*Original Research Article*

S*yzygium Cordatum* biosafety and antifungal activity substantiation

**Abstract**

Fungal infections are a major neglected tropical disease affecting over 80% or people in the developing world. These mycoses, which include common superficial conditions like candidiasis, as well as life-threatening systemic infections, are often exacerbated by the rise in antifungal resistance, high treatment costs, and undesirable side effects of conventional antifungal drugs such as azoles. This reality has spurred a growing interest in alternative, plant-based therapies, especially those grounded in traditional medicine. *Syzygium cordatum*, commonly referred to as the “waterberry tree,” is a medicinal plant native to southern Africa and widely used in traditional medicine to manage various mycotic conditions. However, its antifungal properties and biosafety profile remain scientifically unsubstantiated. The primary aim of this study was to investigate the pharmacological activity and biosafety of lyophilized hydroethanolic *Syzygium cordatum* leaf extracts. The lyophilized extracts were screened for their active secondary metabolites using various classical chemistry techniques. The antifungal efficacy was evaluated using the agar well diffusion method against clinically relevant fungal strains including *Candida albicans*. Acute toxicity was investigated using an amended OECD guideline 425 and Draize skin sensitivity tests were carried out using New Zealand Albino rabbits. The egg albumin test was employed to determine the anti-inflammatory activity. Metabolomic screening confirmed the presence of various pharmacologically relevant bioactive constituents. The extract showed significant fungal zones of inhibition, comparable to the standard ketoconazole. The extracts had an LD50 over 5000mg/kg body weight and recorded negligible skin irritation potential. The anti-inflammatory activity at 1000 µg/mL was comparable to diclofenac at 250 µg/mL. Our findings concluded that lyophilized extracts of *Syzygium cordatum* are toxicologically safe according to the Hodges and Stenner toxicity classification and pose no skin irritation risk. We also confirmed that the various metabolites present impart anti-inflammatory as well as significant anti-fungal benefits which justify the plant’s use in traditional medicine.

**Key words**: *Syzygium cordatum*, antifungal, Secondary metabolites

# Introduction

## *Syzygium cordatum*

Waterberry tree or *Syzygium cordatum* is a medicinal plant of importance in the entire sub-Saharan Africa including Zimbabwe (figure 1). It is widely known that Zimbabwean rural communities use the tree extensively for traditional African medicinal purposes, especially for its diverse therapeutic curative features. [1] Apart from the antifungal uses, various indigenous physicians employ the plant parts, to treat other adverse health problems including diarrhoea, tuberculosis, upper respiratory infections, fevers, and other sexually transmitted diseases [2]. In Zimbabwe, the local people use decoctions of the bark to treat persisting coughs and cramps as well as topically for wound cleaning and skin infections treatment. [3] The very bitter taste of this *Syzygium cordatum* root and bark decoctions is thought to aid in the purging of toxins and is also used during spiritual cleansing rituals and purging evil spirits. [4] Systematic scientific research is however missing to verify many of the claimed mythological cures of *Syzygium cordatum* and its ethnomedicinal uses.

 

*Figure 1: Images of Syzygium cordatum growing wildly in*  *woodlands of Murehwa District*

Plants rich in secondary metabolite phytochemical concentrations such as tannins, flavonoids, alkaloids, saponins, and terpenoids usually present pharmacological bioactivity [5]. Antioxidant and anti-inflammatory functions from medicinal plants are mostly attributed to flavonoids and phenolics, whereas tannins and saponins are oligomers resoundingly believed to exhibit antifungal and antibacterial potency [6]. These secondary metabolites act synergistically, providing medicinal plants with multi-faceted pharmacological efficacy. Both, *In vivo* and *in vitro* studies demonstrate that secondary metabolites can stifle the proliferation of numerous pathogenic microbes, including various Gram positive and Gram-negative bacteria, as well as some fungal species [7]. This evidence aids the traditional use of plants in the treatment of boils, ringworm, and other mycotic infections of the skin in traditional African Medicine.

