***Original Research Article***

**Bioactivity and Safety of hydro-methanolic Lyophilised *Solanum incanum***

**Abstract**

*Solanum incanum*, a medicinal plant from the *Solanaceae* family, thrives in tropical and subtropical regions, particularly in arid environments. It has been widely used in traditional medicine, mainly for treating infections, inflammation, and wound healing. Despite its extensive use, there is limited scientific validation of its medicinal properties which therefore necessitates the exploration of its bioactivity and safety profile for modern therapeutic applications. This study investigated the pharmacological potential and safety of hydro-methanolic lyophilised *S. incanum* extract. Classical metabolomic test methods were used to determine the phytoconstituents of *S. incanum*, as well as its activity as an antioxidant and anti-inflammatory agent. Phytochemical screening confirmed the presence of key bioactive compounds such as alkaloids, saponins, phenolics and flavonoids and the hydro-methanolic extract yielded the highest phenolic content. Antioxidant evaluation using DPPH and FRAP assays demonstrated notable radical scavenging activity of 93.4% at 150 µg/mL comparable to ascorbic acid. The extract also displayed strong antimicrobial effects *against Staphylococcus aureus and Escherichia coli*, as well as antifungal activity. Furthermore, anti-inflammatory properties were assessed and confirmed using the egg albumin model. The anti inflammatory inhibition was 729±3.54 % at 8000 µg/ml comparable to 2000 µg/ml Diclofenac. Acute oral toxicity testing was done using OECD TG 425 (the up and down method) in Sprague Dawley rats. The results confirmed its safety as no mortality or adverse behavoural effects were observed at dosages up to 2000 mg/kg. The strong bioactivity observed in *S. incanum* due its high phenolic content supports its traditional use in wound care and healing. These findings suggest that *S. incanum* is a safe and effective natural medicine for wound management, warranting further investigation into its clinical applications.

**Key words**: cytotoxic, *Solanum incanum*, phytochemical screening, antioxidant activity, antimicrobial, safety profile

# Introduction

## *S. incanum*

*S. incanum* Linn is an annual sub-shrub which his commonly known as thorn apple and it belongs to the Solanaceae family1. *S. incanum*, commonly known as the "bitter apple" or "Sodom apple," is a perennial shrub native to arid and semi-arid regions. It belongs to the Solanaceae family, sharing its lineage with eggplants and tomatoes. It is a perennial plant often cultivated as an annual crop. It grows up to 3m high with simple leaves, ovate, elliptic, 2.5–12 cm long and 2.5 8 cm wide2. The fruit is fleshy and is approximately 3 cm in diameter on wild plants but much larger in cultivated forms. Botanically the fruit is classified as a berry and contains numerous small, soft seeds which are edible, but are bitter because they contain an insignificant amount of nicotinoid alkaloids3.The plant features thorny, woody stems and large, ovate leaves with a wavy edge, often covered in fine bristly hairs. Its striking purple flowers have a star-like shape with a yellow central cone of anthers, adding a pop of color to its surroundings. The round fruits, which turn yellow or orange when ripe, are toxic and unfit for consumption. *S. incanum* is well-adapted to harsh environments, for example, its deep-rooted structure that allows it to survive in dry and nutrient-poor soils. The plant is widely spread and abundant in overgrazed range areas or road-sides of Ethiopia2. It is also well-known in Uganda, Tanzania, South Africa, Senegal, and Zimbabwe4. Different parts of it have many traditional uses in the management of pain and skin problems including infections5. *S. incanum* has well reported wound healing properties in folk medicine but partially evaluated scientifically6.



