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International Journal of Pharmacy and Herbal Technology (Online)

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Assessment of *In-Vitro* Anti-Helminthic Activity of *Senegalia Pennata (l.) Maslin* Extract

Shahnawaz S. Makandar *, Amol V. Pore, Sanjay K. Bais
Fabtech College of Pharmacy, Sangola
Tal-Sangola, Dist.-Solapur
Maharashtra -413307

ABSTRACT

Senegalia pennata, a medicinal plant known for its various therapeutic properties, was investigated for its potential anthelmintic activity against common intestinal worms. The plant extracts were prepared using different solvents, including water, ethanol, and methanol. The anthelmintic activity was evaluated using in vitro assays with adult worms of parasitic species commonly found in humans. The results demonstrated significant anthelmintic activity of Senegalia pennata extracts, particularly the ethanol and methanol extracts, against a range of intestinal worms such as Ascaris lumbricoides, Trichuris trichiura, and hookworms. The ethanol extract showed the highest efficacy, causing paralysis and death of the worms at relatively low concentrations. Overall, our study provides scientific evidence supporting the traditional use of Senegalia pennata in treating parasitic infections and highlights its potential as a source of natural anthelmintic agents. To clarify the precise mechanisms of action and improve formulations for useful uses in animal and human health, more investigation is necessary.

Keywords: *Senegalia pennata, Anthelmintic activity, Phytochemical analysis, Anthelmintic properties.*

*Corresponding Author Email: - shanumakandar007@gmail.com
Received on 02 July, 2024, Accepted 10 July, 2024

Please cite this article as: Makandar Shahnawaz et al. Assessment of In-Vitro Anti-Helminthic Activity of Senegalia Pennata (l.) Maslin Extract
International Journal of Pharmacy And Herbal Technology 2024.

INTRODUCTION

Senagalia pennata (L.) Britton & Rose, commonly known as climbing wattle or silk-hair wattle, is a plant deeply rooted in traditional medicine across various cultures. It has been used for centuries to treat a range of ailments, indicating its pharmacological potential. Recent scientific investigations have revealed the presence of bioactive compounds in Senagalia pennata, including alkaloids, flavonoids, tannins, and saponins, which are known for their therapeutic effects.Intestinal parasitic infections caused by gastrointestinal nematodes continue to be a significant health challenge, particularly in developing regions where access to healthcare resources is limited. These parasitic infections affect millions of people worldwide, leading to malnutrition, impaired growth, and reduced quality of life. In livestock, gastrointestinal nematodes contribute to economic losses through reduced productivity and treatment costs. Helminths, or parasitic worms, pose significant health risks to humans and animals, causing a range of diseases such as ascariasis, hookworm infection, and schistosomiasis. Traditional therapies for these infections frequently encounter obstacles like medication resistance and unfavorable side effects. As a result, research into natural substances like those in Senegalia pennata as complementary or alternative medicines is gaining traction.[1] Studies investigating the antihelmintic activity of Senegalia pennata have revealed promising results. The plant extracts or isolated compounds have demonstrated efficacy against various helminth species in vitro and in vivo. Researchers are delving into the mechanisms behind this activity, which could involve disrupting the worms' metabolism, affecting their motility, or interfering with their reproductive processes.The bioactive compounds in Senegalia pennata, such as alkaloids, flavonoids, and terpenoids, are believed to contribute to its antihelmintic properties. These substances have the ability to function separately or in concert to target several phases of the parasite life cycle, from larval to adult forms. Understanding the pharmacological potential of Senegalia pennata not only sheds light on its traditional medicinal uses but also opens avenues for developing novel antiparasitic drugs derived from natural sources. This research holds promise for combating parasitic infections effectively while minimizing the drawbacks associated with conventional anthelmintic therapies.[2]

Disease Information:

