***Bioactivity and safety evaluation of lyophilized Hydroethanolic Datura stramonium extract***

**Abstract**

 The study investigated the bioactivity and biosafety of lyophilized hydroethanolic *Datura stramonium* seed extracts. The plant commonly known as the Devil's Trumpet, is a widespread weed in Zimbabwe, found in disturbed areas such as roadsides, fallow fields, and waste grounds. The plant, with its large, trumpet-shaped flowers and prickly seed pods, is easily recognizable and is used frequently in traditional Zimbabwean medicine to treat various ailments, including pain, fever, and inflammation. The use of the plant has, however, been associated with adverse toxicity issues including reported fatalities. In the present study, the phytoscreening was done using various classical chemistry techniques, antimicrobial activity was assessed using the agar well diffusion and broth dilution methods and the acute toxicity was evaluated using OECD TG 425. The lyophilized extract demonstrated potent antimicrobial activity against a range of microorganisms, including *Staphylococcus aureus* and *Escherichia coli*, with a minimum inhibitory concentration (MIC) of 12.5 mg/mL. Phytochemical analysis identified the presence of alkaloids, flavonoids, tannins , glycosides and phenolic acids, which may contribute to the observed bioactivities. Acute toxicity studies however confirmed the toxicity issues associated with this plant in folk medicinal practices. Serious adverse effects including mortality were observed from dosages as low as 500mg/kg body weight in laboratory rats. It was therefore concluded that despite the huge potential as a potent antimicrobial agent, the use of the plant in traditional medicine poses a risk to patients and should only be used with caution or excluded from treatment regiments until further studies on sub-acute toxicity profiles at lower doses and mechanisms of action have fully been investigated

**Key words**: *Datura stramonium;* biosynthesis; secondary metabolites; antibacterial

# INTRODUCTION

## *Datura stramonium*

 *Datura stramonium*, commonly known as Jimsonweed or Devil's Trumpet, is a widespread weed in Zimbabwe, found in disturbed areas such as roadsides, fallow fields, and waste grounds. It belongs to the *Solanaceae* family, and it is natively known as *zavazava* [1]. *Datura* refers to a species of shrubby herbaceous plants which produce large, white or purple trumpet-shaped flowers and which is also referred to as the ‘ angel’s trumpet [2].’ The plant, with its large, trumpet-shaped flowers and prickly seed pods, is easily recognizable. In traditional Zimbabwean medicine, *Datura stramonium* (*D. stramonium*) has been used to treat various ailments, including pain, fever, and inflammation [1]. However, its toxic alkaloids, scopolamine and hyoscyamine can cause hallucinations, delirium, and even respiratory failure if ingested in excess. As a result, the handling and consumption of *D. stramonium* are approached with caution in Zimbabwean culture. The pubescent plant can grow to a meter and a half in height [2].

 

Figure 1: Images of Datura stramonium

*Datura stramonium* has been employed in traditional medicine for centuries, particularly in Southern African, cultures. The plant's ethno-pharmacological significance is multifaceted, with various parts of the plant being utilized to treat a range of ailments. In traditional Zimbabwean medicine*, Datura stramonium* has been used to treat fever, rheumatism, and skin conditions, while in Ayurvedic medicine, it is used to treat insanity, epilepsy, and fever [2]. The plant's seeds and leaves are also used in traditional Chinese medicine to treat asthma, bronchitis, and other respiratory conditions. Furthermore, *Datura stramonium* has been used in shamanic rituals in some cultures, where it is believed to possess spiritual and mystical properties. The plant's alkaloids, particularly scopolamine and hyoscyamine, have been isolated and studied for their potential medicinal properties, including their anticholinergic, antispasmodic, and sedative effects [2] [3]. However, the plant's toxicity and potential for abuse have also been noted, highlighting the need for caution and careful use in traditional medicine. Overall, the ethno-pharmacological significance of *Datura stramonium* underscores the importance of preserving traditional knowledge and exploring the potential medicinal properties of plants used in Indigenous cultures. The pod seeds are used as purgative, in cough, fever and asthma and have been reported to be smoked for their narcotic effects [3].The primary biologically active substances in *Datura stramoniu*m are the alkaloids - atropine and scopolamine. Atropine has been used in treating Parkinson’s disease, peptic ulcers, diarrhea and bronchial asthma[2].Recent Insights have outlined their role as potential anticancer nutraceuticals for patients receiving anthracycline based neoadjuvant chemotherapy for breast cancer [4]. Seeds and leaves of *D. stramonium* have also been used to treat psychotic patients, insomnia and depression, relaxing the smooth muscles of the bronchial tube and asthmatic bronchial spasm. *D. stramonium* is a plant with both poisonous and medicinal properties. Studies too have demonstrated that it has great pharmacological potential with excellent value and usage in folklore. The seeds of *Datura* are used in the treatment of analgesic, anti-helmintic and anti-inflammatory, intestinal pain, infestation, toothache, and alleviating fever from inflammation.