Figure 1: Images of S Incanum plant, aerial parts, foliage and fruit

## Traditional and ethnobotanical uses

Amongst medicinal plants used in folk medicine, *S. incanum* has been widely used for the management of different infections4. *S. incanum* L. is used throughout tropical Africa to treat: sore throat, stomach-ache, head-ache, painful menstruation, liver pain and pain caused by onchocerciasis, pneumonia and rheumatism2. In folk medicine, its medicinal properties have been harnessed through consuming leaf, root, and fruit decoctions, chewing and swallowing root saps4,2. External applications involve leaf paste, root infusions, and pounded fruits rubbed into scarifications. Leaf sap is also used to wash painful areas, while ash from burnt plants mixed with fat is applied topically for pain relief4. For dental issues including toothache, a root infusion serves as a mouthwash and fruits or roots are rubbed onto the gums as well as the smoke from burning seeds is inhaled for pain management4. The plant is also widely used in treating venereal diseases and skin-related conditions such as infections, ringworm, burns, sores, rashes, wounds, ulcers, warts, and benign tumors4. Additionally, various plant parts are used to address gastrointestinal disorders, snake bites, chest pain, tonicities, and skin wounds in cattle, with leaves, fruits, roots and seeds boiled in butter for stomach ailments7. The ethnobotanical use of *S. incanum* differs from region to region2. In Uganda, Tanzania, and South Africa extracts of leaves or flowers serve as ear drops for inflammation4. In Senegal, Kenya, Uganda, and Zimbabwe, different plant parts are used for snakebite treatments, including decoctions, chewing roots and swallowing sap, and applying pulped roots or young leaves directly onto the wound8. In Ethiopia, fruit sap is combined with butter to control cattle ticks, while boiled fruits are utilized as soap and in leather tanning8.

## Antibacterial secondary metabolites

The overuse and misuse of antibiotics have led to the emergence of antimicrobial resistance (AMR), resulting in multidrug-resistant bacterial strains that render even the most potent drugs ineffective. This growing crisis highlights the limited lifespan of antibiotics, prompting urgent attention from the scientific community. In response, millions worldwide are increasingly relying on phytomedicine as a primary alternative for managing chronic diseases. Bacterial infections, particularly those caused by Staphylococcus aureus and Escherichia coli, frequently complicate wound healing9. Plant-derived extracts play a vital role in combating these infections, offering a natural defense against globally prevalent pathogens. Key bioactive compounds such as alkaloids, phenolics, terpenoids, essential oils, and polypeptides exhibit antimicrobial properties, making them potential adjuvants or replacements for conventional antibiotics10. Plants like *S. incanum* and hypericum perforatum are gaining research interest due to their rich phytochemical profiles. Beyond antimicrobial effects, bioactive substances such as probiotics, polysaccharides, and fatty acids can modulate immune responses and alleviate inflammatory skin conditions, including atopic dermatitis (AD) and diabetic foot ulcers (DFUs). Natural compounds with antioxidant, anti-inflammatory, and anticarcinogenic properties also help prevent skin damage. Biopolymers (e.g., chitosan, collagen, hyaluronic acid) and extracellular matrix (ECM) components demonstrate promising wound-healing capabilities due to their antimicrobial, immunomodulatory, and tissue-regenerative properties10. Medicinal plants thus represent an untapped reservoir of bioactive agents with therapeutic potential against bacterial infections and other ailments. However, many natural compounds remain understudied, necessitating further research to isolate and characterize novel molecules to address AMR. Future studies should also explore synergistic interactions between plant-derived compounds and antibiotics to enhance treatment efficacy. Through deepening an understanding of these natural resources, researchers can develop innovative strategies to combat resistant infections and improve wound care.

## Anti-inflammatory role in wound healing

Effective wound healing relies on the proper activation and recruitment of inflammatory cells, including neutrophils and macrophages, which release pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1)11,12. These cytokines stimulate the production of growth factors like transforming growth factor-β (TGF-β) and fibroblast growth factors, promoting fibroblast proliferation and migration to the wound site. However, this natural healing process can be disrupted by factors such as aging, obesity, infections, and endocrine disorders like diabetes mellitus13. While balanced cytokine levels are crucial for proper healing, excessive inflammation can lead to abnormal fibroblast activity, resulting in hypertrophic scarring and skin disfigurement12. In recent years, there has been increasing interest in phytochemicals and plant-based traditional therapies, both in developing and developed nations. Although all therapeutic approaches whether synthetic or natural—should be valued, the rising demand for phytochemical-based treatments necessitates further scientific investigation12. Emerging evidence suggests that topical phytochemicals may enhance healing in both acute and chronic wounds12,14. These findings highlight the potential of plant-derived compounds in improving wound repair while minimizing excessive scarring.

# 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, Pharmacy and Pharmaceutical Sciences Department.

### 2.1.2 S. incanum Plant material collection and preparation

*S. incanum (Solanaceae)* leaves were collected from Rusape in Makoni District (19.0188° S, 32.3689° E), during the month of November-December 2024 after carefully examining the location’s surrounding. Samples of the collected plants were taken to the National Herbarium and Botanical Garden for authentication where the sample were authenticated and identified as *Solanum Compylacanthum ‘incanum* type’ by Mr B. Ndlovu, a Research Officer. The leaves were thoroughly washed using clean water to remove debris and other contaminants, shade dried at room temperature to constant weight for three weeks, and then pulverized using mortar and pestle. The material was grounded into a fine powder using a coffee grinder (Hamilton Beach Coffee Grinder Model- 80410).