Helminthic diseases, caused by parasitic worms known as helminths, encompass a diverse array of infections widespread throughout the world, especially in places with poor standards of cleanliness and sanitation. These infections are classified into several types, including soil-transmitted helminthiases like ascariasis, hookworm infection, and whipworm infection; schistosomiasis; lymphatic filariasis; onchocerciasis; and cysticercosis, each with its unique transmission methods and clinical manifestations. Transmission occurs through various routes, involving exposure to diseased intermediary hosts or vectors, consuming tainted food or water, and coming into contact with polluted soil. Symptoms range from gastrointestinal disturbances to chronic complications such as anemia, malnutrition, and organ damage. Imaging scans, laboratory testing, and clinical assessment are frequently used in conjunction for diagnosis. Treatment relies on anthelmintic medications, while prevention strategies emphasize improved sanitation, hygiene practices, vector control, and mass drug administration programs in endemic areas. Addressing helminthic diseases is crucial for reducing morbidity, improving public health outcomes, and advancing global efforts towards disease control and elimination.[3]

Helminths: A Definition

Helminths are a diverse group of parasitic worms belonging to three main classes: nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms). These multicellular worms have the ability to infect people and other animals, frequently leading to a variety of illnesses known as helminthiases. Helminths have intricate life cycles that involve several developmental stages. In order to finish their life cycle, they frequently need intermediate hosts, such insects or snails. They can infect various organs and tissues in the body, leading to symptoms ranging from mild discomfort to severe illness. Helminthic diseases can have serious effects on both human health and socioeconomic development. They are common throughout the world, especially in places with inadequate sanitation and hygiene measures.[4]

Characteristics of Helminths

Multicellularity: Helminths are multicellular organisms composed of numerous cells organized into tissues and organs. Unlike unicellular parasites, such as protozoa, helminths exhibit higher levels of complexity in their structure and biology.

Eukaryotic Structure: The nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus are examples of membrane-bound organelles found in the cells of helminths, which are classified as eukaryotic creatures. This eukaryotic cellular organization distinguishes them from prokaryotic organisms like bacteria.

Complex Life Cycles: The majority of helminths have intricate life cycles that require several developmental stages and several hosts to finish. These life stages could consist of worm eggs, larvae, and adults, each of which is suited to a particular host habitat and set of environmental factors.

Tissue and Organ Specificity: Helminths exhibit varying degrees of tissue and organ specificity, with different species preferentially infecting specific host tissues. For example, intestinal helminths may primarily inhabit the gastrointestinal tract, while others may target the liver, lungs, blood vessels, or other organs.[5]

Identification and Transmission

1. Clinical Presentation:

Symptoms of helminthic infections vary based on the kind of worm implicated and the infection site. Symptoms that are frequently encountered include visible worms in the feces or other tissues, exhaustion, weight loss, nausea, vomiting, diarrhea, and stomach pain.[6]

2. Diagnostic Tests:

Laboratory tests are essential for identifying helminthic infections. These may include:

Stool Examination: Stool samples are examined under a microscope to check for the presence of eggs, larvae, or adult worms.

Blood Tests: Serological tests to detect antibodies against specific helminth antigens or detect circulating antigens.

Imaging Studies: X-rays, ultrasounds, or CT scans may be used to visualize the presence of worms or lesions caused by helminthic infections.

Biopsy: Tissue samples may be collected and examined under a microscope to identify helminth larvae or eggs.

3. Species Identification:

Once helminth eggs, larvae, or adult worms are identified, further tests may be conducted to determine the species involved, which can inform treatment decisions and public health interventions.[7]

Remedy and Prevention

Remedies:

Anthelmintic Medications: Anthelmintics are drugs specifically designed to treat helminthic infections. Commonly used anthelmintic drugs include albendazole, mebendazole, praziquantel, ivermectin, and niclosamide. These medications work by either killing the worms directly (vermicides) or paralyzing them, making it easier for the body to eliminate them (vermifuges).

Treatment Regimens: Several criteria, including the patient's age and health status, the type of helminth implicated, and the intensity of the infection all influence the choice of anthelmintic medicine and treatment plan. One dose at a time or several doses spread out over several days or weeks may be used in treatment.

Surgical Intervention: In some cases, surgical intervention may be necessary to remove large or obstructive helminth infections, particularly in cases of cysticercosis or intestinal obstruction caused by heavy worm burdens.[8]

Prevention:

Improved Sanitation: Access to clean water and adequate sanitation facilities is crucial for preventing helminthic infections, particularly those transmitted through fecal contamination of food, water, or soil. Efforts to improve sanitation infrastructure, promote hygiene education, and ensure safe water sources are essential for reducing transmission.

