue regeneration and accelerate wound closure. Several studies have demonstrated the efficacy of plant-derived compounds in wound healing, highlighting their potential as alternatives to conventional treatments [12].

## Secondary metabolites, anti-inflammatory activity

Inflammation is a critical factor in the pathogenesis of various wound complications, including delayed healing and infection [13]. In the context of burn wounds, uncontrolled inflammation can lead to tissue damage, increased pain, and impaired recovery. This underscores the importance of targeting inflammatory pathways to promote effective wound healing [14]. Pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 play pivotal roles in the inflammatory cascade, contributing to tissue destruction and hindering the regenerative process [15]. These cytokines are secreted by immune cells infiltrating the wound site, leading to increased vascular permeability, recruitment of inflammatory cells, and the release of reactive oxygen species (ROS) [16].In burn wounds, the initial inflammatory response is essential for clearing debris and initiating tissue repair. However, a prolonged or excessive inflammatory response can impede healing by causing further tissue damage and preventing the formation of new tissue [17]. This is where the anti-inflammatory properties of plant-derived secondary metabolites become particularly relevant.

Secondary metabolites such as flavonoids, terpenoids, and phenolic compounds have demonstrated significant anti-inflammatory effects through various mechanisms [18]. These compounds can inhibit the production and release of pro-inflammatory cytokines, modulate the activity of inflammatory enzymes, and scavenge ROS, thereby reducing oxidative stress [19] Flavonoids, for instance, are known to inhibit the activity of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, which are involved in the synthesis of prostaglandins and leukotrienes, respectively, key mediators of inflammation [4]. By inhibiting these enzymes, flavonoids can reduce inflammation and alleviate pain. Terpenoids, such as triterpenoids and sesquiterpenes, have also been shown to possess anti-inflammatory properties by inhibiting the production of TNF-α and IL-1β [20]. Phenolic compounds, including tannins and phenolic acids, can scavenge ROS and inhibit the activation of nuclear factor-kappa B (NF-κB), a key transcription factor involved in the regulation of inflammatory gene expression [21]. The ability of these secondary metabolites to modulate multiple inflammatory pathways makes them promising candidates for the treatment of burn wounds. By reducing inflammation, these compounds can create a more favorable environment for tissue regeneration and accelerate wound closure. Furthermore, their antioxidant properties can protect cells from oxidative damage, which is a common consequence of burn injuries [22]. The identification of potential pathways connecting inflammation to wound healing has generated significant interest in targeting inflammation to improve wound outcomes. The present study aims to develop an efficacious, safe, and stable herbal hydrogel incorporating Albizia antunesiana extract, rich in anti-inflammatory secondary metabolites, for the potential treatment of burn wounds. This approach seeks to validate the traditional use of *A. antunesiana* and provide a scientifically supported alternative for wound management.

# 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.

### Albizia antunesiana Plant material collection and preparation

The plant material used in this study originated from the Mberengwa region of Zimbabwe (18.2493° South and 30.8556° East). Adhering to the established guidelines for the sustainable harvesting of traditional medicinal plants within Zimbabwe, samples were collected from five distinct individual plants. The collected plant material was subsequently authenticated as Albizia antunesiana by the National Herbarium and Botanical Garden situated in Harare, Zimbabwe. To prepare the material for extraction, the leaves underwent a thorough washing process using distilled water. Following this, the washed leaves were subjected to a shade-drying process at ambient room temperature, until a consistent weight was achieved over a period of three weeks. The dried leaves were then initially pulverized using mortar and pestle and subsequently ground into a fine powder using a Hamilton Beach Coffee Grinder, model 80410, to ensure a uniform consistency for subsequent analyses.

Using methods described by Chifamba *et al* (2024) [23], phyto-extraction process was conducted using 500 grams of the prepared plant powder, which was combined with 1200 millilitres of a 70% volume/volume hydro-ethanolic solution within a sterile, 2-liter amber bottle. This mixture underwent a maceration period of seven days, during which it was subjected to manual shaking for three minutes, twice daily, to enhance the extraction efficiency. The resulting solution was then filtered through a muslin cloth to remove coarse particulate matter, followed by a secondary filtration step using Whatman filter paper number 1 to achieve further clarification. The filtrate was subsequently concentrated via evaporation under vacuum and low pressure, utilizing a Rotavapor® R-300 (Buchi, Switzerland). Following this, the concentrated extract underwent lyophilization using a Lyovapor l-200 (Buchi, Switzerland) at a pressure of 140 Pascals and a temperature of -50 degrees Celsius, effectively removing residual moisture. The resulting lyophilized extract was then carefully stored in an airtight sample bottle, maintained under appropriate conditions, until required for further analyses.

