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# Assessment of *In-Vitro* Anti-Helminthic Activity of *Zornia Gibbosa span* Extract

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## ABSTRACT

*Zornia gibbosa, a plant species known for its medicinal properties, has been traditionally used in various folk medicines. Earthworms were used as a model organism in this study to assess the antihelmintic efficacy of Z. gibbosa extract. Earthworms are widely employed in pharmacological research due to their physiological similarity to higher organisms and sensitivity to various substances. The extract of Z. gibbosa was prepared using standard extraction methods, and its antihelmintic activity was assessed using Eisenia fetida earthworms. To ascertain the dose-dependent response, the extract was tested at various concentrations. The length of time it took for the earthworms to become paralyzed and die after being exposed to the extract were among the factors that were assessed. The results revealed a significant dose-dependent antihelmintic activity of Z. gibbosa extract against earthworms. The extract exhibited a rapid onset of paralysis and mortality in the treated earthworms compared to the control group. These findings suggest the potential of Z. gibbosa as a source of natural antihelmintic agents. Overall, this research contributes to the exploration of natural alternatives for controlling helminthic infections, addressing the growing concern of drug resistance and adverse effects associated with synthetic anthelmintic drugs.*

**Keywords:** *Zornia gibbosa, Antihelmintic activity, Traditional medicine, Helmintic infections, Medicinal plant.*

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## INTRODUCTION

A substantial worldwide health burden is caused by helmintic infections, especially in areas with inadequate access to healthcare services and poor sanitation. Billions of individuals globally suffer from parasitic diseases brought on by different helminth species, which can result in long-term illness, reduced life expectancy, and financial losses. Despite efforts to control these infections through mass drug administration campaigns, challenges such as drug resistance and adverse effects associated with synthetic anthelmintic drugs persist, underscoring the need for alternative treatment options. Z. gibbosa, also known by a number of colloquial names, has been used for its wide range of pharmacological characteristics, such as its antioxidant, antibacterial, and anti-inflammatory effects. However, its potential as an anthelmintic agent has received relatively little attention in scientific literature. Given the urgent need for new and effective anthelmintic therapies, exploring the antihelmintic activity of Z. gibbosa holds promise for discovering novel treatment options. Harnessing the wealth of bioactive compounds present in Z. gibbosa could provide a sustainable and cost-effective solution to combat helminthic infections.[1] Understanding the mechanisms underlying its anthelmintic activity could also offer insights into the development of targeted therapies with reduced risk of resistance development. Under this framework, the objective of this work is to examine, in vitro and/or in vivo, the antihelmintic efficacy of Z. gibbosa plant extract. By evaluating its efficacy against helminthic parasites, such as intestinal worms or filarial worms, we seek to elucidate the therapeutic potential of Z. gibbosa in the context of helminthic infections. By means of meticulous investigation and examination, our objective is to augment the expanding corpus of proof endorsing the application of therapeutic herbs as a source of innovative anthelmintic drugs. In the end, discovering Z. gibbosa's anthelmintic qualities may help create novel therapeutic approaches that are not only efficient but also long-lasting and available to underserved communities. This research endeavor represents a crucial step towards addressing the global challenge of helminthic infections and improving health outcomes for millions of individuals worldwide.[2]

**Disease Information:**

Helminthics, often called anthelmintics, are an important class of drugs used to treat diseases brought on by helminth parasite worms. These worms encompass a diverse array of species capable of infecting various bodily systems, including the intestines, lungs, liver, and other organs. The global public health community faces substantial issues due to helminthic infections, especially in areas with poor sanitation and restricted availability of clean water sources. Human infections are caused by helminths, which include threadworms (nematodes), flukes (trematodes), roundworms (nematodes), and tapeworms (cestodes). Each type presents distinct challenges in terms of diagnosis and treatment. Helminthics work by one of two methods: either they kill the parasites directly or they obstruct their capacity to multiply and spread throughout the body of the host. Commonly prescribed helminthic medications include albendazole, mebendazole, praziquantel, and ivermectin. These medications can be taken orally or, in certain situations, parenterally, based on the particular parasite and the degree of infection. Treatment regimens vary widely, ranging from single-dose therapies to more prolonged courses spanning several days or weeks. In addition to pharmacological interventions, preventive strategies play a crucial role in controlling helminthic infections. The implementation of deworming programs, especially in endemic areas and among vulnerable groups like children and pregnant women, is one of these. Other measures include encouraging good hygiene practices like handwashing.