## Antibacterial secondary metabolites

Antibacterial secondary metabolites are compounds produced by plants, microorganisms, or other organisms that can inhibit the growth of, or kill bacteria [5].These metabolites can be classified into several groups, including alkaloids, flavonoids, terpenes, and phenolic acids, each with unique structures and mechanisms of action [6].Alkaloids, such as berberine, found in the Berberis plant, and quinine, found in the Cinchona plant, have been shown to exhibit antibacterial activity against a range of microorganisms, including *Staphylococcus aureus* and *Escherichia coli* [7].Flavonoids, such as quercetin, found in apples and onions, and kaempferol, found in tea and broccoli, have also been found to possess antibacterial properties, inhibiting the growth of bacteria such as *Bacillus subtilis* and *Pseudomonas aeruginosa* [8]. Terpenes, such as tea tree oil (*Melaleuca alternifolia*), which contains the compound cineole, and eucalyptus oil, which contains the compound eucalyptol, have been shown to exhibit antibacterial activity against a range of microorganisms, including *Escherichia coli* [9]. Phenolic acids, such as salicylic acid, found in willow bark, and gallic acid, found in gallnuts, have also been found to possess antibacterial properties, inhibiting the growth of bacteria such as *Staphylococcus aureus* and *Bacillus subtilis [7]*. The mechanisms of action of these antibacterial secondary metabolites can vary, but often involve the inhibition of cell wall synthesis, protein synthesis, or DNA replication, leading to the death of the bacterial cell [5]. For example, penicillin, a secondary metabolite produced by the fungus *Penicillium chrysogenum*, works by inhibiting cell wall synthesis, while streptomycin, a secondary metabolite produced by the bacterium *Streptomyces griseus*, works by inhibiting protein synthesis [6]. The discovery of antibacterial secondary metabolites has led to the development of many antibiotics, including penicillin, streptomycin, and tetracycline, which have revolutionized the treatment of bacterial infections [5].However, the overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria, highlighting the need for the discovery of new antibacterial secondary metabolites [6]. In conclusion, antibacterial secondary metabolites are a diverse group of compounds with unique structures and mechanisms of action and have played a crucial role in the development of antibiotics. Further research is needed to discover new antibacterial secondary metabolites and to develop new antibiotics to combat the growing threat of antibiotic-resistant bacteria.