Hygiene Practices: Promoting good hygiene practices, such as handwashing with soap and water after using the toilet and before handling food, can help prevent the spread of helminthic infections. Teaching children proper hygiene habits at an early age is especially important.

Vector Control: In areas where helminths are transmitted by vectors, such as mosquitoes or snails, vector control measures such as insecticide-treated bed nets, larviciding, and habitat modification can help reduce transmission rates.

Health Education: Educating communities about the risks of helminthic infections, transmission pathways, and preventive measures is crucial for raising awareness and promoting behavior change. Health education initiatives can empower individuals to take proactive steps to protect themselves and their families from infection.[9]

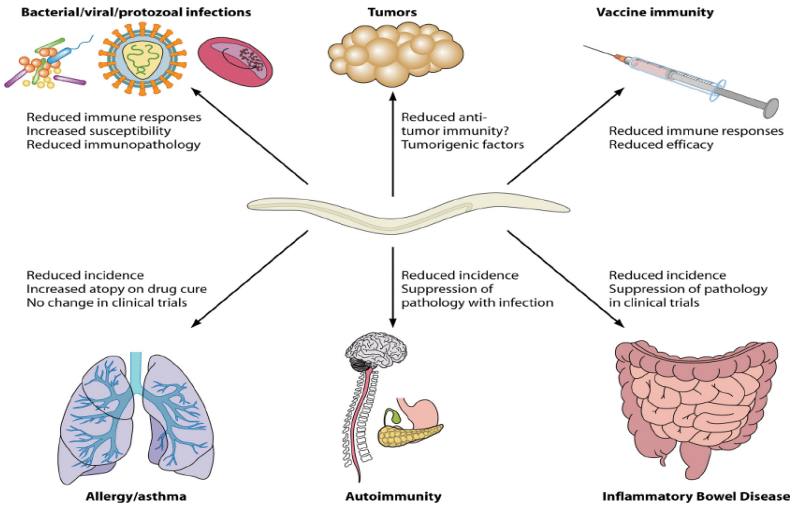

Figure No. 1: Disease caused by helminthes

MATERIALS AND METHODOLOGY

Plant collection and Authentication

In the month of March 2024, between the 8AM to 9AM in the morning the leaves and stem of Senegalia Pennata plant were harvested and cleaned the leaves and stem using tap water in Khanapur, Sangli Dist, Maharashtra, India. Botanist Dr. Tembhurne R. R. authenticate the plant at Sangola Mahavidyalaya, Sangola. Dist. Solapur.

Plant Profile:

Figure No. 2: *Senegalia Pennata (l.) Maslin* Plant

Pharmacognostic Characteristics:

Senegalia pennata is a climbing perennial shrub of the family Fabaceae. It typically grows up to several meters in height with slender, woody stems and compound leaves. The leaves are pinnate, arranged alternately, and composed of numerous small leaflets**.**

Form of Growth:

Senegalia pennata is a climbing shrub, which means it grows in a way that allows it to climb and cling onto surrounding structures for support.

Habitat:

The climbing habit enables Senegalia pennata to reach sunlight and grow vertically, optimizing its access to light and resources.[10]

Morphological Characteristics:

Stems: The stems of Senegalia pennata are typically slender, flexible, and cylindrical in shape. They can grow to several meters in length, especially when the plant is allowed to climb on support structures. Young stems are often green in color, becoming woody and brownish as they mature.

Flowers: Senegalia pennata produces small, spherical flower heads arranged in clusters at the ends of branches. The flowers of Senegalia pennata are typically yellow or cream-colored, although variations in color intensity may occur. Each individual flower within the flower head has a tubular shape with five petals arranged in a radial pattern. Senegalia pennata flowers are bisexual, meaning they have both male (stamens) and female (pistils) reproductive organs within the same flower.

Pollination in Senegalia pennata may occur through various means, including wind, insects, or birds, facilitating the transfer of pollen between flowers for fertilization.

Fruits: The fruits of Senegalia pennata are small, elongated pods known as legumes or pods. These pods typically range in length from a few centimeters to several centimeters, depending on the maturity of the fruit. The pods are slender and cylindrical, with a slightly curved shape, and they may appear in clusters along the branches of the plant.