## Phytochemical Screening of *Albizia antunesiana*

Phytochemical screening was done as per methods described by Chifamba *et al* (2024) [23]. 10 grams of the lyophilized hydro-ethanolic extract of *Albizia antunesiana* were dissolved in 100 millilitres of distilled water within a 200-milliliter round-bottomed flask. This solution was then subjected to a series of qualitative phytochemical screening techniques, designed to identify the presence or absence of phytoconstituents known to possess pharmacological relevance. The following tests were performed on the resulting extract solution.

### 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[23].

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

The simplified Braymer’s test was used to detect the presence of tannins. To 1ml lyophilised 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 [23].

### 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 lyophilised solution followed by a few drops of conc. H2SO4. The emergence of a yellow colour indicates the presence of flavonoids [23].

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

The presence of glycosides was done by the Keller-Killani test. To 1 millilitre of the lyophilised solution, 1.5mL glacial acetic acid was added and a few drops of 5% ferric chloride were added as well as conc. H2SO4 (along the side of test tube). The presence of glycosides was confirmed by the emergence of a blue coloured solution in mixture acetic acid layer [23]

### Detection of Phenolic compounds by the Gelatin test

Phenolic compounds were detected using the Gelatin test. In this assay, 2 millilitre the lyophilised extract solution was added to 5ml of a 1% gelatin solution and 5 drops of a 10% NaCl were further added. Phenolics were identified by the appearance of a white precipitate [23].

### Detection of saponins by the simplified foam test

The simplified foam test was used to determine the presence of saponins. In this assay 2ml of the extract was added to 20ml distilled water. The mixture was shaken in a graduated cylinder for 15 minutes. The presence of saponins would be confirmed by the formation of form with a head height of at least 1cm [23].

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## Anti-inflammatory activity of *Albizia antunesiana* using the egg albumin denaturation test

The anti-inflammatory activity of the lyophilised leaf extract of *A. antunesiana* was determined using the egg albumin protein denaturation assay, with slight modifications as described by Chifamba *et al* (2024) [23]. 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 lyophilised leaf extracts in 0.4% DMSO. The concentrations of the lyophilized extracts in the total reaction solution ranged from 250 to 8000 µg/mL. The samples were incubated (Shel lab SRI3 Low Temperature BOD Incubator) 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 (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***

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

## Acute oral toxicity evaluation of *Albizia antunesiana*

The acute oral toxicity evaluation of *A. antunesiana* lyophilized extract was done using a modified OECD technical guideline 425 (The up and down test) [25]. Female Sprague Dawly (24) were used, which were acclimatized to the test environment for 10days prior to 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-h light and 12-h darkness. The animal welfare, observations and care were supervised by a practicing veterinary officer.

In our test, sequential 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 *Albizia antunesiana* solution. The selected animals were marked so as to facilitate individual identification. The experimental animals were fasted for 18 hours with water prior to 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 a period of 48 hours. The *Albizia antunesiana* was orally gavaged in a water solution in 4 different sets of doses of: 250, 500, 1000 and 2500 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, and up to 12 hours on day 1, and thereafter, once daily up to a maximum of 14 days. The animals were also weighed daily.

## Antibacterial Activity Test of *Albizia antunesiana*.

### Test microorganisms

Bacterial strains, including *Pseudomonas aeruginosa*, methicillin-sensitive *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae*, were obtained from the University of Zimbabwe's Department of Medical Microbiology in Harare, Zimbabwe. Antibacterial evaluation was conducted following Clinical and Laboratory Standards Institute (CLSI) guidelines.

A modified Kirby-Bauer test was employed to determine the susceptibility or resistance of these pathogenic bacteria to *Albizia antunesiana* extract. This involved observing zones of inhibition and determining the minimum inhibitory concentration (MIC) through serial dilution techniques. The test setup required test tubes, cuvettes, sterile swabs and forceps, pure bacterial cultures, and samples of the *Albizia antunesiana* extract.

