***Original Research Article***

***ANTIFUNGAL ACTIVITY AND BIOSAFETY ASSESSMENT OF VERNONIA ADOENSIS***

**Abstract**

Dermatophytes and other pathogenic fungi species are neglected tropical infections that grow on skin, hair, nails, mucous membranes, appendages and other body surfaces, causing various diseases in over a billion people annually. Common treatments are not only inaccessible but there are rising incidences of resistance to these antifungals. *Vernonia adoensis*, a plant indigenous to tropical and subtropical regions and prevalent in Southern Africa has widely been used as an effective treatment for fungal infections in traditional medicine in Southern Africa, however its toxicity profile and effectivity against pathogenic fungi remains unscientifically systematically validated. In this study, the phytochemical constituents of pharmacological relevance were identified through wet chemical analysis techniques, the toxicity profile was determined through OECD guidelines as well as Draize dermal irritation and skin sensitivity tests, the antifungal activity was assessed through the agar well diffusion method against Aspergillus *fumigatus*, with miconazole as a standard. The anti-inflammatory activity was determined through the egg albumin test with diclofenac as a standard. It was confirmed in this study that lyophilized hydroethanolic extracts from *V. adoensis* possess numerous phytoconstituents of pharmacological relevance including flavonoids, alkaloids, and terpenoids. The extracts demonstrated significant inhibitory effects against Aspergillus *fumigatus* comparable to miconazole. Our findings indicate that the lyophilized extracts were non-toxic at doses up to 5000 mg/kg, and the skin irritation tests confirmed that V adoensis has negligible skin irritation potential. The extracts also demonstrated considerable anti-inflammatory activity at doses within the toxicity safety zone. Based on the foregoing it was concluded that Vernonia adoensis possess relevant, pharmacologically active metabolites with regards to common end points of fungal infections. The hydroethanolic extracts possess no adverse dermal sensitivity issues and is toxicologically safe according to the Hodge and Sterner toxicity scale. *V.adoensis* therefore presents a safer, efficacious alternative as an antifungal agent for pharmacological use.

**Key words**: *Vernonia adoensis*, anti-inflammatory, antifungal, fungal infection, secondary metabolites

# Introduction

## Vernonia adoensis

The *Vernonia adoensis (V. adoensis)* plant belonging to the *Asteraceae* family is native to the Sub Sahara, African region in general, being more prevalent in Southern Africa. This plant is commonly located in grasslands and savannas and has the potential to grow up to a meter in height [1]. A distinctive feature of the plant is its purple flowers, which aid in attracting various forms of pollinators. Adaptability is one of the plant’s strongest attributes and it allows the species to be one of the most dominant and familiar plants in traditional medicine. *Vernonia adoensis* is hugely popular amongst traditional medicinal practitioners in Zimbabwe due to its vast medicinal properties [2]. Locals have devised plant-based cures to treat malaria, fever, gastrointestinal complications, and inflammation, utilizing different components of the plant such as leaves, stems, and roots. These medicinal practices, though lacking rigorous scientific investigation, endorse the rich cultural heritage rooted in traditional flora knowledge. “Conservation of *Vernonia adoensis* is crucial because habitat loss, climate change, and spear harvesting pose serious threats. Increased use of sustainable harvesting practices and educational initiatives focusing on ecological and medicinal significance can help protect the species for future generations. [3] This is an excellent example where integrating native wisdom alongside rigorous scientific study could facilitate biodiversity management and conservation in Zimbabwe.

  

*Figure 1: Images of Vernonia adoensis growing wildly in the Murehwa rural areas of Zimbabwe*

## Secondary metabolites and fungal infection

Fungal infections pose a significant health risk globally, particularly in immune-compromised individuals. Fungal pathogens such as *Candida*, *Aspergillus*, and *Cryptococcus* are responsible for a wide range of infections, from superficial skin diseases to life-threatening systemic conditions. These infections are especially prevalent in regions with elevated levels of HIV/AIDS, diabetes, and other chronic illnesses, which weaken the immune system and increase susceptibility to fungal colonization and dissemination. In Africa, where access to medical care and antifungal treatments can be limited, fungal infections represent a serious public health issue [4]. The global burden of fungal infections is compounded by the emergence of resistance to conventional antifungal drugs, such as azoles, polyenes, and echinocandins. This resistance is partly attributed to the overuse and misuse of antifungal agents in both clinical and agricultural settings, leading to mutations and the development of drug-resistant strains. Moreover, many of the available antifungal drugs are expensive and have significant side effects, further exacerbating their limited accessibility in low-income settings. As a result, there is an increasing demand for alternative, cost-effective, and safe antifungal treatments that can be used in resource-limited environments [5]. This has prompted significant interest in the exploration of natural products, especially plant-derived secondary metabolites, as potential antifungal agents.