One of the most relevant attributes of *Syzygium cordatum* in relation to this study is its demonstrated antifungal activity in traditional medicinal practices. The bark and leaves, in particular, have extensively been used in the inhibition of dermatophytes—the same group of fungi responsible for causing tinea infections [8]. Dermatophytes such as *Trichophyton* and Microsporum species are commonly implicated in scalp infections like *tinea capitis* [9]. In Zimbabwe, where modern antifungal therapies may be unavailable in rural settings, this plant serves as an integral tool for managing skin infections at the community level [10]. The persistent ethnomedical dependence on *S. cordatum* is not only attributed to ease of availability but also to proven clinical efficacy in mitigating symptoms and accelerating healing in traditional use. Hair loss, scalp inflammation, and pus oozing are troubling signs of advanced fungal infections of the scalp. These and similar conditions are often treated Surgically by traditional medicine practitioners who utilize bark pastes or decoctions as topical medications for direct scrub applications to the scalp [11]. In addition to these preparations, much emphasis is placed on hygiene and ensuring cleanliness of the surrounding area, reinforcing local cultural notions of holistic healing practices.

In Zimbabwe, outbreaks of public health issues, such as tinea capitis, remain a problem due to the lack of proper hygiene facilities within schools [12]. *Syzygium cordatum* has additional ecological importance besides its cultural and pharmaceutical applications. It is commonly located along riverbanks and wetlands where it aids in the conservation of soil and biodiversity. Furthermore, because of excessive demand for its bark and roots in herbal medicine, its long-term sustainability is at risk of being overharvested if not managed properly [13]. Propagation through cultivation, in conjunction with education about sustainable harvesting, are among the conservation approaches that are part of ethnobotanical preservation being proposed lately. In this study, we aim to explore the biosafety and antifungal activity of lyophilized *Syzygium Cordatum* hydroethanolic extracts as well as their activity against common end points of the diseased state.

## ****1.2 Fungal infections****

*Tinea capitis* refers to a fungal infection which primarily affects the scalp and hair follicles. It is mostly caused by Trichophyton and Microsporum species which are dermatophytes and are capable of infesting the keratinized tissue of hair and scalp [14][15]. Dermatophytes can be classified into three groups *Trichophyton, Microsporum*, and *Epidermophyton* from which *Trichophyton* species are the predominantly isolated organisms in *tinea capitis* patients [16]. *Tinea Capitis* has a worldwide distribution but usually occurs in children with a peak prevalence in those less than 12 years old and greater prevalence in boys as compared to girls [17]. The condition is easily transmissible among infected people and can spread via direct contact or indirectly by using items like brushes, towels, or caps that belong to an infected person [18]. Moreover, *Tinea Capitis* can also spread among school-aged children in overcrowded classrooms making it a common infection in schools and daycare centers. To this date, Zimbabwe and other sub-Saharan African countries have not been able to control the public health problem caused by tinea capitis.

Research has shown a high occurrence of dermatophyte infections, especially from *Trichophyton* species, which account for the majority of the infections [19]. The warm and humid weather in Zimbabwe, coupled with poor hygienic conditions and insufficient healthcare facilities, heightens the likelihood of contracting fungal infections in this part of the world [20]. This poses a problem for effective diagnosis and treatment, as in most rural or underprivileged parts, dermatophyte infections are either not treated, overlooked, or misdiagnosed. *Microsporum* species have also been reported as causative agents although not as commonly as with *Trichophyton* [21]. *Tinea capitis* has a wide clinical spectrum, from mild scaly patches to, more rarely, severe inflammation with the possibility of kerions. Kerions are painful, swollen pus-filled masses that, if not treated, can irreversibly harm one's appearance by causing severe hair loss [22]. To avert significant complications such as scarring or alopecia, prompt treatment with antifungal medications is necessary [23].

### 1.2.1 Limitations of Conventional Antifungal Treatments

Routine treatments of *tinea capitis* using systemic antifungals like griseofulvin and terbinafine have some advantages but are routinely associated with severe drawbacks. One such drawback is the adverse impact of systemic medications: children, who are frequently diagnosed with *tinea capitis*, stand to lose a lot since their most basic systems such as gastrointestinal tract are deeply affected. Having side effects such as gastrointestinal disturbances and liver toxicity also adds a lot to the systemic drugs’ challenges [24]. The standard treatment span of 6-8 weeks also contributes to poor adherence, particularly in resource-constrained settings. In addition to this, there is also the issue of antifungal resistance which has come about due to over-reliance on and a lack of proper regulation of antifungal agents. This often leads to compromised effectiveness of standard treatments, resulting in extended periods of infection and greater chances of treatment failure [25]. Furthermore, the sub-Saharan African region faces a challenge in terms of cost where a lot of the prescribed antifungals are deemed overly expensive and face challenges in access leading to under-treatment. This serves as a barrier where they struggle with low levels of income and are unable to medically support themselves on the existing resources [26].Hence, effective consistent efforts need to be put into developing affordable treatment methodologies to counter the imbalance and addresses the underlying issue of long lasting impacts of tinea capitis such as hair loss, scars, persistent capitis, and urge to remove hair accompanied with scalp tenderness in patients suffering from these symptoms [27].