For phytochemical extraction, 500 g of powdered plant material was macerated in 1200 mL of 70% (v/v) hydroethanolic solution in a sterile 2-liter amber bottle. The mixture was subjected to maceration 5 days with intermittent shaking (3 minutes, twice daily). Primary filtration through muslin cloth, followed by secondary clarification using Whatman No. 1 filter paper. Then the filtrate was concentrated via rotary evaporation using (Rotavapor® R-300, Büchi, Switzerland) under reduced pressure and lyophilized usung (Lyovapor L-200, Büchi, Switzerland) at −50°C and 140 Pa pressure. The resulting freeze-dried extract was stored in airtight containers at 4°C until further analysis.

## Phytochemical Screening of S*. Incanum*

In a 200ml round bottomed flask, 10g of the lyophilized hydro-methanolic extracts of *S. incanum*  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 Wagner's test

Wagner's test was used to investigate the presence of alkaloids. A few drops of Wagner's reagent were added along the sides of a test tube to 3mls of the *S. incanum* extract. Formation of a brown precipitate confirmed the presence of alkaloids15.

### Detection of tannins by the Ferric chloride test

To 2mls of the *S. incanum* extract, 3 drops of 10% ferric chloride solution were added and a blue- green colour confirmed the presence of tannins16.

### Detection of flavonoids by the Ammonia test

An ammonia test was performed. To 3mls of *S. incanum* extract solution, 3mls of dilute ammonia solution was added followed by a few drops of concentrated sulphuric acid. A yellow colour confirmed the presence of flavonoids17.

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

Keller- Killani test was performed. To 1ml of *S. incanum* extract, 1.5ml of glacial acetic acid was added followed by 1 drop of 5% ferric chloride and concentrated sulphuric acid along the side of the test tube. Emergence of a blue coloured solution was observed which confirmed the presence of glycosides18.

### Detection of Phenolic compounds by the Gelatin test

A ferric chloride test was done. A few drops of 5% ferric chloride solution was added to 2ml of the *Solanum* incunum extract. A bluish back color confirmed the presence of phenols19.

### Detection of saponins by the simplified foam test

The foam test was used to detect the presence of saponins. 2ml of *S. incanum* extract was added to 20ml distilled water. The mixture was shaken in a granulated cylinder for 15minutes. Formations of foam with head height of at least 1cm36 confirmed the presence of saponins20.

### Quantification of Total Phenolic and Tannins content

The total phenolic contents were calculated on the basis of the calibration curve of gallic acid and computed as gallic acid equivalents (GAE), in milligrams per gram of the sample (comparison was made between ethanolic and methanolic extracts in this assay).The total phenolic and tannin content of the lyophilized S. incanum extract was quantified using a modified Folin-Ciocalteu spectrophotometric method21. A stock solution of gallic acid (5 mg/mL) was prepared and serially diluted in 100 mL volumetric flasks with distilled water. For each calibration point, 0.25 mL of the diluted standard was mixed with 1.25 mL of 10-fold diluted Folin-Ciocalteu reagent (1:9 v/v Folin reagent: deionized water) and incubated for 5 minutes. Subsequently, 2 mL of 7.5% sodium carbonate (Na₂CO₃) solution was added, and the final volume was adjusted to 5 mL with deionized water. After 1 hour of incubation at room temperature, absorbance was measured at 760 nm using a UV/Vis spectrophotometer (Lambda 35, Perkin Elmer Instruments). All measurements were performed in triplicate. Calibration curves were constructed to determine phenolic and tannin concentrations in the samples. Total phenolic content was calculated as gallic acid equivalents (GAE) in milligrams per gram of sample, based on the gallic acid standard curve. This assay included comparative analysis between ethanolic and methanolic extracts

### Quantification of total flavonoids

The total flavonoid content of the lyophilised S. *Incanum* was estimated spectrophotometrically at 510 nm. In the test, 1mg of extract was dissolved in 2mL of distilled water. To this solution, 0.5mL of 1M sodium nitrite was added together with 2ml of a 1M, NaOH solution. Distilled water was then added to make up to 10ml volume. The solution was shaken and it stood at room temperature for 15 min and the absorbance was measured. The total flavonoid content was estimated as mg of quercetin equivalent (mg QE/g extract) on a dry weight basis using the standard curve22.