Plants are a rich source of bioactive compounds known as secondary metabolites, which are not directly involved in the plant’s growth, development, or reproduction but are essential for survival in their natural environment. These compounds often play a defensive role, protecting the plant from pathogens, herbivores, and environmental stresses. Among the most studied secondary metabolites are alkaloids, flavonoids, terpenoids, phenolic acids, and Saponins, which have been shown to possess antimicrobial, including antifungal, properties [6].

Flavonoids, which are widely distributed in the plant kingdom, have demonstrated significant antifungal activity. These compounds exert their effects by disrupting the integrity of fungal cell walls, inhibiting spore germination, and interfering with the synthesis of ergo sterol, a critical component of fungal cell membranes. For instance, studies have shown that flavonoids such as quercetin can inhibit *Candida albicans* growth by affecting its cell membrane structure and reducing its ability to form biofilms. Furthermore, flavonoids have been reported to enhance the activity of conventional antifungal drugs, suggesting their potential as adjunctive therapy in treating fungal infections [7].

 Alkaloids are another group of secondary metabolites with potent antifungal properties. These nitrogen-containing compounds have been shown to interfere with various cellular processes in fungi, such as nucleic acid synthesis, mitochondrial function, and membrane integrity. For example, berberine, an alkaloid found in *Berberis* species, has been demonstrated to inhibit *Candida* species, including *Candida albicans* and *Candida tropicalis*, by disrupting their cellular structures and inducing oxidative stress [8]. Similarly, alkaloids from *Papaver somniferum* (opium poppy) have shown antifungal activity against *Aspergillus* species, including *A. fumigatus*, one of the most common pathogens causing invasive aspergillosis in immune-compromised individuals [9]. Terpenoids, another class of secondary metabolites, are also known for their antifungal properties. Terpenoids, such as *thymol*, carvacrol, and ginsenosides, have demonstrated activity against a broad range of fungal pathogens. *Thymol* and *carvacrol*, which are found in essential oils of thyme and oregano, act by disrupting the fungal cell membrane, increasing membrane permeability, and ultimately leading to cell death [8]. Ginsenosides, saponins found in ginseng, have shown antifungal effects through the inhibition of fungal growth and spore germination in various fungi, including *Aspergillus* and *Penicillium* species [10]. These compounds, due to their relatively low toxicity in humans, present an attractive alternative to conventional antifungal treatments, especially in regions where drug resistance is becoming a growing concern. Phenolic acids, such as gallic acid and caffeic acid, are widely distributed in plants and have been found to possess potent antifungal activity. These compounds exert their effects by generating reactive oxygen species (ROS), which cause oxidative damage to the fungal cell membrane and other critical cellular components.

Studies have shown that phenolic acids can inhibit the growth of *Candida* species and *Aspergillus fumigatus* by interfering with cell wall biosynthesis and inducing apoptosis in fungal cells [11]. In addition, phenolic acids have been shown to work synergistically with other antifungal agents, enhancing their effectiveness and reducing the potential for resistance development [12]. Saponins, another diverse group of secondary metabolites, are known for their ability to disrupt fungal cell membranes by binding to sterols such as ergosterol. This leads to increased membrane permeability, cell leakage, and cell death. Saponins derived from plants like *Panax ginseng* (ginseng) and *Quillaja saponaria* (soapbark) have demonstrated antifungal activity against a variety of fungi, including *Candida* species and *Aspergillus* species [13]. Saponins have also been found to enhance the immune response, further boosting their potential as therapeutic agents for fungal infections [14].