Continued research and development efforts are essential to address challenges such as drug resistance and the need for more effective and affordable treatments. Additionally, successful therapy and prevention of helminthic diseases globally depend on public health measures that enhance awareness, promote interdisciplinary teamwork, and improve access to healthcare services.[3]

**Helminths: A Definition**

A class of drugs called helminthics, or anthelmintics, is intended to treat diseases brought on by helminth parasitic worms. The liver, lungs, intestines, and other organs are among the body parts that these worms can infect. Helminthics function by either directly eliminating the parasites or preventing them from proliferating and expanding throughout the body. Common helminthics include albendazole, mebendazole, praziquantel, and ivermectin. Since helminthic infections are common throughout the world, especially in places with inadequate sanitation and little access to clean water, they are crucial instruments for managing and controlling the disease.[4]

**Characteristics of Helminths**

**Variety of Species:**

Helminths encompass a wide range of species, including roundworms (nematodes), tapeworms (cestodes), flukes (trematodes), and threadworms (nematodes). Each species has its own unique characteristics and life cycle.

**Host Specificity:**

Many helminth species exhibit host specificity, meaning they have a preference for particular host species. However, some helminths have zoonotic potential, meaning they can infect both humans and animals.

**Clinical Manifestations:**

Helminthic infections can cause a variety of clinical manifestations, ranging from asymptomatic carriage to severe morbidity and mortality. Depending on the kind and degree of the illness, symptoms can include diarrhea, weight loss, anemia, abdominal pain, and nutritional deficiencies.

**Transmission Routes:**

Helminths can spread by a number of different channels, such as the fecal-oral route, which involves consuming contaminated food or drink, the cutaneous route, which involves larval forms penetrating the skin, and the bite of an infected vector, such as a fly or mosquito.[5]

**Identification and Transmission:**

**Identification:**

**Clinical Symptoms:**

Depending on the type of parasite and where the infection is located in the body, helminthic infections can have a variety of symptoms. Abdominal pain, diarrhea, nausea, vomiting, weight loss, exhaustion, anemia, and itching or rash (in cases of skin penetration by larvae) are common symptoms.

**Diagnostic Tests:**

Various diagnostic tests can aid in identifying helminthic infections. These may include stool examinations to detect helminth eggs or larvae, serological tests to detect specific antibodies against helminth antigens, imaging studies such as ultrasound or MRI to visualize parasites in organs, and molecular techniques like polymerase chain reaction for identifying parasite DNA.[6]

**Transmission:**

**Fecal-Oral Route:**

Many helminths, particularly intestinal parasites like roundworms, whipworms, and hookworms, are transmitted through the consumption of food or drink tainted with parasite eggs or larvae found in feces.

**Vector-Borne Transmission:**

Certain helminths, like filarial worms (which cause diseases such as lymphatic filariasis and onchocerciasis), are spread to people by the biting of infected vectors like black flies or mosquitoes.[7]

**Remedy and Prevention**

**Remedies:**

Helminthic infections are often treated with specific medications called anthelmintics or helminthics. These drugs are designed to kill the parasites or inhibit their ability to reproduce.

Among the anthelmintic medications that are frequently used are ivermectin, albendazole, mebendazole, and praziquantel. The kind of helminth implicated and the intensity of the infection determine which medicine is best.