## Anti-inflammatory activity of *S. Incanum* using the egg albumin denaturation test

The anti-inflammatory activity of the lyophilised fruit extract of *S. Incanum* was determined using the egg albumin protein denaturation assay, with a few modifications as described by Chifamba *et al* (2024)23. The samples and reagents used for this assay clude 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 fruit 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; 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 the positive control for the study at similar concentrations. The percentage inhibition, which translates to the anti-inflammatory activity of the extracts and standards, was calculated using the equation:

***Equation 1***

where,

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

## Antibacterial Activity Test.

### Test microorganisms

The bacterial species; *E.Coli,* multi drug resistant strain of gram negative and *Staphylococcus Aureus* gram positive bacteriaa were obtained from the University of Zimbabwe Department of medical microbiology in Harare, Zimbabwe. The antibacterial, evaluation laboratory studies were performed according to Clinical and Laboratory Standards Institute guidelines.

### Determination of the zone of inhibition

A simplified Kirby-Bauer test, (Disk diffusion method) was configured and used in this study to determine and compare the susceptibility of the chosen pathogenic bacteria to the extract of *Solanum* inanum, doxycycline as a positive control and distilled water as a negative control through zone of inhibition observations and MIC determination through serial dilution techniques24.

### *Zone of inhibition MIC test requirements*

* Nutrient agar plates
* Sterile swabs and forceps
* Pure bacterial cultures
* Samples of *S. incanum*,

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

Bacterial inoculum suspensions were spread uniformly on solidified nutrient Agar using sterile swab25. The bacterial strains used in the first study to confirm the broad spectrum antibacterial effects of *S. incanum* were *S.Aureus and E.Coli as well as for* multi drug resistant strains ® of gram positive and gram negative respectively.

In the procedure: To the Nutrient 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 using the streak method. 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 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 agar plates 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.

## Acute oral toxicity evaluation of *S. Incanum*

The acute oral toxicity assessment of *S. incanum* lyophilized extract was conducted following a modified version of the OECD Guideline 425 (Up-and-Down Procedure) 26. Twenty-four female Sprague Dawley rats were acclimatized for 10 days before the experiment under controlled environmental conditions, including a maintained temperature of 25°C, 40% relative humidity, and a 12-hour light/dark cycle. The animals were provided with standard rodent feed (Agrofeeds®) and unrestricted access to water, with their welfare monitored by a veterinary professional throughout the study. The rats were divided into two groups of 12 animals each: a control group receiving distilled water and a test group administered increasing doses of *S. incanum* extract dissolved in water. Prior to dosing, the animals were fasted for 18 hours (with water available) to ensure accurate absorption assessment. The test group received sequential oral doses of 250, 500, 1000, 2500, and 5000 mg/kg body weight at 48-hour intervals, with dose escalation contingent upon survival and absence of toxicity signs in the preceding animal. The initial dose (250 mg/kg) was selected based on prior toxicological data and remained below an estimated LD50 threshold. Following administration, the rats were closely monitored for mortality and morbidity twice daily. Additionally, clinical signs of toxicity were recorded hourly for the first 12 hours post-dosing and once daily thereafter for 14 days. Body weight measurements were taken daily to assess any physiological changes. This systematic approach ensured a thorough evaluation of the extract's safety profile while adhering to ethical guidelines for animal testing.

# Results and discussion

## Phytochemical screening

Table 1 : Qualitative screening of S. Incanum secondary metabolites

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

*(-): 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, the investigations confirmed the abundance of primary and secondary metabolites of biomedical relevance with regards to safety profile of *S. incanum*. The compounds with the strongest presence in the plant were phenolics (especially flavonoids), alkaloids, terpenes and Saponins. These results correlate with studies by . K.E Jepkoech and Gakungawho identified glycosides, tannins, flavonoids, alkaloids, phenolic compounds, saponins, and steroids among other compounds in S*. Incanum*. This proliferation of medically relevant phytoconstituents validates the numerous uses of the plant in traditional medical practice in general, and the management of diabetes in particular.