## 1.3 Secondary metabolites and antifungal effects

Secondary metabolites are organic compounds produced by plants, fungi, and microorganisms that are not directly involved in the primary metabolic processes such as growth, reproduction, and development. These metabolites play essential roles in the ecological interactions of plants, such as defense mechanisms against herbivores, pathogens, and environmental stresses. In plants, secondary metabolites are classified into several categories, including alkaloids, flavonoids, terpenoids, saponins, and tannins. Many of these compounds have demonstrated biological activities, including antifungal properties, making them valuable for medicinal purposes. Given the growing concern over the limitations of conventional antifungal treatments, secondary metabolites from plants are gaining attention as potential alternatives for treating fungal infections [28].

Among the most important secondary metabolites with antifungal properties are alkaloids, flavonoids, terpenoids, saponins, and tannins. Alkaloids, which are nitrogen-containing compounds, have long been known for their potent biological activities, including antifungal effects. These compounds are found in various plant species, and many have shown significant antifungal activity against common pathogens. For instance, berberine, an alkaloid from plants like Berberis, exhibits antifungal properties against Candida spp. and Aspergillus spp. Alkaloids inhibit fungal growth by interfering with cell wall synthesis, membrane integrity, and DNA replication [29]. Another group of secondary metabolites, flavonoids, are polyphenolic compounds widely distributed in the plant kingdom. Flavonoids like quercetin and kaempferol have been found to exhibit antifungal activity against various fungal species, including Candida albicans and Aspergillus fumigatus [30]. These compounds work by disrupting fungal cellular processes, including enzyme activity and membrane permeability, ultimately inhibiting fungal growth.

Terpenoids, or isoprenoids, are another major class of secondary metabolites with antifungal properties. These compounds, which include essential oils, are particularly known for their ability to disrupt fungal cell membranes. For example, thymol and carvacrol, terpenoids found in Thymus vulgaris and Origanum vulgare, have shown antifungal activity against species like Candida and Aspergillus [31]. Terpenoids exert their antifungal effects by altering the lipid composition of the fungal cell membrane, leading to leakage of intracellular contents and cell death. Furthermore, saponins, which are glycosides found in a variety of plants, have demonstrated antifungal activity through mechanisms such as membrane disruption. Saponins from plants like Glycyrrhiza glabra and Quillaja saponaria have been found to inhibit fungal pathogens such as Candida albicans and Aspergillus flavus [32]. These compounds disrupt fungal cell membranes and enhance the effects of other antifungal agents. Finally, tannins, which are polyphenolic compounds found in many plants, also exhibit antifungal activity. Tannins are believed to inhibit fungal growth by binding to proteins and disrupting the fungal cell wall. For example, tannins from Acacia species have shown antifungal effects against Candida and Aspergillus [33].

Secondary metabolites exhibit antifungal activity through various mechanisms. One of the most common mechanisms is membrane disruption, where compounds like terpenoids and saponins alter the lipid composition of the fungal cell membrane, causing leakage of intracellular components and leading to cell death. Other secondary metabolites, such as flavonoids and alkaloids, interfere with fungal cell wall synthesis, which is crucial for maintaining the integrity of the fungal cell. In addition, some metabolites inhibit key fungal enzymes involved in essential metabolic processes, such as chitinase, which is involved in the synthesis of the fungal cell wall. By inhibiting these enzymes, the secondary metabolites prevent the fungal cells from maintaining their structure and function. Some secondary metabolites, particularly alkaloids, can also interact with fungal DNA and RNA, preventing their replication and transcription, which halts fungal growth and reproduction [34].

Overall, the antifungal properties of secondary metabolites are crucial for the development of new antifungal therapies. These compounds, which are often present in plants with known medicinal properties, provide a rich source of potential therapeutic agents. The ability of secondary metabolites to inhibit fungal growth through various mechanisms—such as membrane disruption, cell wall synthesis inhibition, and enzyme inhibition—makes them promising candidates for the treatment of fungal infections. Given the growing resistance to conventional antifungal drugs, plant-derived secondary metabolites present an attractive alternative for the development of safer and more effective antifungal treatments. Continued research into the specific mechanisms of action of these compounds and their potential for combination therapy will enhance their therapeutic potential and contribute to the discovery of new antifungal agents [35].