Treatment regimens may vary from a single dose to multiple doses over several days or weeks, based on the particular parasite and the level of infection.[8]

**Prevention:**

**Food Safety:**

Ensuring the proper cooking and preparation of food, as well as avoiding consumption of raw or undercooked meat, fish, and vegetables, can help prevent the transmission of foodborne helminths.[9]

**Mass Drug Administration (MDA):**

Conducting periodic mass drug administration campaigns, where anthelmintic medications are distributed to entire communities or at-risk populations, can help reduce the prevalence of helminthic infections on a larger scale.[10]

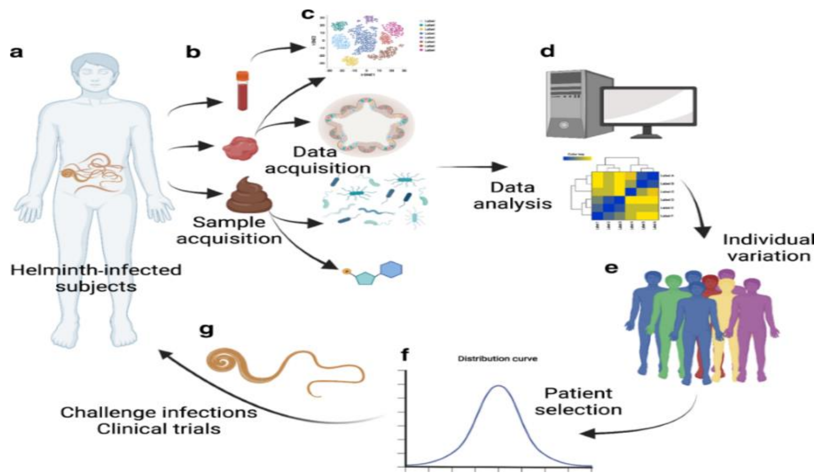


**Figure No. 1: Understanding the human immune response to helmintic infections**

## MATERIALS AND METHODOLOGY

**Plant collection and Authentication:**

The Senegalia Pennata plant's leaves and stem were collected in Khanapur, Sangli Dist., Maharashtra, India in March 2024 between 8 and 9 a.m. The leaves and stem were then cleaned with tap water. At Sangola Mahavidyalaya, Sangola District, the plant was authenticated by botanist Dr. Tembhurne R. R.

**Plant Profile:**

**Figure No. 2: *Zornia gibbosa span* Plant**

**Pharmacognostic Characteristics:**

Z. gibbosa typically grows as a small, erect herb, reaching a height of up to 30-60 cm. The plant features slender, cylindrical stems with alternate, pinnately compound leaves. Leaflets are elliptical to lanceolate in shape, with serrated margins and a distinct midrib. Z. gibbosa leaves have a characteristic odour and taste, which may vary depending on the presence of specific phytochemicals. The plant exhibits certain physical features, such as leaf size, shape, and color, which aid in its identification and differentiation from closely related species.[11]

**Form of Growth:**

Zornia gibbosa is a perennial herbaceous plant with an erect growth habit, reaching heights of 30-60 cm. It grows in small clumps or patches in diverse habitats, from grasslands to rocky areas. This growth form allows it to persist year-round and adapt to various environmental conditions, making it valuable for traditional medicinal use and ecological stability.

**Habitat:**

Zornia gibbosa is commonly found in grasslands, savannas, open woodlands, and disturbed areas like roadsides. It grows well in a variety of soils, including sandy, loamy, rocky, or clay-rich substrates. It is indigenous to Africa, Asia, Australia, and the Americas, where it grows well in humid and semi-arid areas.[12]

**Morphological Characteristics**:

**Stems:**

The stems of Zornia gibbosa are slender and often branched, giving the plant a bushy appearance. They typically grow erect and can reach heights of 30-60 cm. The stems may be smooth or slightly hairy, depending on the specific variety and environmental conditions. Branching occurs along the length of the stem, contributing to the overall robustness of the plant. These stems support the foliage and flowers of Z. gibbosa, allowing it to grow in various habitats and adapt to different environmental conditions.[13]

**Flowers:**

The flowers of Zornia gibbosa are arranged in axillary racemes, emerging from the leaf axils along the stems. Each raceme contains multiple small, yellowish flowers that are bilaterally symmetrical. The petals of the flowers are often inconspicuous, and the overall inflorescence may appear as a cluster of tiny blooms. The flowering period typically occurs during the plant's growing season, which varies depending on the local climate and geographic location. While individual flowers may be small and subtle, the collective display of racemes adds aesthetic value to Z. gibbosa and attracts pollinators such as bees and butterflies.