## Anti-inflammatory secondary metabolites

Anti-inflammatory secondary metabolites are compounds produced by plants, microorganisms, or other organisms that have anti-inflammatory properties, reducing or inhibiting the inflammatory response [6]. These metabolites can be classified into several groups, including alkaloids, flavonoids, terpenes, and phenolic acids, each with unique structures and mechanisms of action [7]. Numerous alkaloids have been shown to possess anti-inflammatory activity, inhibiting the production of pro-inflammatory cytokines and enzymes, such as TNF-α and COX-2 [6]. Flavonoids, such as quercetin and kaempferol, have been found to possess anti-inflammatory activity, inhibiting the production of pro-inflammatory cytokines and enzymes, thereby reducing inflammation. [8]. Terpenes, such as boswellic acid and ursolic acid, have been shown to possess anti-inflammatory activity, inhibiting the production of pro-inflammatory cytokines and enzymes, and reducing inflammation in various models of inflammation [9]. Phenolic acids, such as salicylic acid and gallic acid, have also been found to possess anti-inflammatory activity, inhibiting the production of pro-inflammatory cytokines and enzymes. [7].The mechanisms of action of these anti-inflammatory secondary metabolites can vary but often involve the inhibition of pro-inflammatory cytokines and enzymes, antioxidant activity, and modulation of immune cells [6]. Other examples include, curcumin, a polyphenol found in turmeric, which also has potent anti-inflammatory activity. [10]. Resveratrol, a polyphenol found in grapes and berries, has anti-inflammatory activity, inhibiting the production of pro-inflammatory cytokines and enzymes, and reducing inflammation in various models of inflammation[11]. Gingerols, found in ginger, have anti-inflammatory activity, inhibiting the production of pro-inflammatory cytokines and enzymes, and reducing inflammation in various models of inflammation [12].The discovery of anti-inflammatory secondary metabolites has led to the development of many anti-inflammatory drugs, including aspirin, ibuprofen, and celecoxib, which have revolutionized the treatment of inflammatory diseases [6]. However, the overuse and misuse of these drugs have led to the emergence of side effects and toxicity, highlighting the need for the discovery of new anti-inflammatory secondary metabolites[7]). In conclusion, anti-inflammatory secondary metabolites are a diverse group of compounds with potent anti-inflammatory activity and have played a crucial role in the development of anti-inflammatory drugs. Further research is needed to fully understand the mechanisms of action of these metabolites and to explore their potential as therapeutic agents for the treatment of inflammatory diseases.

# Materials and methods

## Materials, equipment and facilities

All chemicals, associated reagents, equipment and facilities for the acute toxicity evaluations and the bioactivity assays were obtained from the University of Zimbabwe, Faculty of Medicine and Health Sciences laboratories, and the Harare Institute of Technology, Pharmaceutical Technology Department.

### Datura stramonium plant material collection, identification and authentication

*Datura stramonium* plant was obtained from Beatrice district, in Mashonaland east, Zimbabwe with the coordinates 18.2493° S, 30.85. Identification and authentication were carried out by a qualified Botanist at the Herbal Botanical Institute, Harare, Zimbabwe. The seeds were collected and handled as per ICH guidelines and Good Agricultural Practices(GAPs).

###  Plant preparation

The seeds were thoroughly washed with distilled water to remove dirt and other contaminants, then shade-dried at ambient temperature with adequate ventilation for three weeks until a constant weight was obtained. The *Datura stramonium* seeds were ground into fine powder using an electric mill. Weighed 300g of fine powder using an electronic balance. Hydroethanolic extraction was done by adding 300g plant powder into 3000ml of 70%(v/v) ethanol and macerated for 7 days at room temperature. Filtered the extracts using whatmann filter paper number 1 and evaporated to concentrate the extracts using a rotary evaporator under low pressure. The extracts were freeze-dried using a freeze dryer and the mass of the powdered extracts obtained will be recorded. The yield was calculated using the following equation.

Equation 1:

*Yield (%w/w) = [Obtained mass of plant extract (g)/amount of plant material soaked (g)]×100*

## Phytochemical Screening of Datura stramonium

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2g of the lyophilized hydro-ethanolic extracts were dissolved in 100ml of distilled water and qualitative preliminary screening was carried out using various techniques as described by Cherie Melaku (2020) [14].

### Detection for alkaloids by the Dragendorff test

Alkaloids were tested using the Dragendorff test.1ml Dragendorffs reagent was added to 0.5ml extract. A positive test result was identified by formation of reddish-brown precipitate indicating the presence of alkaloids.