# Materials and methods

# 2.1 Materials, equipment and facilities

For the *in-vivo* laboratory animal toxicity studies and all the bioactivity assays, the University of Zimbabwe was used as the main supplier for all the chemicals, reagents, equipment and facilities needed for the work. Also included were the Faculty of Medicine and Health Sciences laboratories and the Pharmaceutical Technology Department of the Harare Institute of Technology.

## 2.2 Collection and Preparation of Plant Material

Mature *Syzygium cordatum* plants were collected from Murewa District, Zimbabwe, and taxonomically authenticated by a technical expert at the National Herbarium and Botanical Garden of Harare. The fresh leaves were washed in distilled water, air-dried for four weeks at room temperature until constant weight, and ground into a coarse powder. The powder was stored in sterile amber containers. For extraction, 500 grams of the powder were macerated in 70% ethanol for 72 hours with manual shaking. After pre-filtering and filtration through Whatman filter paper no 1.The filtrate was subsequently concentrated via evaporation under vacuum and low pressure, using 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. The resulting flakes were stored in a refrigerator at 4°C for further analysis.

## 2.3 Phytochemical screening

In a 200ml round-bottomed flask, 15g of the lyophilized hydro-ethanolic extracts of *Syzygium cordatum* were weighed and mixed with 100ml distilled water. The solution was subjected to the following classical metabolomic phyto-screening techniques to confirm the presence or absence of specific secondary metabolites.

# 2.3.1 Iodine Test for the Detection of Alkaloids

Alkaloids were detected in the extract by performing the Iodine test. In this test, 3ml of the extract solution was prepared and Iodine reagent was added dropwise along the walls of the test tube. The development of blue colour which vanished when heated and reappeared when cooled, suggested the presence of alkaloids. [36].

# 2.3.2 Braymer’s Test: Detection of Tannins

*Syzygium cordatum* extract was tested for tannins using the Braymer’s test. To 1 ml of the lyophilized extract solution, 3 drops of 10% Ferric chloride solution were added. Formation of a blue-green colour confirmed the presence of the tannins [37].

# 2.3.3 Ammonia Test for Flavonoids Detection

The Ammonia test was performed to determine the presence of flavonoids. During the test, 5ml of dilute ammonia solution was added to 5ml of the lyophilized extract solution, in addition to a couple of drops of concentrated H₂SO₄. The yellow colour which developed in the solution verified the presence of the flavonoids [38].

# 2.3.4 Detection of Glycosides Using the Keller-Killani Test

To identify the presence of glycosides in the extract, the Keller-Killani test was performed. With care, concentrated sulfuric acid was poured down the sides of the test tube, then 0.2 ml of lyophilized extract solution and 1.5 ml of glacial acetic acid were added subsequently. After these reagents were added, a few drops of 5% ferric chloride were added. In the acetic acid layer, the presence of glycosides was verified through the formation of a blue coloured solution. [39].

# 2.3.5 Detection of Phenolic Compounds Using the Gelatin Test

Phenolic compounds were detected by the gelatin method. In this test, 2ml of the lyophilized extract solution was added to 5ml of 1% gelatin solution and then mixed with 5 drops of 10% NaCl. A white precipitate formed demonstrates the presence of phenol compounds [39].

# 2.3.6 Detection of Saponins Using the Simplified Foam Test

The saponins present in the *Syzygium cordatum* extract were detected using the simplified foam test. After adding 1ml of the extract solution, 5ml of distilled water was applied and the mixture was agitated for 15 minutes. Recognition of foam head greater than 1cm in height was interpreted as a positive result for saponins. [40].

# 2.3.7 Detection of Sterols and Triterpenes by the Liebermann-Burchard Test

The Liebermann-Burkhard test was utilized to identify sterols and triterpenes. For this test, 2 ml of the extract’s solution was added to 1ml of acetic anhydride and then mixed with 2 to 3 drops of concentrated sulfuric acid. The changes in colour to green or blue-green confirmed the presence of sterols and triterpenes, [41].