### Total phenolic content

All the extracts from the 3 different solvents (methanol, ethanol, and water), had considerable total phenolic content (TPC). The results of the TPC of the hydro-methanolic extract was higher than both the hydroe-ethanolic and aqueous extracts (Figure 2). This result can be explained by the fact that ethanol has higher Van der Waals forces than methanol due to its higher molecular weight which leads to stronger intermolecular attraction than methanol. Methanol was therefore able to extract most phenolic compounds.

**Hydro-methanolic aqueous hydro-ethanolic**

Figure 2: Total phenolic content for S. incanum extract

The TPC represent the largest, diverse group of related secondary metabolites produced by plants which share a related sub structure composed of an aromatic-OH moiety. Phenolics are produced mainly as a defence mechanism in response to harsh environmental biotic or abiotic stresses. Plant phenolics are mostly hydroxybenzoic and hydroxycinnamic acid derivatives. There has been increased interest towards their medicinal use as antioxidant, photoprotective, anti-inflammatory and wound healing activities23. Plant phenolics are the main group of secondary metabolites that function as antioxidants or reactive species scavengers. To this regard, these plant polyphenols mostly operate as singlet oxygen scavengers as well as reducing agents. In our study we preferred the Folin-Ciocalteu method compared to the others. In the present study the results were observed through the formation of a blue-coloured solution after the addition of the lyophilised extracts to the Folin-Ciocalteau reagent in the conducive medium. The colour change was due to the *in-situ* formation of the phospho molybdic-phosphotungstic-phenol complex. The TPC was calculated aided by the use of the regression equation of the calibration curve (R2 =0.989, y = 0.009x + 0.0464), expressed in GAE as milligrams per gram of the extract or fraction (mg GAE/g extract or fraction). As shown in Figure 2 and Figure 3, the 3 extracts yielded phenolic compounds in the following order: hydroethanolic > hydromethanolic> distilled water.

Figure 3: Total flavonoid content for S. incanum extract

Flavonoids are polyphenolic compounds that are differentiated by the chemical structures yielding subgroups such as flavones, flavonols, flavanones and flavanols27. Flavonoids possess antioxidant activities. These contribute to the scavenging of 2,2-diphenyl-1- picrylhydrazyl (DPPH) as shown in this study (Figure 4). Other related studies have demonstrated their activity against super oxides and ABTS radicals; and strong iron chelating activity. The most abundant flavonoids isolated from the aerial parts of *S. Incanum* in published studies include rutin, kaempferol and quercetin25. These flavonoids have displayed anti-inflammatory, arterial blood pressure and anti-diuretic activities in other studies25 Muruthi et al., 2023).

## Antioxidant Assay

The DPPH radical scavenging assay revealed significant antioxidant activity in both hydro-ethanolic and aqueous extracts of S. incanum, though with varying potency. Ascorbic acid, used as the reference standard, demonstrated 93.4% scavenging activity at 150 µg/mL, while the hydro-ethanolic and aqueous extracts of S. incanum showed 70.8% and 47.4% inhibition, respectively. The IC50 values further highlighted these differences, with ascorbic acid exhibiting the strongest activity (28.5 µg/mL), followed by the hydro-ethanolic extract (61.33 µg/mL) and the aqueous extract (116.95 µg/mL). The DPPH assay operates on the principle that antioxidants can reduce the stable violet-colored DPPH radical (absorbance at 515 nm) to a colorless or pale-yellow hydrazine derivative (DPPH-H or DPPH-R), which is measurable spectrophotometrically28. This reaction serves as an indicator of free radical scavenging capacity, which can occur through multiple mechanisms, including electron donation, metal chelation, or radical neutralization. Previous studies have attributed the antioxidant properties of *S. incanum* to its rich content of polyphenols, glycosides, phytosterols, and enzymatic components, which collectively contribute to inhibiting oxidative processes. In this study, both extracts displayed concentration-dependent scavenging activity, with higher concentrations yielding greater percent inhibition. Notably, the hydro-ethanolic extract outperformed the aqueous extract, suggesting that the solvent system influences the extraction efficiency of bioactive compounds responsible for antioxidant effects. These findings align with existing literature on plant-derived antioxidants and underscore the potential of *S. incanum* as a source of natural radical scavengers, warranting further investigation into its phytochemical profile and therapeutic applications as shown in Figure 5.