### Detection of tannins

### Plant extract of Datura stramonium, 0.5ml was mixed with 5ml of 10% NaOH and agitated. The gelatinous precipitation confirmed the presence of tannins.

### Detection of flavonoids

To 0.5ml of the *Datura stramonium* plant extract, 1ml of 2% NaOH and a few drops of dilute H2SO4 were added. The color initially turns into an intense yellow color with the addition of NaOH solution and later becomes colorless with Sulphuric acid. This indicates the presence of flavonoids [14].

### Detection of Glycosides by the Libberman’s test

Glycosides were tested using Libberman's test. In this assay,0.5ml of the crude extract was mixed with 1ml of chloroform and 1ml of acetic acid. Positive tests were identified by the presence of a yellow precipitate indicating the presence of glycosides [14].

### Detection of Phenolic compounds by the Ferric chloride test

The Ferric Chloride test was used to determine the presence of phenolic compounds. In this assay, a few drops of neutral 5%ferric Chloride solute ion were added into 0.5ml of the filtrate extract. Formation of a bluish-black colour indicates the presence of phenolic compounds [15].

### Detection of saponins by the Frothing test

The frothing test was used to determine the presence of saponins. In this assay 1ml of the extract was added to 10ml of distilled water. The mixture was shaken vigorously for 15 minutes in a graduated cylinder. Formation of honeycomb froth indicates the presence of saponins[14].

### Total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu spectrophotometric method. The stock solution was prepared by diluting 0.5g of gallic acid in 10ml ethanol and then added distilled water up to 100ml.To prepare a calibration curve ,different concentrations of gallic acid 50, 100, 125,250, 500 mg/ml were prepared.0.25ml of each calibration solution ,was mixed with 1.25ml of the Folin-Ciocalteau reagent .The mixture was allowed to react for 5 minutes. Then, 2ml of 7.5% Na2CO3 solution and 1.25ml of distilled water were then added to make up a total volume of 5ml. The mixture was then placed in a dark cabin for an hour at room temperature. It was then transferred into a cuvette and measured the absorbance at 760 nm in triplicate using a Shimadzu UV-Vis spectrophotometer(UV-160) .The results were quantified as mg gallic acid equivalents per g of the extract (GAE,mg/g).

## Antibacterial inhibition evaluation of lyophilized *Datura stramoniu*m

### Test microorganisms

Pure cultures of (ATCC 25923) and *Klebsiella pneumoniae* were obtained from the University of Zimbabwe, Microbiology laboratory. To obtain a stock solution, 500mg of lyophilized plant extract was dissolved in 5ml of sterile distilled water to yield an extract concentration of 100mg/ml. Stock portions were obtained by sterile dilutions to obtain concentration of 50mg/ml,25mg/ml,12.5mg/ml and 6.25mg/ml. A standard stock of doxycycline was prepared at a concentration of 100mg/ml in sterile water. The doxycycline stock solution was used as a positive and distilled water as the negative control.

### Broth preparation and culturing

The broth was prepared by dissolving 1.6g of nutrient broth in 200ml of distilled water and heated to 60°C, then 10ml aliquots of the prepared nutrient broth were autoclaved at 120°C for 15 minutes in polypropylene bottles. After cooling, the bacterial strains were introduced into the nutrient broth using inoculating loops and the media was inoculated at 37°C for 48 hours.

### Agar well diffusion

To prepare the agar solution, 8g of nutrient Agar was dissolved in 200ml of distilled water and mixed thoroughly at boiling temperature. The prepared agar solution was divided into 20ml cutting bottles and the medium was sterilized by autoclaving at 121°C for 15minutes. After cooling to roughly 45oC, 0.1 ml of a bacterial suspension and 20ml of molten agar were mixed and poured into petri dishes. The plates were titled to ensure the evenly spread and allowed to settle in an aseptic environment. The stock solution to be tested was prepared by dissolving the extract to a concentration of 50mg/ml, 25mg/ml,12.5mg/ml, 6.25 mg/ml and 3.12mg/ml distilled water. A sterile cock borer was used to perforate the agar, and the holes were filled with 0.05ml of stock solutions. The petri dishes were held at room temperature for an hour and kept in an incubator for 48 hours at 37oC .After the incubation period, the bacterial inhibition zones were quantified.