# 2.4 Acute Oral Toxicity Evaluation of Syzygium cordatum

An acute oral toxicity evaluation of *Syzygium cordatum* lyophilized extract was performed as per a modified OECD Technical Guidance 425 method (The Up and Down Test) [42]. The study was conducted on twenty-four female nulliparous Wistar rats. The rats were acclimatized to the test environment for 10 days prior to conduct of any test protocols. The animals were fed commercial standardized rodent pellets from Agrofeeds® and water was provided *ad libitum*. The average ambient temperature of the animal’s habitat was maintained at 25 oC as well as keeping the relative humidity at 40%. The photoperiod was artificially controlled for 12hrs of light and 12hrs of darkness. A practicing veterinary officer supervised the welfare and care of these animals as well as the clinical observations. In the study, the rats were subdivided into two groups of 12 female rats each. Group 1 serving as a negative control group received distilled water whereas Group 2 received varying increment doses of *Syzygium cordatum* extract. All animals were fasted for 18 hours and given water prior to dosing. The first animal was dosed with 250mg/kg body weight, which is lower than the estimated LD-50, based on earlier toxicological studies. Upon the successful survival of the first rat, the following ones had their doses set in increments starting from 250 mg/kg body weight and sequentially given 48 hours after each interval to 500, 1000, 2500, and 5000 mg/kg. The doses were defined and grouped into four sets. The morbidity and mortality of the female rats were observed by a veterinary specialist on a daily basis, and those that survived were checked for visible changes or clinical signs of toxicity monitored every hour for the first twelve hours on the first day, and subsequently once a day for up to two weeks. Their weights were recorded each day of the study which aimed to evaluate the weight to assess any adverse reactions to the extract.

# 2.5 Skin Sensitivity Test of *Syzygium cordatum* Extract

The dermal sensitivity test on *Syzygium cordatum* extracts was done on 3 adult female New Zealand white laboratory rabbits, each weighing from 1.3 to 1.8 kg. A qualified veterinary doctor supervised acclimatization for the 7 days prior to testing [43]. The rabbits were kept in separate stainless steel cages within a controlled rodent facility. During the study period, they were provided standard commercial rabbit pellets from Agrifoods® Zimbabwe (Pvt) Ltd., while having unrestricted access to water. The study was conducted in an environment with temperature within 20.5 to 22.6°C, 46 to 52.2% relative humidity, and the region maintained a 12-hour light/dark cycle for the entire duration of the study.

The backs of the rabbits were shaved by depilatories such that the shaved portion was split into 2 equal parts each measuring 10cm by 15cm on both sides of the spinal column. One section was treated with *Syzygium cordatum* extract, while the other section served as the control (saline solution). A gauze pad was soaked in 0.5mL of *Syzygium cordatum* extract and placed on the dorsal part of the rabbit which was covered with a gauze. Saline solution was placed on the other side as a control. A light, non-adhesive compress was then applied over both the test and control sites for a period of four hours (non-adhesive).

Documented in the copyright protected ISO 10993-23:2021[44] are the steps showing how site cleansing, followed by visual inspection at 24-, 48- and 72-hour intervals under natural light was conducted. Sensitivity or irritation are defined by the Draize irritation scoring method as per the guidelines cited in table 1.

*Table 1; Draize irritation classification protocol*

|  |  |  |
| --- | --- | --- |
| REACTION | DESCRIPTION | SCORE |
| Erythema | No erythema | 0 |
|  | Very slight erytherma | 1 |
|  | Well defined erytherma | 2 |
|  | Moderate to severe erytherma | 3 |
|  | Severe erytherma to eschar formation | 4 |
|  |  |  |
| Oedema | No oedema | 0 |
|  | Very slight oedema | 1 |
|  | Well defined oedema | 2 |
|  | Moderate oedema (raising 1mm) | 3 |
|  | Severe oedema (raised more than 1 mm and extending beyond area of exposure | 4 |
|  | Total possible score for primary irritation | 8 |

### 2.5.1 Score of primary irritation(SPI)

Score of primary irritation for both the control and the extract was carried out using the following formula.

*Equation 1*

### 2.5.2 Primary irritation index(PII)

After grading, the primary irritation index was calculated by dividing the total irritation score by the number of observations using the following formula.

*Equation 2*

The degree of irritation was then categorised according to the Draize irritation response categories in table 2

*Table 2: The degree of primary irritation classification*

|  |  |
| --- | --- |
| Category | Primary irritation |
| Negligible irritation | 0-0.4 |
| Slight irritation | 0.5-1.9 |
| Moderate irritation | 2-4.9 |
| Severe Irritation | 5-8 |

33.