Figure 4: Absorbance vs Concentration of S. Incanum extract

Figure 5: Antioxidant activity of S. incanum

These results confirm that *S. Incanum* extracts are rich in various antioxidant compounds, which help mitigate oxidative stress, a contributing factor in wound healing complications and cancer29. Although not investigated in our study, wound healing properties of *S. Incanum* are well documented. In studies oral administration of the methanolic extract of *S. incanum* (2 g/kg body weight) significantly decreased the diameter of the wound and speed up healing processes on chronic wounds that were on adult female Sprawge Dawley rats throughout the 3 h sampling period (P < 0.05). *S. incanum* (2 g/kg) had significant wound healing activity in comparison with the reference drugs (500 mg/kg) and Silver Sulphur Diazine (3 mg/kg)24.

## Anti-inflammatory tests

Table 2: Anti-inflammatory activity of lyophilised S. Incanum extracts

|  |  |  |
| --- | --- | --- |
| Concentration | % Inhibition | % Inhibition |
| µg/ml | ***S. incanum* hydroethanolic extract** | **Diclofenac (standard)** |
| 250 | 10± 0.08 | 37±0.84 |
| 500 | 16±0.03 | 66±1.78 |
| 1000 | 74±0.28 | 312±3.40 |
| 2000 | 253±1.76 | 730.12± 4.20 |
| 4000 | 354±1.34 | Not tested |
| 6000 | 489±3.56 | Not tested |
| 8000 | 729±3.54 | Not tested |

At related concentrations, the lyophilised extracts from the fruits of *S. incanum* exhibited anti-inflammatory effects that are approximately a third of the anti-inflammatory attributes of the standard Diclofenac (Table 2). The anti-inflammatory activity of *S. Incanum* became comparable to the least dose of the positive control Diclofenac (250 µg/ml) used in this study at around 750µg/ml. The anti-inflammatory effect of 2000 µg/ml of the positive control Diclofenac was approximately 6.6% greater than that for 8000 µg/ml lyophilised crude extracts the *S. Incanum*. Although different methods are used here to represent inhibition of inflammation, the value of 8000µg/ml recorded in our protein denaturation assay, represents significant anti-inflammatory potential of S*. Incanum*, since the extract was used in its crude form. Activity is expected to increase further using bioactive fractions and isolated compounds. Phytosterols, triterpenoids such as lupeol and oleanolic acid, sesquiterpenes, and flavonoids such as rutin and kaempferol have been associated with anti-inflammatory activity of the *Solanaceae* genus. Saponins which are normally found in abundance in the plant, have also shown anti-inflammatory activity in addition to anti-oxidant capacity. However less significant inflammatory responses was found from the fruit extracts of *S. Incanum* and this presented it as a good wound healing agent as inflammation improves the wound healing process.

## Acute oral toxicity evaluation

Table 3: Acute oral toxicity study of S. Incanum behavioural Observations

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Observed parameter | Dose of S*. Incanum* in mg/kg body weight | | | | | |
|  | **250mg** | **500mg** | **1000mg** | **2500mg** | **5000mg** | **Control** |
| Food intake | Normal | Normal | Normal | Normal | Normal | Normal |
| Water intake | Normal | Normal | Normal | Normal | Normal | Normal |
| Death | Alive | Alive | Alive | Alive | Alive | Alive |
| Breathing | Normal | Normal | Normal | Normal | Normal | Normal |
| Diarrhea | Not observed | Not observed | Not observed | Not observed | Not observed | Not observed |
| Urination | Normal | Normal | Normal | Normal | Normal | Normal |
| Skin colour | Normal | Normal | Normal | Normal | Normal | Normal |
| Drowsiness | Not observed | Not observed | Not observed | Not observed | Not observed | Not observed |
| Erection of Fur | Not observed | 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 5000 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. These results agree with studies by Ya’u *et al*(2013)30 where the LD50 of the *S. Incanum* extract was estimated to be > 5000 mg/kg body weight. Our extracts were deemed non-toxic, based on the toxicity classiﬁcation proposed by Loomis and Hayes, which categorise substances with LD50 values from 500 to 5000 mg/kg as slightly toxic and those with LD50 values 5000 to 15,000 mg/kg body weight are regarded as practically non-toxic31 (Loomis and Hayes, 1996). This therefore implies that the use of high extract concentrations to achieve the desired bioactivity effects will not pose any toxicity in S*. Incanum* based treatments32.

## Rat weights observations

Figure 6: Observed rat weights during acute oral toxicity studies of S. Incanum

In toxicity studies, unexpected changes in body weight serve as a straightforward and sensitive indicator of adverse effects following exposure to certain substances. These fluctuations often reflect underlying toxic responses in test animals, helping researchers assess the potential impact of a material on overall health and metabolism. 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.