 Given the traditional use of indigenous plants in sub-Saharan Africa for treating fungal infections, there is a growing interest in scientifically validating their efficacy and safety. In Zimbabwe, *Vernonia adoensis*, commonly known for its use in traditional medicine, has been employed for a range of ailments, including microbial infections. However, despite its widespread use, the antifungal properties and biosafety profile of *Vernonia adoensis* have not been well studied. Preliminary ethno botanical surveys suggest that the bark of this plant is used for treating fungal infections, and its secondary metabolites may play a role in this therapeutic activity [15]. The present study seeks to investigate the biosafety and bioactivity of the hydro-ethanolic extract of the barks of *Vernonia adoensis*, in order to validate its use in the prevention of fungal infection in traditional medicine in Zimbabwe.

## Anti-inflammatory role in fungal treatment

Fungal infections are a significant health challenge worldwide, contributing to high morbidity and mortality rates, particularly among immune-compromised individuals. While fungi directly invade tissues to cause infections, an equally crucial factor in the pathogenesis of these infections is the host's immune response, which triggers an inflammatory cascade. Inflammation, a natural defense mechanism, plays a pivotal role in combating pathogens. However, when this response is exaggerated or poorly regulated, it can lead to tissue damage and exacerbate disease progression, particularly in fungal infections. This dual role of inflammation—as both a protective response and a contributor to disease pathology—has sparked increasing interest in understanding its role in fungal infections and the potential benefits of modulating this response to improve treatment outcomes [16].During a fungal infection, the body’s immune cells, including macrophages, neutrophils, and dendritic cells, are activated upon recognizing fungal pathogens through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs). Upon activation, these immune cells release a variety of pro-inflammatory cytokines, including interleukins (IL-1β, IL-6), tumor necrosis factor-alpha (TNF-α), and interferons (IFN-γ).

These cytokines are essential for orchestrating the immune response and recruiting additional immune cells to the site of infection. However, excessive or prolonged production of these cytokines can result in chronic inflammation, leading to tissue damage and an inability to clear the infection effectively [17]. Infections caused by *Candida albicans*, *Aspergillus* species, and *Cryptococcus* are prime examples where inflammation significantly contributes to pathogenesis. In *Candida albicans* infections, for instance, the host’s immune response triggers the release of IL-1β, IL-6, and TNF-α from activated macrophages and other immune cells. These cytokines help in recruiting neutrophils, which are crucial for controlling fungal growth. However, the overproduction of IL-1β and TNF-α can cause persistent inflammation, leading to tissue damage and immune system exhaustion. Chronic inflammation in Candida infections is particularly problematic in immune compromised individuals, as the immune system becomes unable to resolve the infection and, instead, the inflammation persists, worsening the infection [18]. Similarly, in invasive *Aspergillus* infections, the inflammatory response is characterized by the release of TNF-α and IL-6, both of which play a critical role in the body ’s attempt to control the fungal pathogen. However, in cases of prolonged infection, these cytokines not only fail to clear the pathogen but also contribute to tissue injury.

inflammation exacerbates lung damage in individuals with conditions like chronic obstructive pulmonary disease (COPD) and cystic fibrosis, further complicating the infection [19]. The overactivation of these inflammatory pathways leads to a vicious cycle where tissue destruction impairs the host's ability to clear the infection, allowing it to persist. Moreover, free fatty acids (FFAs) have emerged as important mediators of inflammation in fungal infections. FFAs are released from adipose tissue, particularly in individuals with obesity or metabolic dysfunction. These fatty acids can activate TLRs, which then trigger the release of pro-inflammatory cytokines, including IL-1β and TNF-α, amplifying the inflammatory response. Elevated FFAs have been shown to worsen the inflammatory environment during Candida infections, thus contributing to persistent fungal growth and making the infection more difficult to treat [20].

The link between inflammation and fungal infection severity has spurred interest in modulating the inflammatory response as a potential therapeutic approach. Traditional medicine has long recognized the role of inflammation in the progression of various diseases, including infections. In particular, secondary metabolites from plants have demonstrated significant anti-inflammatory properties, offering a promising avenue for reducing the inflammatory response associated with fungal infections. These compounds can modulate immune cell activity, reduce the production of pro-inflammatory cytokines, and alleviate tissue damage caused by excessive inflammation [21]. Flavonoids, such as quercetin, have been shown to exert anti-inflammatory effects by inhibiting the production of TNF-α and IL-1β, two cytokines that play critical roles in fungal infections. By reducing the inflammatory response, these compounds can help the immune system focus on clearing the fungal infection, while minimizing the collateral damage to host tissues [22]. Alkaloids and terpenoids, found in plants like *Berberis vulgaris* and Thymus vulgaris, also possess anti-inflammatory properties.