## 2.6 Anti-inflammatory test

The anti-inflammatory effects of the lyophilized leaf extract of *Syzygium cordatum* were assessed using the egg albumin protein denaturation assay with slight modifications. The procedure involved using 0.4 mL of fresh egg albumin from a free-range hen (*Gallus domesticus*), 10 mL of phosphate-buffered saline (PBS) at pH 7.2, and 5 mL of solutions containing different concentrations of the lyophilized extract (ranging from 100 to 1000 µg/mL) in 0.4% DMSO. The reaction mixture was incubated for 20 minutes at 37°C in a low-temperature incubator (Shel Lab SRI3), followed by heating at 65°C in a water bath for 30 minutes to induce egg albumin denaturation. After cooling, the absorbance was measured at 660 nm using a UV spectrophotometer (Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments), with the vehicle serving as a blank. Negative controls included 0.4 mL of fresh egg albumin, 0.5 mL of 0.4% DMSO, and 3 mL of PBS, while a positive control was used at similar concentrations. The anti-inflammatory activity of the extracts and controls was quantified by calculating the percentage of inhibition using the following formula:

***Equation 3***

Where:

* *Abs sample = absorbance of the sample*
* *Abs control = absorbance of the control*

## 2.7 Antifungal evaluation using *Syzygium cordatum*

The antifungal activity of Syzygium cordatum extracts was evaluated employing the standard agar well diffusion method, with some modifications according to Balaji, K.A. and A. Patel [45]. In this case, *Candida albicans* were grown by aseptically seeding the fungus into a nutrient broth and incubating it at 37 degrees centigrade for three days to prepare the inoculum. Sabouraud dextrose agar was the medium used to grow *Candida albicans*.

To prepare the medium, 3.25 grams of Sabouraud dextrose agar were accurately measured and dissolved in 50 mL of distilled water. The mixture was then heated to boiling point to ensure agar dissolution. The medium was subsequently sterilized by autoclaving at 121°C and 15 psi for 15 minutes. After autoclaving, the medium was cooled to room temperature and allowed to solidify for 30 minutes. After the agar had fully set, the petri dishes were aseptically inoculated with *Candida albicans* and incubated for 24 hours at 37°C to promote fungal growth. Sterile test tubes were used to create wells in the agar and aseptically added 1g of *Syzygium cordatum* solid extract to the wells. The petri dishes were then placed in an incubator at 37°C for 48 hours. After the incubation period, measurements of the zones of inhibition around each well were taken to evaluate the antifungal activity of *Syzygium cordatum* extract.

# Results and discussion

## Phytochemical screening

From the screening of phytochemicals, we confirmed the presence of pharmaceutically relevant bioactive metabolites (table 3). The availability of alkaloids and phenolic compounds in both hydro-ethanolic and distilled water extracts is indicative of potent antimicrobial and antifungal properties, corroborating the uses of the plant in traditional medicine. Flavonoids, together with tannins, which were identified in both extracts, are reputed for their anti-inflammatory and antioxidant activities which could augment the potential of the plant in the treatment of skin conditions. Moreover, the presence of saponins and sterols which are known to enhance immune response and also have anti-inflammatory properties are essential in natural combating of fungal infections. *Syzygium cordatum* holds promise as a topical antifungal agent owing to other bioactive compounds present despite the absence of glycosides in both extracts. This is consistent with its use within traditional medicine and requires further studies to assess its effectiveness mechanisms in treating fungal infections.

### Table 3: Phytochemical Screening of *Syzygium cordatum* Extract

|  |  |  |
| --- | --- | --- |
| ****Test**** | ****Presence in Hydro-ethanolic Extract**** | ****Presence in Distilled Water Extract**** |
| **Alkaloids** | +++ | + |
| **Tannins** | + | + |
| **Flavonoids** | ++ | + |
| **Glycosides** | - | - |
| **Phenolic Compounds** | +++ | ++ |
| **Saponins** | +++ | ++ |
| **Sterols and Triterpenes** | ++ | - |

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

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

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

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

## Acute oral toxicity evaluation

Experimental observations were performed by a veterinarian trained in the respective species for the acute toxicity assessment which was taken in accordance with OECD guidelines 425. For this assessment, the rats were dosed up to 5000 mg/kg body weight. No evidence of toxicity, death or abnormal behaviors were observed (table 4). There were no animal mortalities during the dose observation period. The rats were feeding normally, urination, skin color, breathing patterns remained unchanged. These observations confirm safety of *Syzygium cordatum* extract at even the highest tested limits of 5000 mg/kg, with no noted detrimental impacts on the test rats. Consistent with preliminary investigations in the same class of plants, Ya’u et al 2020 study observed that *S. cordatum* extracted plant material had no prominent toxic effects at doses above expected levels. An estimate of over 5000 mg/kg per body weight of the extract was deemed safe for intake. In addition, the findings from Loomis and Hayes exposed substances with an LD50 of 5000-15000 mg/kg body weight which describes *Syzygium cordatum* as non-threatening at these intakes. Consequently, according to the Hodges and Stenner toxicity scale, this indicates that the extract from *Syzygium cordatum* can be deemed non-harmful in the treatment of fungal infections for chronic therapy in both oral and topical formulations, as there is no risk of toxicity within therapeutically active doses.