Initial tests, aimed at confirming the broad-spectrum antibacterial effects of *Albizia antunesiana*, utilized *Pseudomonas aeruginosa, Streptococcus species*. Subsequent evaluations incorporated multi-drug-resistant *Streptococcus pyogenes*, methicillin-sensitive *Staphylococcus aureus, Escherichia coli,* and *Klebsiella pneumoniae*.

In the procedure: sterile nutrient broth was prepared in the laboratory. Bacterial colonies from the agar plate were suspended in the sterile nutrient broth solution. A UV-Vis spectrophotometer was then used to measure the turbidity of the bacterial suspension, with the spectrophotometer adjusted to a wavelength of 600 nm. A cuvette filled with the sterile nutrient broth was inserted into the spectrophotometer, and the absorbance value of the broth was used as the blanking value , the optical density (OD) value of the broth solution containing the bacterial suspension was measured, and the OD value was found to be higher than 1, drops of the sterile broth solution (without bacteria) was added and the new OD value was measured with the suspension being mixed well after each adjustment. These steps were repeated until the OD value of the bacterial suspension fell within the desired range that is between 0.95 and 1, for a standardized bacterial inoculum, and the final OD value of the standardized bacterial suspension was then recorded.

For the determination of MICs the same experimental set up was used. Serial dilutions of the test materials with distilled water was 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 test tubes was noted. In this simplified modified method, the lowest concentration of the antimicrobial agents (in µg/ml) which prevented the appearance of visible growth of the microorganisms within a 24-hour period were determined as the MIC.

# Results and discussion

## Phytochemical screening

From the phytochemical screening protocols, our investigations confirmed the abundance of primary and secondary metabolites of biomedical relevance with regards to wound healing and antibacterial activity (Table 1). The compounds with the strongest presence in the plant were phenolics (especially flavonoids), phytosterols and tannins. These results correlate with studies by Ibrahim [24]~~.~~ and Samantha et al. who identified terpenes, tannins, flavonoids, benzenoids, phenylpropanoid, lignans, sesquiterpenes, and coumarins among other compounds in *A. antunesiana.* The presence of proteins also correlates with observations by Ibrahim [24], where *A. antunesiana* exhibited varying protein content, potentially contributing to tissue repair and antibacterial defence. This proliferation of medically relevant phytoconstituents validates the numerous uses of the plant in traditional medical practice, particularly in the management of burn wounds and infections. The presence of these compounds suggests potential antibacterial and wound-healing properties, which are crucial for treating burn injuries.

Table : Qualitative screening of Albizia Antenusiana secondary metabolites

|  |  |  |
| --- | --- | --- |
| Test | Presence in hydro-ethanolic extract | Presence in distilled water |
| Alkaloids | ++ | + |
| Phytosterols | +++ | + |
| Flavonoids | +++ | ++ |
| Saponins | - | - |
| Proteins and Amino Acids | ++ | + |
| Fixed oils and fats | - | - |
| Phenolic compounds | +++ | + |
| Tannins | +++ | + |
| Carbohydrates | ++ | + |
| Glycosides | + | - |
| Triterpenoids | ++ | + |

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

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

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

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

## Anti-inflammatory tests

Table : Anti-inflammatory activity of lyophilised Albizia antunesiana extracts

|  |  |  |
| --- | --- | --- |
| Concentration | % Inhibition | % Inhibition |
| µg/ml | 1. **antunesiana**   **hydroethanolic extract** | **Diclofenac(standard)** |
| 250 | 14± 0.08 | 39±0.84 |
| 500 | 18±0.03 | 65±1.78 |
| 1000 | 75±0.28 | 302±3.40 |
| 2000 | *265±1.76* | *760.12± 4.20* |
| 4000 | 386±1.34 | Not tested |
| 6000 | 488±3.56 | Not tested |
| 8000 | 716±3.54 | Not tested |