These compounds can suppress the production of inflammatory cytokines and, in doing so, support the body’s ability to combat fungal infections without causing excessive tissue damage [23]. Given the role of inflammation in the pathogenesis of fungal infections and the growing interest in plant-based treatments, there is a need for further investigation into the efficacy of natural products that combine both anti-inflammatory and anti-inflammatory activities. For example, extracts from plants like *Vernonia amygdalina* (bitter leaf) and *Cucumis edulis* (cucumber) have shown promise in reducing inflammation while also exhibiting antifungal effects. These plants contain secondary metabolites such as flavonoids and alkaloids, which not only help modulate the immune response but also target fungal pathogens directly, offering a potential dual-action treatment approach for fungal infections [24].Aiming to expand the scope of natural treatments for fungal infections, the present study was carried out to confirm the bioactivity of the hydro-ethanolic extract of *Vernonia adoensis* against known biological endpoints associated with fungal infections for example, inflammation.

# 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, and the Harare Institute of Technology, Pharmaceutical Technology Department.

### 2.1.1. Animal use approval

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

### 2.1.2 Vernonia adoensis Plant material collection and preparation

Naturalised, wildly growing *Vernonia Adoensis* was obtained from Murehwa rural forest, Murehwa District in Mashonaland East Province (17.6502° S, 31.7787° E). The plant was taxonomically authenticated by the National Herbarium and Botanical Garden in Harare, Zimbabwe. The plant materials were washed separately with fresh water to remove dirt and other contaminants, shade-dried for three weeks until constant weight. The dried roots were ground to fine powder using manual grinder. The hydro-ethanolic extraction was done by adding 400g plant powder into 1000ml of 70% (v/v) ethanol in a 2-litre sterile amber bottle and macerated for 3 days with 3minute physical shaking twice a day. The extracts were filtered firstly using a Matton cloth then (Whatman filter paper number 1) and evaporated under low pressure (Rotavapor® R-300, Buchi, Switzerland), followed by lyophilisation (Lyovapor l-200, Buchi, Switzerland) under 140Pa pressure and -50 °C.

## 2.2 Phytochemical Screening of Vernonia adoensis

In a 200ml round bottomed flask, 7.5g of the lyophilized hydro-ethanolic extracts of *Vernonia Adoensis* was 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.

### 2.2.1 Detection for alkaloids by the Iodine test

The Iodine test was used to determine the presence of alkaloids. In this assay, to 3ml of the lyophilized extract solution, a few drops of iodine solution were slowly added along the sides of the test tube. The presence of alkaloids was then identified by the appearance of a blue colour, which disappears on boiling and reappears on cooling [25].

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

The simplified Braymer’s test was used to detect the presence of tannins. To 1ml lyophilised extract solution, 3 drops of a 10% Ferric chloride solution were added. The presence of tannins was confirmed by conversion of the solution to a blue-green colour. [26]

### Detection of flavonoids by the Ammonia test

Flavonoids were detected by means of the Ammonia test where 5ml dilute ammonia solution was added to 5ml of the lyophilised solution followed by a few drops of conc. H2SO4. The emergence of a yellow colour indicates the presence of flavonoids. [27]

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

The presence of glycosides was done by the Keller-Killani test. To 1mL of the lyophilised solution, 1.5mL glacial acetic acid was added and a few drops of 5% ferric chloride were added as well as conc. H2SO4 (along the side of test tube). The presence of glycosides was confirmed by the emergence of a blue coloured solution in mixture acetic acid layer. [28]

### Detection of Phenolic compounds by the Gelatin test

Phenolic compounds were detected using the Gelatin test. In this assay, 2ml the lyophilised extract solution was added to 5ml of a 1% gelatin solution and 5 drops of a 10% NaCl were further added. Phenolics were identified by the appearance of a white precipitate [29].

### Detection of saponins by the simplified foam test

The simplified foam test was used to determine the presence of saponins. In this assay 2ml of the extract was added to 20ml distilled water. The mixture was shaken in a graduated cylinder for 15 minutes. The presence of saponins would be confirmed by the formation of form with a head height of at least 1cm [30].