## Acute oral toxicity evaluation of Datura stramonium

## The acute oral toxicity evaluation of *Datura stramonium* lyophilized extract was conducted as per OECD technical guideline 425 (The Up and Down Test) [15]. Thirty female Sprague Dawley rats were used, and they were acclimatized to the test environment a week before the initiation of the experiment.

### Animal care

## The animals were kept at the animal house of the pharmacy department ,University of Zimbabwe. They were fed standard pellet laboratory diet and water ad libitum. The animal habitat was maintained at a standard temperature of 25°C, with a relative humidity level of 40% and a 12-hour light-dark cycle[19]. A practicing veterinary officer supervised the welfare, observations and care of the animals.

### Experimental design

### Twenty-four( 24 )female Sprague Dawley rats were used and were divided into two groups . The first group (Group 1) served as the control which comprised of three rats and was administered distilled water only, while the second group (Group 2) received varying doses of Datura stramonium extract starting from 50mg/kg up to 2000mg/kg body weight by oral gavage using a stomach tube for 14 days. Each animal was marked for individual identification. The rats were fasted prior to dosing, with water provided. The fasted body weights were determined and used to calculate the dose depending on the body weight.

### Dosing and observation

## The *Datura stramonium* extract was administered by oral gavage in a water solution in different sets of doses for 14 days[19]. The doses used were 50,200,500 ,1000,and 2000mg/kg body weight with the female rats were monitored for any behavioral changes by a veterinary specialist twice daily. The animals were observed for any visible changes or clinical signs of toxicity every hour for the first 12 hours on day 1 with special attention to the first four hours[15] and then once daily for up to a maximum of 14 days. Furthermore, various parameters were assessed including body weight, behavioral changes, movement and mortality rates. The rats were weighed, and body weight was recorded throughout the study to see if there were any changes

# RESULTS AND DISCUSSION

## Percentage yield of the hydroethanolic seed extract of D*atura stramonium*

A yield of 3.7% was recorded for the hydroethanolic extraction of *Datura stramonium* seed, with 11,1g being obtained after extraction and lyophilization which was lower compared to yields obtained in other studies, for instance 7.14%[16], extraction by methanol. However, methanol being cytotoxic was dropped as an unsafe choice of solvent[13]. Studies by other scholars emphasize that the success of extraction of biologically active compounds depends on the type of solvent used in the extraction procedure. A study by Dhawan and Gupta in 2017 on *Datura* showed that methanol had the highest extraction percentage compared to other solvents, namely : chloroform, distilled water[17] .The reduced yield might have been due to differences in geographical location of the plant. The possible explanation for these results could be that the solvent polarity influenced the efficiency of the extraction process. Altemimi (2017) emphasized that solvent polarity influences extraction efficiency, with methanol’s higher polarity extracting more polar compounds like phenolics and alkaloids compared to the ethanol-water mix ratio [20].This finding is supported by Pandey and Tripathi, who also suggested that solvent choice affects the recovery of biologically active compounds, with methanol outperforming ethanol in Datura species due to its ability to dissolve both polar and non-polar constituents[13]. While it can be argued that solvent polarity affects yield obtained, Rayees *et al* (2017) demonstrated that yields are mostly influenced by the extraction duration[21], which is supported by another study that was carried out on *Mormodica charantia L*. The results confirmed that the maximum number of bioactive compounds was obtained at a duration of 12 hours which was the medium duration[21]. These findings suggest that the number of phytochemicals obtained is also influenced by the extraction time. Based on this, the seven-day extraction period used in this study may have resulted in the number of phytochemicals identified .It is possible that extracting the *Datura stramonium* seed extract at a shorter period such as four days might increase the phytochemicals extracted. However, there is a possibility of extension of extraction duration enhancing the breakdown of the seed cell walls which may have limited penetration of the solvent as explained by Azwanida, in the compendium on medicinal extractions [22].