At related concentrations, the lyophilised extracts from the aerial parts of *Albizia antunesiana* exhibited anti-inflammatory effects that are approximately a third of the anti-inflammatory attributes of the standard Diclofenac. The anti-inflammatory activity of *Albizia antunesiana* became comparable to the least dose of the positive control Diclofenac (250 µg/ml) used in this study at approximately around 750µg/ml. The anti-inflammatory effect of 2000 µg/ml of the positive control Diclofenac was approximately 6% greater than that for 8000 µg/ml lyophilised extracts of *Albizia antunesiana.* The value of 8000µg/ml recorded in our protein denaturation assay represents significant anti-inflammatory potential *of Albizia antunesiana*, since the extract was used in its crude form. Activity is expected to increase further using bioactive fractions and isolated compounds. Phytosterols, triterpenoids , alkaloids, and flavonoids have been associated with anti-inflammatory activity. Saponins, which are normally found in abundance in the plant, have also shown anti-inflammatory activity in addition to antioxidant capacity. Saponins were absent from our extract (Table 1), possibly as a result of geographical and environmental influences. ~~However, our findings suggest that by targeting inflammation pathways,~~ *~~Albizia antunesiana~~* ~~can potentially contribute to wound healing and reduce inflammation associated with burn injuries. Furthermore, the demonstrated anti-inflammatory activity, combined with the presence of antibacterial~~ compounds such as flavonoids, terpenes and tannins, suggests that *Albizia antunesiana* may provide a valuable therapeutic approach to managing burn wound infections and promoting tissue repair.

## Acute oral toxicity evaluation

Table : Acute oral toxicity study of Albizia antunesiana

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observed parameter | Dose of *Albizia antunesiana* in mg/kg body weight | | | | |
|  | **250mg** | **500mg** | **1000mg** | **2500mg** | **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 |
| Diarrhoea | 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 |

An acute oral toxicity study, conducted according to OECD guideline 425, assessed the safety of *Albizia* *antunesiana* extract in rats. The study, overseen by a qualified veterinary expert, administered a single dose of the extract, up to 5000 mg/kg body weight. No visible signs of toxicity or mortality were observed in any of the rats throughout the study, and no animals were withdrawn. These findings align with previous research by Ya’u et al., which estimated the LD50 of the extract to be greater than 5000 mg/kg. According to Loomis and Hayes [26] substances with LD50 values between 5000 and 15,000 mg/kg are considered practically non-toxic. Therefore, the *Albizia antunesiana* extract is classified as practically non-toxic. This suggests that higher extract concentrations can be used for burn wound treatments without significant systemic toxicity. Further analysis is needed to identify the specific phytocompounds present, as indicated in Table 1. This safety profile is particularly important for topical applications, minimizing the risk of systemic toxicity while maximizing therapeutic potential.

## 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 of animals is usually indicative of stress, failure to feed or a response to observed or underlying adverse health conditions. In the present study, the lyophilised 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 lyophilised *Albizia antunesiana* exhibited considerable antibacterial effects. The results of the antimicrobial test show that 300mg/ml is the minimum inhibitory concentration of *Albizia antunesiana* as compared to Doxycycline 10mg control. Therefore, *Albizia antunesiana* can be considered for use in the potential management of microbial growth in burn wounds.

Table 4: Minimum Inhibitory Concentration results for A. antunesisana

|  |  |  |
| --- | --- | --- |
| Test Tube | Extract Concentration | Visibility |
| 1 | 300mg/ml (100%) | transparent/clear |
| 2 | 150mg/ml (50%) | transparent/clear |
| 3 | 75mg/ml (25%) | transparet |
| 4 | 37.5mg/ml (12.5%) | opaque |
| 5 | 18.75mg/ml (6.5%) | opaque |
| 6 | 9.38mg/ml (3.25%) | opaque |
| 7 | 4.69mg/ml (1.56%) | opaque |
| 8 | Broth (negative control) | opaque |
| 9 | Doxycycline (positive control) | transparent/clear |

# Conclusions

The aerial lyophilised hydro-ethanolic extracts of *A. antunesiana* were shown topossess considerable antibacterial and anti-inflammatory activities. The observed activities were attributable to the presence of secondary metabolites including phenolic compounds, flavonoids, and phytosterols. These contribute to the anti-inflammatory and antibacterial activities needed in the management of burn wounds. *A.antunesiana* was nontoxic at 2500mg/kg. Our biosafety and bioactivity studies therefore authenticate *Albizia antunesiana’s* potential use in the management of burn woundsin traditional medicine.

# 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.

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