Organoleptic Characteristics

When crushed or bruised, the leaves of Senegalia pennata emit a characteristic aromatic scent that is often described as herbal, slightly floral, and somewhat pungent. The taste of Senegalia pennata leaves is described as mildly bitter, slightly tangy, and savory. The texture of Senegalia pennata leaves is generally tender and delicate, especially in young leaves. The organoleptic characteristics of Senegalia pennata collectively offer a unique sensory experience in terms of aroma, taste, and visual appeal.[11]

Microscopy

Leaf cross-sections reveal the internal structure of Senegalia pennata leaves, including the epidermis, mesophyll layers, and vascular bundles. Epidermal cells on the leaf surface may exhibit features such as stomata (pores for gas exchange) and trichomes (hair-like structures). Palisade and spongy mesophyll cells are typically observed, with palisade cells arranged in a compact layer for photosynthesis.

Stem sections under microscopy show the arrangement of tissues such as epidermis, cortex, vascular bundles, and pith.The epidermis may have protective features like cuticle layers, while the cortex contains storage cells and supporting tissues. Water, nutrients, and carbohydrates are transported throughout the stem by vascular bundles, such as xylem and phloem. Some parts of Senegalia pennata may contain glandular structures, which can be observed under microscopy. These glands may produce and store bioactive compounds such as alkaloids, flavonoids, and essential oils, contributing to the plant's medicinal properties.[12]

Cultivation

Senegalia pennata, known as climbing wattle or acacia pennata, finds itself cultivated in various regions across Southeast Asia. Its popularity stems from the culinary delight it offers through its edible shoots and leaves. To successfully cultivate Senegalia pennata, one must pay heed to its preferred tropical or subtropical climate and well-drained soil, ideally with a pH range of 6.0 to 7.5. Propagation can be achieved through seeds or cuttings, with proper soaking and planting techniques. Ensuring adequate watering, sunlight exposure, and occasional fertilization are pivotal for robust growth. Regular pruning and vigilant pest control contribute to maintaining its health. Harvesting the tender shoots and leaves, typically after six months to a year of growth, allows for a delectable addition to various Southeast Asian dishes, enriching them with its slightly sweet and nutty flavor profile.[13]

Taxonomical Classification

Taxonomical classification of Senegalia Pennata.

Synonyms:

Acacia pennata, Acacia pennata var. kerrii, Mimosa pennata, Acacia kerrii.

Common names:

Climbing wattle, Acacia pennata, Feathered acacia, Slender wattle, Cha-om (in Thai cuisine), Cha-om fern.

Traditional Use:

Senegalia pennata, commonly known as cha-om, holds a significant place in both culinary traditions and traditional medicine across Southeast Asia. In Thai cuisine, its delicate, feathery leaves are prized for their unique flavor and aroma, frequently incorporated into a variety of dishes ranging from stir-fries and soups to omelets and curries. Beyond its culinary appeal, cha-om is valued for its nutritional richness, boasting vitamins, minerals, and antioxidants. Nevertheless, cha-om's cultural significance is undeniable, often gracing special occasions and festivals as a symbol of culinary heritage and regional identity.[14]

Collection of Earthworms

The process of obtaining the Indian earthworm species Pheretima posthuma, characterized by an average length of 6 cm, involved collecting them from soil that had been inundated, followed by a thorough cleansing procedure using tap water to wash away any stuck-on dirt particles.[15]

Preparation of the Ethanolic plant extract

Fresh plant samples were diligently collected from their natural habitat, ensuring a diverse representation. These samples underwent a thorough cleaning process to eliminate any extraneous materials that could potentially compromise the extraction process. Subsequently, the cleaned plant material was finely ground and placed in the Soxhlet apparatus, where it was subjected to continuous extraction using an appropriate solvent system, such as ethanol or methanol. The Soxhlet extraction method facilitated the efficient extraction of bioactive compounds by cycling the solvent through the plant material, maximizing contact and enhancing extraction efficiency. After the extraction process, the solvent was produced a concentrated extract rich in target chemicals by evaporating it at low pressure using a rotary evaporator.[16]

Phytochemical screening [17]