### 3.2 Secondary metabolite qualitative phytochemical screening of Datura stramonium

The results obtained confirmed the efficiency of 70% ethanol in extracting a wide range of phytochemicals namely saponins, alkaloids, flavonoids, carbohydrates, glycosides and tannins. However, results showed that ethanol extracted more alkaloids and flavonoids compared to other phytochemicals such as carbohydrates [23]. Furthermore ,the results obtained in this study align with previous findings by Damergi (2023), which showed that seed extract contained the highest amount of flavonoids with 84.79 ± 3.62 mg CE/g DW compared to other aerial parts of the plant namely leaves and flowers[24].

 ***Table 1 : Qualitative screening of Datura stramonium secondary metabolites***

|  |  |  |
| --- | --- | --- |
| Bioactive compounds | Observation | Ethanol |
| Alkaloids | A reddish-brown precipitate was observed | +++ |
| Polyphenols | The appearance of bluish black colour was observed | +++ |
| Flavonoids | The colour initially turned to an intense yellow with the addition of NaOH solution and later become colourless with sulphuric acid. | +++ |
| Glycosides | A yellow precipitate was observed | +++ |
| Tannins | A brown greenish coloration was observed for both plants | ++ |
| Saponins | A layer of foam was formed and persisted for about 30 minutes | ++ |
| Carbohydrates | A brick red precipitate was formed in a greenish solution showing the presence of carbohydrates. | + |
| Terpenoids | A reddish-brown interface observed | + |

*(+): Indicates low concentration of phytochemical*

*(++): Indicates medium concentration of phytochemical*

*(+++): Indicates high concentration of phytochemical*

## Total phenolic content

Phenolic compounds in *Datura stramonium* were quantified using the Gallic acid calibration curve as the standard. The calibration curve (Figure 2) had an equation$ y=0.003x+0.509$ ,with an R2 value of 0.9965. There was an increase in the average absorbance as the concentration of Gallic acid increased.

Figure 2: Total phenolic content graph

In this current study ,the total phenolic content in the hydroethanolic seed extract of *Datura stramonium* was 168.21 GAE(mg/ml).This value indicates high antioxidant activity of the plant, which can enhance the wound healing process, and it attributes to antimicrobial activities. This value is lower compared to that from another study where the total phenolic content was 219.65±10.3mg GAE/g [24].These findings demonstrate the potential of *Datura stramonium* as a source of naturally occurring antioxidant agents[25]. Phenolic compounds include certain phytochemicals such as phenolic acids, flavonoids and tannins which aligns to the moderate to abundant amounts in *Datura stramonium* plant. These findings explain the large total phenolic content quantifications. Flavonoids have been reported to be responsible for chemo-preventive action, particularly, quercetin which induces cell death of cancerous cells and prevent proliferative growth[26][27].

## 3.4 Antibacterial assays

### 3.4.1 Agar well diffusion

The agar well diffusion test is a method used to evaluate the antimicrobial activity of substances. The plant extracts have been found to produce a zone of inhibition of 10mm and 8mm in *Staphylococcus aureus* and *Klebsiella pneumonia* at 50mg/ml respectively (Table 2, Figure 3). The minimum observable inhibition of both species indicates a reduced antibacterial activity at this concentration. This suggests that higher doses are required to achieve effective antibacterial activity. Hydroethanolic extract was found to be more efficient in inhibiting *Staphylococcus aureus* compared to the *Klebsiella pneumoniae* with a zone of inhibition of 17mm at 100mg/ml. The maximum inhibition against *K.pneumonia* was observed at 100mg/ml .In addition, a study conducted by Al-Snafi (2017) showed that the ethanolic extract of *Datura stramonium* gave a maximum zone of inhibition against *K.pneumonia*, which suggests potent antibacterial properties and indicates its potential as a plant-based antimicrobial agent [18].