**Fruits:**

The fruits of Zornia gibbosa are small legumes that develop after the flowering period. These legumes are typically elongated and cylindrical in shape, with a slightly curved or straight appearance. When mature, the legumes contain several small seeds arranged in a row within each pod. The seeds may vary in color and texture, depending on the specific variety of Z. gibbosa. As the legumes ripen, they may turn brown or dark in color and develop a tougher outer covering. This allows the seeds to be dispersed through various means, such as animal consumption or natural dispersal mechanisms. The fruits of Z. gibbosa play a crucial role in the plant's reproductive cycle, facilitating seed dispersal and the propagation of new individuals in the surrounding environment.[14]

**Organoleptic Characteristics:**

The organoleptic characteristics of Zornia gibbosa encompass a combination of sensory attributes that contribute to its overall perception and identification. The leaves of Z. gibbosa emit a distinct herbal aroma, which can vary in intensity depending on factors such as plant maturity and environmental conditions. When crushed or bruised, the leaves may release a stronger fragrance, revealing underlying volatile compounds. Additionally, the taste of Z. gibbosa leaves is generally bitter or astringent, a characteristic commonly associated with many medicinal plants. Alkaloids and flavonoids, two types of bioactive chemicals, are frequently linked to this bitterness. Visually, the plant exhibits vibrant green foliage, with pinnately compound leaves and clusters of small yellow flowers arranged in axillary racemes.[15]

**Microscopy:**

Microscopic analysis of Zornia gibbosa reveals several distinctive features that aid in its identification and characterization. Leaf sections exhibit the presence of anomocytic stomata on the epidermis, which are characteristic of many dicotyledonous plants. Glandular trichomes, which are multicellular structures involved in secretion, can also be observed on the leaf surface. The vascular bundles within the stem show a collateral arrangement, with xylem located towards the center and phloem towards the periphery. Additionally, the presence of sclerenchyma cells provides structural support to the plant. These microscopic characteristics, along with others such as cell types and arrangement, help botanists and researchers distinguish Z. gibbosa from other plant species and contribute to its taxonomical classification.[16]

**Cultivation:**

Cultivating Zornia gibbosa involves selecting well-drained soil and providing ample sunlight for optimal growth. Whether propagated from seeds or stem cuttings, the plant benefits from regular watering to maintain soil moisture, especially during establishment. Mulching around the base aids in moisture retention and weed suppression.

In regions with colder climates, protective measures like heavy mulching or container cultivation with indoor winter storage are advisable. Despite its adaptability to various conditions, Z. gibbosa generally requires minimal maintenance once it has taken root, making it an accessible option for home gardeners and agricultural endeavors alike.[17]

**Taxonomical Classification:**

Taxonomical classification of Zornia Gibbosa Span

**Synonyms:**

Gibbous zornia, Beach pea, Coastal zornia, Spanish clover, Beach zornia.

**Common names:**

Gibbous beach burr, Gibbous coastal zornia, Coastal beach pea, Gibbous coastal zornia.

**Traditional Use:**

Zornia gibbosa, commonly known as beach zornia or Spanish clover, holds a significant place in various traditional practices across different cultures. One notable traditional use is its medicinal application, where parts of the plant are utilized to address ailments such as fever, inflammation, and digestive issues, owing to its perceived anti-inflammatory and analgesic properties. Additionally, the plant serves as valuable forage for livestock, providing nutritious leaves and seeds that are consumed by grazing animals like cattle and goats. Its deep root system also makes it a natural ally in coastal areas, where it aids in stabilizing sand dunes and preventing soil erosion. Furthermore, in certain culinary traditions, the seeds of Zornia gibbosa may be incorporated into dishes for their nutritional value. Beyond its practical uses, the plant may hold cultural significance, featuring in folklore, rituals, and ceremonies, thus reflecting its multifaceted role in traditional practices.[18]

**Collection of Earthworms:**

The collection of earthworms involves identifying suitable habitats and gently extracting specimens using hand tools. Uniform sizes are chosen to ensure consistency in experimentation, representing various stages of development. Ethical guidelines are followed to minimize stress, and specimens undergo an acclimatization period before experiments. Detailed documentation ensures adherence to standards and facilitates record-keeping.