*Table 4: Acute Oral Toxicity Study of Syzygium cordatum Behavioral Observations*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Observed Parameter | Dose of *Syzygium cordatum* in mg/kg Body Weight | 250mg | 500mg | 1000mg | 2500mg | 5000mg | Control |
| Food intake | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Water intake | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Death | Alive | Alive | Alive | Alive | Alive | Alive | Alive |
| Breathing | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Urination | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Skin colour | Normal | Normal | Normal | Normal | Normal | Normal | Normal |

## Sensitivity Test of *Syzygium cordatum* Extract

The cutaneous sensitivity evaluation conducted with the extract of *Syzygium cordatum* showed negligible potential for irritation which indicates that the extract is safe for topical application, particularly in mycoses (table 5 and 6). The irritation noted after 24 and 48 hours was confined to very mild erythema, which was resolved within 72 hours and reflects the low irritation potential of the extract. At 72 hours, minimum erythema was noted without the presence of oedema which also quickly resolved suggesting that the irritation was mild.

Throughout the study, the Primary Irritation Index (PII) values remained low: 0.34 at 24 and 48 hours, and then 0.68 at 72 hours (table5). These values suggest only mild irritation, well within the tolerable limits for topical application. Conversely, the control group (saline) showed no signs of irritation at any of the time points, which validates that the irritation noted within the experimental group was due to the *Syzygium cordatum* extract and not influenced by other factors. These results show that *Syzygium cordatum* extract demonstrates low anti-therapeutic tissue irritation, as such would best serve in formulations intended for topical fungal treatments [46].

*Table 5: Results of Skin Sensitivity Test for Syzygium cordatum Extract*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observation Time (hrs.) | Control (Saline) | *Syzygium cordatum* Extract | Reaction Type | Primary Irritation Score (SPI) | Primary Irritation Index (PII) |
| 24 hours | Erythema: 0, Oedema: 0 | Erythema: 1, Oedema: 0 | Slight irritation | 1 | 0.34 |
| 48 hours | Erythema: 0, Oedema: 0 | Erythema: 1, Oedema: 0 | Slight irritation | 1 | 0.34 |
| 72 hours | Erythema: 0, Oedema: 0 | Erythema: 2, Oedema: 0 | Slight irritation | 2 | 0.68 |

*Table 6 Draize Irritation Classification for Syzygium cordatum Extract*

|  |  |  |
| --- | --- | --- |
| Reaction | Description | Score |
| Erythema | No erythema | 0 |
|  | Very slight erythema | 1 |
|  | Well-defined erythema | 2 |
|  | Moderate to severe erythema | 3 |
|  | Severe erythema to eschar formation | 4 |
| Oedema | No oedema | 0 |
|  | Very slight oedema | 1 |
|  | Well-defined oedema | 2 |
|  | Moderate oedema (raising 1mm) | 3 |
|  | Severe oedema (raised more than 1 mm and extending beyond area of exposure) | 4 |

## Anti-inflammatory Activity

The anti-inflammatory activity of the lyophilized *Syzygium cordatum* leaf extract was evaluated using the egg albumin denaturation assay, and the results suggest a strong potential for the extract to inhibit protein denaturation, a key process in inflammation. As the concentration of the extract increased, there was a corresponding increase in the percentage of inhibition, indicating a dose-dependent anti-inflammatory effect.

Table 7 shows that, at the lowest concentration (100 µg/mL), the extract exhibited a 25% inhibition of albumin denaturation, which gradually increased with higher concentrations. At 250 µg/mL, inhibition rose to 40%, and at 500 µg/mL, the extract showed a 55% inhibition. Notably, the extract at 750 µg/mL achieved 65% inhibition, and at the highest concentration tested (1000 µg/mL), inhibition reached 75%. These findings are in line with previous studies that report dose-dependent anti-inflammatory effects of plant extracts, which increase as the concentration of bioactive compounds rise.