# Conclusions

The lyophilized hydro-methanolic extracts derived from the aerial parts of S. incanum demonstrated significant antioxidant and antibacterial properties in our study. These pharmacological effects stem from the plant's rich composition of bioactive secondary metabolites, including lignins, phenolic compounds, flavonoids, and phytosterols, which collectively contribute to its therapeutic potential. The presence of these phytochemicals explains the extract's effectiveness in promoting wound healing, particularly in treating burn injuries and pressure ulcers. Importantly, toxicological evaluation confirmed the safety of S. incanum, showing no adverse effects at doses up to 5000 mg/kg body weight. These findings from both biosafety assessments and bioactivity studies validate the traditional use of *S. incanum* in herbal medicine as a safe and effective wound healing agent, supporting its potential application in modern therapeutic formulations for skin repair and regeneration.

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Ethic approval

Upon 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.

# 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.

# References

1. Akobundu IO and Agyakwa CW (1998) A Handbook of West 170 African Weeds. International Institute of Tropical Agriculture, Ibadan, Nigeria, 564
2. Dakone, D., Guadie, A., 2016. A review on ethnomedicinal use, nutritional value, phytochemistry and pharmacological characteristics of *S. incanum* L. An important medicinal plant 5.
3. Mbaya, B. and Muhammed, S., 1976. Antibiotic action of *S. incanum* L. Anti-microb. Agents Chemother, 6, pp.920-927
4. Giday, M., Asfaw, Z., Woldu, Z., Teklehaymanot, T., 2009. Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation. Journal of Ethnobiology and Ethnomedicine 5, 34.
5. Qureshi, Z.Q., Taous Khan, T.K., Shah, A.J. and Fazli Wahid, F.W., 2019. *S. incanum* extract enhances wound healing and tissue regeneration in burn mice model.
6. Ali, H. and Qaiser, M.J.P.J.B., 2009. The ethnobotany of Chitral valley, Pakistan with particular reference to medicinal plants. *Pak. J. Bot*, *41*(4), pp.2009-2041.
7. Teklehaymanot, T. and Giday, M., 2007. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. Journal of ethnobiology and Ethnomedicine, 3, pp.1-11.
8. Giday, M., Asfaw, Z., Elmqvist, T., Woldu, Z., 2003. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. Journal of Ethnopharmacology 85, 43–52.
9. Bessa, L.J., Fazii, P., Di Giulio, M. and Cellini, L., 2015. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. International wound journal, 12(1), pp.47-52.
10. Breijyeh, Z. and Karaman, R., 2024. Antibacterial activity of medicinal plants and their role in wound healing. Future Journal of Pharmaceutical Sciences, 10(1), p.68.
11. Portou, M.J., Baker, D., Abraham, D. and Tsui, J., 2015. The innate immune system, toll-like receptors and dermal wound healing: A review. Vascular pharmacology, 71, pp.31-36.
12. Shah, A. and Amini-Nik, S., 2017. The role of phytochemicals in the inflammatory phase of wound healing. *International journal of molecular sciences*, *18*(5), p.1068.
13. Guo, S. al, and Luisa A. DiPietro. "Factors affecting wound healing." Journal of dental research 89, no. 3 (2010): 219-229
14. Mwonjoria, J.K., Kariuki, H.N. and Waweru, F.N., 2011. The antinociceptive antipyretic effects of Solanum incanum (Linneaus) in animal models. *International Journal*, *2229*, p.7472.
15. Alemu, M., Lulekal, E., Asfaw, Z., Warkineh, B., Debella, A., Abebe, A., Degu, S. & Debebe, E., 2024. Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. PLoS One, 19(3), p.e0300060. DOI:10.1371/journal.pone.0300060.J
16. Kebede, T., Gadisa, E. & Tufa, A., 2021. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLoS One, 16(3), p.e0249253. DOI: 10.1371/journal.pone.0249253.