Table 2 : the zone of inhibition

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Organism | 0mg/ml | 3.12 mg/ml | 6.25 mg/ml | 12.5 mg/ml | 25 mg/ml | 50 mg/ml | 100 mg/ml | Doxy (100 mg/ml) |
| *S.aureus* | - | - | - | - | - | 10 ± 0.5 | 17 ± 0.8 | 10 ± 0.4 |
| *K. pneumoniae*  | - | - | - | - | - | 8 ± 0.4 | 10 ± 0.5 | 8 ± 0.3 |

Figure 3: Bar graph showing Zone of inhibition

### 3.4.2 Broth microdilution

Table 3 : Minimum inhibitory concentration of the ethanolic seed extract of Datura stramonium against selected bacteria

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ORGANISM | 0.00mg/ml | 3.12 mg/ml | 6.25 mg/ml | 12.5 mg/ml | 25 mg/ml | 50 mg/ml | 100 mg/ml | Positive control |
| *S.aureus* | - | - | - | - | - | - | + | + |
| K. pneumoniae | - | - | - | - | - | - | + | + |

***Key:*** *(+) = present ; (-) = absent*

## 3.5 Acute oral toxicity evaluation

Table 4: Acute oral toxicity study of Datura stramonium behavioural Observations

|  |  |  |
| --- | --- | --- |
| Dose(mg/kg) | Observable effects | Toxicological implications  |
| 50 | No clinical signs of toxicity. Normal feed and water intake. | Safe  |
| 200 | Normal behavior in most rats. Slight decrease in movement | Marginal signs of stress .Still within safe range |
| 500 | Reduction in body weight. No mortality observed  | Safe with mild physiological changes |
| 1000 | Sedation ,tremors, respiratory distress, reduced appetite | Mild toxicity |
| 2000 | Severe tremors, reduced movement, respiratory stress, lethargy, mortality in some rats | Lethal |

The rats maintained their behavior, normal feed and water intake after being administered distilled water while the oral dose of 500mg/kg of *Datura stramonium* extract was found to be non-toxic but not entirely safe since some there were some observable changes in body weight. In this study, there were no significant observable changes in behavioral, body weight and feeding at lower doses up to 200mg/kg. Slight changes in weight were recorded for 500mg/kg but there was no mortality observed. However, at doses from 1000mg/kg body weight to 2000mg/kg, toxicity effects were observed, including sedation ,severe tremors, lethargy, respiratory stress, reduced feeding rate, reduced body weight and in some cases mortality. Moreover, it was observed that there was a decrease in body weight with an increase in the number of days the rats were exposed. This study has also shown that the plant extract that *Datura stramonium* seed extract is unsafe at higher doses and caution should be taken when using the plant .A corresponding study by Ogunmoyole *et al* suggests that these various effects of the plant’s extract differ depending on the organ involved and the solvent used for extraction[19]. These results can be further explained through the use of several biomarkers of toxicity such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lipid peroxidation (MDA) were estimated in the liver, brain, kidney and heart homogenates. These findings align with other studies that recorded a median lethal dose(LD5) of 3185.25mg/kg of ethanolic extract of *Datura stramonium* [28].

# Conclusions

The hydroethanolic extracts of *Datura stramonium* demonstrated significant antibacterial properties making the plant a promising candidate for the treatment of vaginal candidiasis. Phytochemical screening revealed the presence of key bio-active compounds that contribute to its therapeutic effects. The antibacterial assays showed that the hydroethanolic seed extract of *Datura stramonium* moderately inhibited and *Klebsiellae pneumoniae* growth, in comparison to doxycycline ,a standard antibacterial agent for both bacterial species. However, our acute toxicity evaluations confirmed that *Datura stramonium* hydroethanolic extracts are not biologically safe and should be used with caution at all concentrations. There is therefore a need to further carry out sub scute toxicities tests at doses below 500mg so as to determine chronic organ effects before this plant can be recommended as an adjunct treatment in traditional medicine

**Ethical 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.

COMPETING INTERESTS

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

# 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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