## Anti-inflammatory activity of Vernonia adoensis using the egg albumin denaturation test

 The anti-inflammatory activity of the lyophilised leaf extract of *V. adoensis* was determined using the egg albumin protein denaturation assay, with slight modifications [31]. Samples and reagents used include 0.4 mL of egg albumin (fresh) from a free-range domesticated hen (*Gallus* *domesticus*), 10 mL of phosphate-buffered saline (PBS) at pH 7.2, and 5ml solutions of varying concentrations of the lyophilised root extracts in 0.4% DMSO . The concentrations of the lyophilized extracts in the total reaction solution ranged from 100 to 1000 µg/mL. The samples were incubated (Shel lab SRI3 Low Temperature BOD Incubator) for 20 minutes at 37°C and then heated at 65 degrees in a water bath for 30 minutes to denature 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: 0.4 mL of fresh egg albumin, 0.5 mL of 0.4% DMSO, and 3 mL of PBS were included in the experiment.

 Positive control was used as a positive control for the study at similar concentrations.

 The percentage of inhibition, which translates to the anti-inflammatory activity of the extracts and standards, and calculated by the following equation

***Equation 1***

$$Inflamation inhibition percentage effect=\frac{Abs\_{sample}}{Abs\_{control}-1} x 1$$

where,

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

## Acute oral toxicity evaluation of Vernonia adoensis

The acute oral toxicity evaluation of *Vernonia adoensis* lyophilized extract was conducted using The Up and Down Test [32]. Twenty-four female nulliparous Wistar rats were used, and they were acclimatized to the test environment for 10 days prior to the initiation of the test protocols. The animals were fed commercial standardized rodent pellets from Agrofeeds® and provided with water ad libitum. The animal habitat was maintained at an average ambient temperature of 25°C, with a relative humidity level of 40% and an artificially controlled photoperiod of 12 hours of light and 12 hours of darkness. A practicing veterinary officer supervised the welfare, observations, and care of the animals. In this study, sequential doses were orally administered to the animals at 48-hour intervals. The animals were divided into two groups of 12 female rats each. The first group (Group 1) received distilled water and served as the control, while the second group (Group 2) received incremental doses of the *Vernonia adoensis* solution [33]. Each animal was marked for individual identification. Prior to dosing, the experimental animals were fasted for 18 hours, with water provided. The initial doses were selected based on related toxicological studies. The first animal received a dose of 250 mg/kg body weight, which was below a randomly selected estimated LD50. After surviving the initial dose, the subsequent dose was doubled, based on the observations of the test animals over a 48-hour period. The *Vernonia adoensis* extract was orally administered in a water solution in four different sets of doses: 250, 500, 1000, 2500, and 5000 mg/kg body weight [34]. The female rats were monitored for morbidity and mortality by a veterinary specialist twice daily. In the absence of mortality, the rats were further observed for any visible changes or clinical signs of toxicity every hour for the first 12 hours on day 1, and then once daily for up to a maximum of 14 days. The animals were also weighed daily throughout the study.

## Sub-acute oral toxicity evaluation of Vernonia adoensis

 The sub-acute oral toxicity evaluation of *Vernonia Adoensis* lyophilized extract was done using a modified OECD-TG 425 (The up and down test) [35]. A total of 24 Female nulliparous Wistar rats were used, which were acclimatized to the test environment for 10 days before the commencement of the test protocols. The participating animals were fed with a commercial standardized rodent pellet from Agrofeeds® and were given water ad libitum [41]. The animal habitat was kept at an average ambient temperature of 25°C throughout the study with a relative humidity level of 40% and an artificially controlled photoperiod of 12-h light and 12-h darkness. [36]

In the test, sequential ordered progressions of doses were orally administered to the animals at 48-hour intervals. The animals were divided into 2 groups of 12 female rats each; the first group (group 1) received distilled water and served as the control group. The second (group 2) received incremental doses of the *Vernonia Adoensis* solution. The selected animals were marked so as to facilitate individual identification. The experimental animals were fasted for 18 hours with water prior to dosing. Initial starting doses were chosen based on related toxicological studies. The first animal received a dose of 250mg/kg body weight, which was below a randomly selected estimated LD50. When animals survived the dose, the next dose was doubled, subject to our observations of the test animals over a period of 48 hours. The *Vernonia Adoensis* was orally gavaged in a water solution in 4 different sets of doses of: 250, 500, 1000, 2500 and 5000 mg/kg body weight [37]. The female rats were observed by a veterinary specialist for morbidity and mortality twice daily. In the absence of mortality, the rats were observed for any visible changes and clinical signs and symptoms of toxicity every 1 hour, and up to 12 hours on day 1, and thereafter, once daily up to a maximum of 14 days. The animals were also weighed daily*.*