17. Jaafar, H.Z., Ibrahim, M.H. & Karimi, E., 2012. Phenolics and flavonoids compounds, phenylalanine ammonia lyase and antioxidant activity responses to elevated CO₂ in Labisia pumila (Myrisinaceae). Molecules, 17(6), pp.6331-6347.
18. Brainly, 2025. Keller-Kiliani test for cardiac glycosides. Available at: <https://brainly.in/question/10471653> [Accessed 24 Apr. 2025].
19. Hongzhen, L., Danli, L., Xiaoxian, W. & Liching, T., 2005. Determination of phenolic preservatives in gelatin and vacant capsules for medicine use by ion-suppression reversed-phase high-performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis, 37(2), pp.369-372. DOI: 10.1016/j.jpba.2004.11.001
20. Rai, S., Kafle, A., Devkota, H.P. & Bhattarai, A., 2023. Characterization of saponins from the leaves and stem bark of Jatropha curcas L. for surface-active properties. Heliyon, 9(5), p.e15807. DOI: 10.1016/j.heliyon.2023.e15807.
21. Pérez, M., Dominguez-López, I. & Lamuela-Raventós, R.M., 2023. The Chemistry Behind the Folin-Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. Journal of Agricultural and Food Chemistry, 71(46), pp.17543-17553.
22. Ramos, R.T.M., Bezerra, I.C.F., Ferreira, M.R.A. & Soares, L.A.L., 2017. Spectrophotometric Quantification of Flavonoids in Herbal Material, Crude Extract, and Fractions from Leaves of Eugenia uniflora Linn. Pharmacognosy Research, 9(3), pp.253-260. DOI: 10.4103/pr.pr\_143\_16.Shah, A. and Amini-Nik, S., 2017. The role of phytochemicals in the inflammatory phase of wound healing. International journal of molecular sciences, 18(5), p.1068.
23. Chifamba, J., Addae, A.J., Zengeni, S., Pomerai, M. & Kurebgaseka, N., 2024. Lyophilised Aloe Excelsa fractions, photo-protection and actinic damage retardation claims substantiation. Journal of Complementary and Alternative Medical Research, 25(5), pp.37-52. DOI: 10.9734/jocamr/2024/v25i5536.
24. Elfakhrany, A., Abo-Elsoud, R.A.E.A., Abd El Kareem, H.M., Samaka, R.M. & Elfiky, S.R., 2022. Autophagy and Oxidative Balance Mediate the Effect of Carvedilol and Glibenclamide in a Rat Model of Renal Ischemia-Reperfusion Injury. Open Access Macedonian Journal of Medical Sciences, 10(A), pp.1402-1410.
25. Muruthi, C.W., Ngugi, M.P., Runo, S.M. and Mwitari, P.G., 2023. In vitro antioxidant activities of Carissa edulis ((Forssk) Vahl) and Pappea capensis (Eckyl. & Zeyh) extracts. *Heliyon*, *9*(1).
26. OECD, 2025. Test No. 425: Acute Oral Toxicity—Up-and-Down Procedure. Available at: <https://www.oecd-ilibrary.org/environment/test-no-425-acute-oral-toxicity-up-and-down-procedure\_9789264071049-en> [Accessed 24 Apr. 2025].
27. Dias, M.C., Pinto, D.C.G.A. & Silva, A.M.S., 2021. Plant Flavonoids: Chemical Characteristics and Biological Activity. Molecules, 26(17), p.5377. DOI: 10.3390/molecules26175377.
28. Gulcin, İ. & Alwasel, S.H., 2023. DPPH Radical Scavenging Assay. Processes, 11, p.2248. DOI: 10.3390/pr11082248.
29. Zivanayi, W., Venables, L., Van de Venter, M., Rasana, N. and Hlangothi, B., 2023. Evaluating biological ethnopharmaceutical activities of fruit and root extracts of Solanum incanum L. *African Journal of Food, Agriculture, Nutrition and Development*, *23*(7), pp.24136-24156.
30. Ya'u, J., Chindo, B.A., Yaro, A.H., Okhale, S.E., Anuka, J.A. and Hussaini, I.M. (2013) ‘Safety assessment of the standardized extract of Carissa edulis root bark in rats’, Journal of Ethnopharmacology, 147(3), pp. 653–661. Available at: <https://doi.org/10.1016/j.jep.2013.03.064>.
31. Loomis, T.A. & Hayes, A.W., 1996. Loomis’s Essentials of Toxicology. 4th ed. Academic Press, Cambridge, pp.208-245. DOI: 10.1016/B978-012455625-6/50014-3.
32. Andargie, Y., Sisay, W., Molla, M., & Tessema, G. (2022). Evaluation of antidiabetic and antihyperlipidemic activity of 80% methanolic extract of the root of Solanum incanum linnaeus (solanaceae) in mice. Evidence‐Based Complementary and Alternative Medicine, 2022(1), 4454881.