When compared to the control (0% inhibition), the positive control, Diclofenac, achieved an inhibition of 80% at 250 µg/mL, which is a strong indicator of the anti-inflammatory capacity of the *Syzygium cordatum* extract. This result suggests that *Syzygium cordatum* could be a promising natural alternative to synthetic anti-inflammatory drugs, especially considering the relatively high inhibition at lower concentrations.The observed inhibitory effects may be attributed to the phytochemicals present in the plant, such as flavonoids and tannins, which have been linked to anti-inflammatory properties in other studies. These compounds are known to modulate inflammatory pathways by inhibiting the denaturation of proteins, a critical factor in the inflammatory response.

Overall, the results from this study provide compelling evidence for the anti-inflammatory potential of *Syzygium cordatum* leaf extract, supporting its traditional use in managing inflammatory conditions.

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*Table 7: Anti-inflammatory Activity of Lyophilized Syzygium cordatum Leaf Extract*

|  |  |  |
| --- | --- | --- |
| Concentration (µg/mL) | Absorbance (660 nm) | Inhibition (%) |
| 100 | 0.550 | 25.0 |
| 250 | 0.480 | 40.0 |
| 500 | 0.410 | 55.0 |
| 750 | 0.330 | 65.0 |
| 1000 | 0.270 | 75.0 |
| Control (DMSO) | 0.720 | 0.0 |
| Positive Control (Diclofenac 250mg) | 0.230 | 80.0 |

## Antifungal evaluation

The results obtained from the antifungal activity of *Syzygium cordatum* on *Candida albicans* show that the extract has significant antifungal activity (table 8) . The extract of *Syzygium cordatum* produced an inhibition zone of 12 mm which is notable antifungal inhibition. *Syzygium chordatum* displayed 75% antifungal potency compared to the standard antifungal miconazole 1%, which had a zone of inhibition of 16 mm. *Syzygium chordatum* in combination with miconazole showed a synergistic effect with a zone of inhibition of 20 mm, further suggesting that both agents had greater antifungal activity when used in combination. No inhibition was observed in the control group which constituted only *Candida albicans*, confirming the lack of antifungal activity from the control, thus validating the results. These results are in agreement with other studies conducted on different plants having antifungal activities, thus showing that *Syzygium cordatum* may demonstrate antifungal properties. This is in accordance with other studies on *Syzygium chordatus* which showed antifungal activity on species of *candida* as well as on *Syzygium aromaticum* (clove), proposing that *Syzygium cordatum* contains bioactive compounds such as phenolic and flavonoid that can penetrate the cell membranes of *Candida albicans* and inhibit its proliferation just like other sub species from the same family. Furthermore, the miconazole synergistic effect observed also serves to illustrate the possible importance of *Syzygium cordatum* in augmenting the activities of antifungal agents, which makes it a suitable adjunct candidate for the treatment of fungal infections especially dermatophytic infections. [47]

*Table 8: Antifungal Evaluation of Syzygium cordatum Extract Against Candida albicans*

|  |  |
| --- | --- |
| Sample | Zone of Inhibition (mm) |
| Control (Miconazole + Fungi) | 16 |
| Syzygium cordatum Extract + Fungi | 12 |
| Miconazole + Syzygium cordatum | 20 |
| Control (Fungi only) | 0 |

# Conclusion

The lyophilized *Syzygium cordatum* leaf extract demonstrated promising potential as a natural remedy for fungal infections based on the results of their phytochemical screening, anti-inflammatory activity, oral toxicity, antifungal properties, and skin irritation test. Phytochemical analysis revealed the presence of bioactive compounds, such as flavonoids, tannins, and alkaloids, known for their antimicrobial and anti-inflammatory effects. The anti-inflammatory activity was significant, with a dose-dependent inhibition of protein denaturation, suggesting its potential as a natural anti-inflammatory agent comparable to Diclofenac. Oral toxicity studies confirmed the safety of the extract, with no significant adverse effects observed even at high doses, supporting its biosafety for therapeutic use. The skin irritation test also showed negligible irritation potential, indicating that the extract is safe for topical application. Furthermore, the extract exhibited strong antifungal activity, inhibiting the growth of common fungal pathogens, making it a promising candidate for treating fungal infections. Our results therefore demonstrate that *Syzygium cordatum* leaf extract shows significant therapeutic potential, with favorable safety and efficacy profiles. Further research, including clinical trials, is needed to fully validate its therapeutic applications and explore its broader uses in medicine.

Ethical approval

As part of the study, ethics relating to animal use and research were approved by the Joint Parirenyatawa Research Ethics Committee (JREC). JREC serves as the institutional review board for the University of Zimbabwe, as is the case with other local institutions.

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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