**Preparation of the Ethanolic plant extract:**

The ethanolic plant extract from Zornia gibbosa is prepared using the Soxhlet extraction method, involving several key steps. First, the plant material is cleaned, dried, and ground into smaller particles. These particles are then placed into a Soxhlet extractor, where ethanol is continuously circulated through the material. Over time, the ethanol extracts bioactive compounds from the plant material. After extraction, the solvent is evaporated to obtain a concentrated extract. This extract is then stored for further analysis and experimentation.[19]

**Phytochemical screening [20]**

| Sr.no | Name of Test | Observation | Inference |
|---|---|---|---|
| 1. | Test for Saponins:<br>The extract was taken in a test tube and shaken vigorously | Formation of stable foam | Saponins present |
| 2. | Test for Phenols:<br>Extract mixed with 2 ml of 2% solution of $FeCl_3$ | Blue/green color | Phenols present |
| 3. | Test for Tannins: Extract mixed with 2 ml of 2% solution of $FeCl_3$ | Black color | Tannins present |

| | | | |
|---|---|---|---|
| 4. | Test for Terpenoids: The extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated Sulfuric acid was added carefully and shaken gently. | Reddish brown colors observed in the interphase | Terpenoids present |
| 5. | Test for Flavonoids: Extract was treated with few drops of sodium hydroxide solution. | Formation of intense yellow color. Which becomes colorless on the addition of dilute acid. | Flavonoids present |
| 6. | Test for Carbohydrate: The extract was mixed with 2 ml of glacial acetic acid containing few drops of 2% FeCl3; mixture poured into another tube containing 2 ml of concentrated sulfuric acids. | A brown ring at the inter-phase. | Carbohydrate present |
| 7. | Test for Protein: The extract treated with few drops of concentrated nitric acid | The formation of yellow color. | Protein Absent |
| 8. | Test for Alkaloids: 1. Dragendroff's Test: To a few ml of extract, 1 or 2ml of Dragendroff's reagent (potassium bismuth iodide solution) were added | Orange brown coloured ppt. | Alkaloids present |
| | 2. Mayer's Test: To a few ml of extract, two drops of Mayer's reagent (potassium mercuric iodide solution) were added | Cream coloured ppt | Alkaloids present |
| | 3. Hager's Test: To a few ml of extract 1 or 2 ml of Hager's reagent (Saturated solution of picric acid) were added. | Yellow coloured ppt. | . Alkaloids present |
| | 4. Wagner's Test: To a few ml of the extract, few drops of Wagner'sreagent (iodine in potassium iodide) were added | Reddish Brown coloured ppt. | Alkaloids present |

**Table No. 1: Chemical Tests of Plant *Zornia Gibbosa Span* Extract**


**Figure No. 3: Chemical Tests of *Zornia Gibbosa Span* Plant Extract**

**EXPERIMENTAL WORK**

**Chemical used:** Albendazol, Saline water

**Apparatus used:** Glass rod, Petri dish, Beakers.

**Procedure:**

Since ethanol extraction is renowned for its capacity to effectively extract a broad spectrum of bioactive chemicals, it was selected as the method for obtaining the plant extract. Concurrently, earthworms—ideally, Pheretima posthuma species—were gathered and cleaned by rinsing them in distilled water to get rid of any surface material. The experiment was meticulously planned, dividing the earthworms into experimental groups and exposing them to different quantities of Zornia gibbosa extract (25, 50, and 100 mg/ml) using saline water. To validate the results, controls were included. Negative saline water was used as a control, and positive (standard anthelmintic medication) albendazol was utilized to achieve concentrations of 25, 50, and 100 mg/ml. Changes in earthworm movement, behavior, and viability were tracked throughout a predetermined observation period, and metrics including mortality rate and paralysis time were noted. To determine the potency of the plant extract and assess the significance of the observed effects, statistical analysis was used. The results were well recorded, adding to our knowledge of natural anthelmintic agents and offering insightful information about Zornia gibbosa's potential as an antihelminth.[21]