## Skin sensitivity tests of Vernonia adoensis

The skin sensitivity test was carried out on the *Vernonia adoensis* extracts using 3 adult female New Zealand breed white laboratory rabbits, weighing between 1.3 to1.8 kg. The rabbits were acclimatized for a period of 8 days prior to the test, under the guidance of a qualified veterinary doctor [38]. The rabbits were kept separately in stainless steel cages in a rodent facility. They fed on a typical commercial rabbit pellets from Agrifoods Zimbabwe (pvt) ltd. The rabbits also had unlimited access to drinking water. The microbial contamination levels were controlled, temperature ranging from 20.5 to 22.6 degrees, humidity was 46% to 52.2 % and a 12; 12-hour light/dark cycle throughout the experiment. The backs of the rabbits were shaved by depilatories and the shaved area was divided into two parts measuring 10cm by 15cm area at either side of the spine. The first area was used for the application of the *Vernonia adoensis* extract and the second area was used as the control for testing the irritation [39]. During Extract application, a gauze was soaked in 0.5 ml of the *Vernonia adoensis* extract and applied at one side of the rabbit’s back. Saline was applied as the control on the other side of the rabbit’s back. Both areas were lightly covered with non-sticky bandages for 4 hours. The sites were cleaned and visually observed at 24, 48 and 72 hours under natural light [40]. Sensitivity or reactions were evaluated by the following criteria as per Draize documented method and presented in the table 1 below.

*Table 1: Draize irritation classification protocol*

|  |  |  |
| --- | --- | --- |
| 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 the area of exposure |  4 |
|  | Total possible score for primary irritation |  8 |

### 2.6.1 Score of primary irritation (SPI)

A score of primary irritation for both the control and the extract was carried out [35]

### 2.6.2 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 1

 

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

*Table 2. Draize primary irritation scale*

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

## Antifungal evaluation using Vernonia adoensis

The antifungal activity of the *V. adoensis* extracts was performed by the standard agar well diffusion method against *Aspergillus fumigatus* with slight modifications [41].

In the experiment, Aspergillus fungi were added to a nutrient broth aseptically to cultivate the fungi. It was placed in an incubator at 37 degrees for 3 days for inoculum formation. Potato dextrose agar was used as the culture medium for cultivating the fungi. Through the use of ratios, 3.25 grams of potato dextrose agar was dissolved in 50 milliliters of distilled water. Heated to boil and dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure. (121°C) for 15 minutes [42]. Afterwards, the agar was removed from the autoclave and allowed to cool and solidified for 30 minutes. The Aspergillus inoculums were aseptically transferred onto the solidified agar and was left for 24 hours in an incubator at 37 degrees to allow for further growth of the Aspergillus in the petri dishes. Wells were made using small sterile test tubes at appropriate positions. The 1g of solid extract of *V. adoensis* was added into the agar wells, aseptically. The plates were incubated at 37 degrees for 48 hours, and thereafter the zone of inhibition was investigated. [43]

# Results and discussion

## Phytochemical screening

##  *Table 3: Qualitative screening of Vernonia Adoensis 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 | +++ | + |
| Lignans | +++ | +++ |

*(-): 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, our investigations confirmed the abundance of primary and secondary metabolites of biomedical relevance with regards to fungi (Table 3). The compounds with the strongest presence in the plant were phenolics (especially flavonoids), saponins, alkaloids and lignans. These results correlate with studies by [44] who identified alkaloids, saponins, flavonoids, benzenoids, phenylpropanoid, lignans, sesquiterpenes, and coumarins among other compounds in *Vernonia adoensis.* The presence of terpenoids where *Vernonia adoensis*exhibited varying terpenoid content and glutathione reductase activity, thereby indicating enzymatic activity potential [45]. This proliferation of medically relevant phytoconstituents validates the numerous uses of the plant in traditional medical practice in general, and the management of fungal infections.

## Anti-inflammatory tests

Table 4: Anti-inflammatory activity of lyophilised Vernonia adoensis extracts

|  |  |  |
| --- | --- | --- |
| Concentration | % inhibition | % inhibition |
| mg/ml | ***Vernonia adoensis* hydro-ethanolic extract** | **Diclofenac (standard)** |
| 1.5 | 12 ± 0.08 | 39 ± 0.84 |
| 2.5 | 18 ± 0.03 | 65 ± 1.78 |
| 5 | 76 ± 0.28 | 302 ± 3.40 |
| 10 | 265 ± 1.76 | 760.12 ± 4.20 |

At the concentrations tested, the hydro-ethanolic extract of *Vernonia adoensis* displayed increasing anti-inflammatory activity, with the results suggesting its potential for managing inflammation in the context of fungi infection. At the lowest concentration tested (1.5 mg/ml), the extract exhibited a 12% inhibition, which is a moderate effect compared to the positive control Diclofenac, which showed 39% inhibition at the same dose (Table 4). As the concentration of *Vernonia adoensis* extract increased, so did its inhibitory effect. At 5 mg/ml, the extract exhibited a significant 76% inhibition, indicating its growing anti-inflammatory potential. At higher concentrations, such as 10 mg/ml, *Vernonia adoensis* displayed even stronger inhibition, with values reaching 265% which suggests a potent dose-dependent anti-inflammatory effect. These findings are consistent with the extract’s potential to alleviate symptoms of inflammation associated with fungi infections, a condition where immune response and inflammation are critical factors in disease progression. Research has shown that inflammation can exacerbate the symptoms of fungal infections including discomfort, itching, and pain [46]. The anti-inflammatory effects observed with *Vernonia adoensis* could help reduce these symptoms, making it a potential adjunct to antifungal therapy. Notably, the increasing inhibition with higher concentrations of *Vernonia adoensis* extract aligns with the dose-dependent response typical of many plant extracts known for their anti-inflammatory properties. Similar studies on plants from the *Vernonia* genus have shown that compounds like flavonoids, triterpenoids, and phenolic acids are responsible for their anti-inflammatory effects [47], [48]. These bioactive compounds may target the inflammatory pathways involved in fungal infections, potentially helping to reduce both the acute and chronic inflammation associated with the infection.

## Acute oral toxicity evaluation

Table 5: Acute oral toxicity study of Vernonia adoensis behavioural Observations

|  |  |
| --- | --- |
| Observed parameter |  Dose of *Vernonia adoensis* 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 |
| Urination | Normal | Normal | Normal | Normal | Normal | Normal |
| Skin colour | Normal | Normal | Normal | Normal | Normal | Normal |

The acute toxicity study was carried out in line with the OECD technical guideline 425. A qualified veterinary expert oversaw all observations, results, and interpretations. Our findings revealed that the *Vernonia adoensis* extract, even at doses as high as 5000 mg/kg body weight, did not result in any noticeable signs of toxicity or death in rats, indicating it is safe. (Table 5). No animals were withdrawn from the study during the observation period. These results are consistent with the findings [43], where the LD50 of the *Vernonia adoensis* extract was estimated to exceed 5000 mg/kg body weight. According to the toxicity classification outlined by Loomis and Hayes, our extract was classified as non-toxic. Substances with LD50 values ranging from 5000 to 15,000 mg/kg are considered practically non-toxic, while those with LD50 values between 500 and 5000 mg/kg are slightly toxic [49] [50]. This suggests that using high doses of *Vernonia adoensis* extract to achieve the desired bioactive effects should not lead to significant toxicity in treatments.

## Skin sensitivity test

*Table 6: Results of Skin Sensitivity Test for Vernonia adoensis Extract*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observation Time (hours) | Control (Saline) | Vernonia Adoensis Extract | Reaction Type | Primary Irritation Score (SPI) | Primary Irritation Index (PII) |
| 24 | Erythema: 0, Oedema: 0 | Erythema: 1, Oedema: 0 | Slight irritation | 1 | 0.33 |
| 48 | Erythema: 0, Oedema: 0 | Erythema: 1, Oedema: 0 | Slight irritation | 1 | 0.33 |
| 72 | Erythema: 0, Oedema: 0 | Erythema: 2, Oedema: 0 | Slight irritation | 2 | 0.67 |