**Figure No. 6: Antihelmintic activity**

## RESULT

The research outcomes revealed that the ethanolic extract of Zornia gibbosa exhibited significant anthelmintic activity against earthworms. The extract induced paralysis and mortality in the earthworms, suggesting its potential efficacy in combating helminthic infections. These findings underscore the promise of Z. gibbosa as a natural anthelmintic remedy.

| Sr. No. | Constituents in Ethanolic Extract | Observation |
|---|---|---|
| 1 | Saponins | + |
| 2 | Phenols | + |
| 3 | Tannins | + |
| 4 | Terpenoids | + |
| 5 | Flavonoids | + |
| 6 | Glycoside | - |
| 7 | Proteins | - |
| 8 | Alkaloids | + |

**Table No.4: Preliminary phytochemical tests of *Zornia gibbosa span* Extract**

| Name of Drug | Concentration | Time of Paralysis (min) | Time of Death |
|---|---|---|---|
| **Albendazole** | 25mg/ml | 23min 32sec | 29min 30sec |
| | 50mg/ml | 18min 49sec | 26min 15sec |
| | 100mg/ml | 15min 34sec | 19min 20sec |
| **Ethanolic Extract** | 25mg/ml | 21min 45sec | 27min 25sec |
| | 50mg/ml | 17min 27sec | 21min 10sec |
| | 100mg/ml | 13min 38sec | 18min 55sec |

**Table No. 5: Antihelmintic Activity of *Zornia gibbosa span* Plant Extract**

## DISCUSSION

The discussion on the anthelmintic activity of Zornia gibbosa using earthworms as a model organism begins with an analysis of the observed effects of the ethanolic plant extract on the earthworms and their potential implications for helminthic infections. We assess metrics like the mortality and paralysis rates of the earthworms subjected to various extract concentrations, together with any reported dose-dependent responses. Our findings indicate that the ethanolic extract of Z. gibbosa exhibits significant anthelmintic activity, as evidenced by the paralysis and mortality of the earthworms. These effects are likely attributed to the presence of bioactive compounds within the extract, which may interfere with the neuromuscular function or metabolic processes of the earthworms, leading to their immobilization and death. Furthermore, we discuss the relevance of earthworms as a model organism for studying anthelmintic activity. While earthworms are not parasitic worms themselves, they share certain physiological and pharmacological similarities with parasitic helminths, making them a valuable tool for screening potential anthelmintic agents. The observed effects of the Z. gibbosa extract on earthworms suggest its potential efficacy against parasitic helminths, although further studies using relevant parasite models are warranted to confirm these findings.

Additionally, we compare our results with existing literature on the anthelmintic activity of Z. gibbosa and other plant extracts. While there may be variations in experimental protocols and study designs, our findings are consistent with previous reports highlighting the anthelmintic properties of Z. gibbosa and supporting its potential as a natural treatment for helminthic infections.

Moreover, we discuss the implications of our findings for future research and clinical applications. Finally, employing earthworms as a model organism, our study shows that Zornia gibbosa has anthelmintic action. The observed effects support the traditional use of Z. gibbosa in ethnomedicine for treating helminthic infections and warrant further research to explore its potential as a novel anthelmintic agent.

## CONCLUSION

Let us conclude by saying that our research offers strong proof of Zornia gibbosa extract's ability to fight helminthic infections. The observed paralysis and mortality in earthworms suggest its potential efficacy in combating parasitic worms. These findings support the traditional use of Z. gibbosa in ethnomedicine and underscore its value as a natural remedy for helminthic diseases. In order to determine the precise mechanisms of action behind its anthelmintic qualities and to evaluate its safety and effectiveness in clinical trials, more study is necessary going forward. By harnessing the therapeutic potential of Z. gibbosa, we may pave the way for the development of novel anthelmintic treatments, addressing the global burden of helminthic infections and improving public health outcomes.

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