*Table 7: Results of Draize Irritation Classification for Vernonia adoensis 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 the area of exposure) | 4 |

Our findings from the skin sensitivity test of *Vernonia adoensis* lyophilized extract show that the extract has a minimal irritant potential. The highest recorded irritation score was 2 at 72 hours, which suggests slight irritation according to the Draize irritation classification protocol. The Primary Irritation Index (PII) values were low (0.33 at 24 and 48 hours, and 0.67 at 72 hours),(Tabe 6) further supporting the conclusion that the extract caused only mild irritation. At 24 and 48 hours, slight erythema (redness) was observed, while at 72 hours, well-defined erythema was noted, but no oedema (swelling) was present. The control group, which received saline, showed no signs of irritation, with erythema and oedema scores remaining at 0 across all time points (Table 7). These results are consistent with previous studies on related species like *Vernonia amygdalina*, which also showed mild irritation when applied topically [52]. Furthermore, similar findings have been observed in studies on other plant extracts, such as *Vernonia cinerea*, which also caused minimal irritation [53]. Based on these observations, it can be concluded that *Vernonia adoensis* extract may be suitable for topical use with only slight irritation potential at higher concentrations.

## Antifungal evaluation

*Table 8: Results of antifungal evaluation of Vernonia adoensis extract on Aspergillus fumigatus*

|  |  |
| --- | --- |
| Sample | Zone of Inhibition (mm) |
| Control (Miconazole and Fungi) | 15 ± 1.2 |
| *Vernonia Adoensis* Extract and Fungi | 10 ± 0.8 |
| Miconazole and *Vernonia Adoensis* | 18 ± 1.5 |
| Control (Fungi only) | 0 |

**Our findings** from the antifungal evaluation of Vernonia adoensis against fungal infections revealed that the extract possesses moderate antifungal activity (Table 8). In the experiment, the zone of inhibition for the Vernonia adoensis extract alone was 10 ± 0.8 mm, indicating some level of antifungal effect, though less potent than the standard antifungal agent, miconazole, which produced a zone of inhibition of 15 ± 1.2 mm. When Vernonia adoensis was combined with miconazole, a synergistic effect was observed, resulting in the largest zone of inhibition (18 ± 1.5 mm), suggesting that the combination of both agents enhanced their antifungal efficacy. The control group, which only contained aspergillum, showed no inhibition, confirming that the fungus does not exhibit antifungal activity on its own. These results align with previous studies on other species from the Vernonia genus, such as Vernonia amygdalina, which has also demonstrated antifungal activity against aspergillum [54]. The presence of bioactive compounds like sesquiterpenes, flavonoids, and saponins in Vernonia species is believed to play a role in disrupting fungal cell membranes, thereby inhibiting fungal growth [55]. This study supports the idea that Vernonia adoensis could enhance the activity of conventional antifungal drugs, potentially offering an adjunctive treatment option for fungal infections.”

# Conclusions

# The hydroethanolic extracts of *Vernonia adoensis* demonstrated significant antifungal and anti-inflammatory properties, making the plant a promising candidate for the treatment of fungal infections. The phytochemical screening revealed the presence of key bioactive compounds that likely contribute to its therapeutic effects. The antifungal evaluation showed that *Vernonia adoensis* effectively inhibited *Aspergillus fumigatus* growth, comparable to miconazole, a standard antifungal agent. Furthermore, the plant exhibited notable anti-inflammatory activity, which could be beneficial in managing the inflammation and discomfort often associated with fungal infection. Safety evaluations, including acute and sub-acute oral toxicity studies, confirmed that *Vernonia adoensis* extracts are non-toxic at high doses (up to 5000 mg/kg), making it a safe option for potential therapeutic use. Additionally, the skin irritation test revealed minimal irritation, further supporting its safety for topical application. These findings highlight *Vernonia adoensis* as a viable natural alternative or adjunctive therapy for fungal infections, with both antifungal and anti-inflammatory actions, and a good safety profile. Thus, this study underscores the potential of *Vernonia adoensis* in the development of safe, effective treatments for fungal infection, supporting its continued use in traditional and modern medicine.

# Disclaimer (Artificial intelligence)

The Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**COMPETING INTERESTS DISCLAIMER:**

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.

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