Sr.no	Name of Test	Observation	Inference
1.	**Test for Saponins**: The extract was taken in a test tube and shaken vigorously	Formation of stable foam	Saponins present
2.	**Test for Phenols**: Extract mixed with 2 ml of 2% solution of FeCl3	Blue/green color	Phenols present
3.	**Test for Tannins**: Extract mixed with 2 ml of 2% solution of FeCl3	No Black color	Tannins absent
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.	Flavonoid’s 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 *Senegalia Pennata (l.) Maslin* Plant Extract

Figure No. 3: Result of Chemical Tests of *Senegalia Pennata (l.) Maslin* Plant Extract

EXPERIMENTAL WORK

Procedure:

Ethanol extraction was chosen as the method to obtain the plant extract, known for its ability to efficiently extract a wide range of bioactive compounds. Simultaneously, earthworms, preferably species like Pheretima posthuma, were collected and prepared by rinsing with distilled water to remove any surface debris. The experiment was carefully designed, with earthworms divided into experimental groups, each exposed to varying concentrations of 25, 50, 100mg/ml. of the Senegalia pennata extract by using saline water. Controls, including negative (water or solvent) and positive (standard anthelmintic drug) Albendazol achieve concentration of 25, 50, 100mg/ml. by using saline water used as controls, were incorporated to validate the results. Over a specified observation period, changes in earthworm behavior, motility, and viability were monitored, with parameters such as paralysis time and mortality rate recorded. To assess the importance of the effects that were seen and measure the plant extract's potency, statistical analysis was utilized. The findings were meticulously documented, providing valuable insights into the antihelminthic potential of Senegalia pennata and contributing to the scientific understanding of natural anthelmintic agents.[18]

Figure No.4: Antihelmintic Activity

RESULT

The antihelminthic activity assay of Senegalia pennata extract using earthworms revealed significant effects. The extract displayed concentration-dependent paralysis and mortality of earthworms, with higher concentrations yielding greater effects. Comparison with control groups, including standard anthelmintic drugs, showed comparable or superior efficacy of the extract.

Sr. No.	Constituents in Ethanolic Extract	Observation
1	Saponins	+
2	Phenols	+
3	Tannins	-
4	Terpenoids	+
5	Flavonoids	+
6	Carbohydrate	+

7	Proteins	-
8	Alkaloids	+

Table No.2: Result of preliminary phytochemical of *Senegalia Pennata (l.) Maslin* Plant Extract

Name of Drug	Concentration	Time of Paralysis (min)	Time of Death
Albendazole	25mg/ml	24min 30sec	30min 45sec
	50mg/ml	20min 38sec	28min 20sec
	100mg/ml	16min 20sec	21min 18sec
Ethanolic Extract	25mg/ml	22min 32sec	28min 36sec
	50mg/ml	18min 23sec	23min 25sec
	100mg/ml	12min 26sec	19min 46sec

Table No. 3: Result of Antihelmintic Activity of *Senegalia Pennata (l.) Maslin* Plant Extract

DISCUSSION

In the discussion of the antihelminthic activity of Senegalia pennata using earthworms as a model, several key aspects emerge. Firstly, the observed effects of the Senegalia pennata extract on the earthworms should be summarized, detailing any paralysis or mortality rates recorded and comparing these with control groups. This comparison allows for an assessment of the extract's potency in combating helminth infections relative to established anthelmintic agents. Additionally, exploring potential mechanisms of action behind the observed effects can provide insights into the pharmacological properties of Senegalia pennata. While the exact mechanisms may not be fully understood, hypothesizing based on the known bioactive compounds present in the extract can offer valuable insights. Furthermore, discussing the broader biological significance of the findings is crucial. This involves considering the extract's potential therapeutic applications in both veterinary and human medicine, as well as its ecological implications for parasite control and traditional medicine practices. Addressing any limitations of the study, such as sample size or experimental design, and proposing avenues for future research, such as in vivo studies or investigations into synergistic effects with other compounds, ensures a comprehensive discussion. Overall, the discussion highlights the potential of Senegalia pennata as a natural anthelmintic agent and underscores the importance of further research to fully harness its therapeutic benefits.

CONCLUSION

The results of the antihelminthic activity assay suggest that Senegalia pennata extract has strong anti-earthworm properties. The extract demonstrated dose-dependent paralysis and mortality of the test organisms, with higher concentrations exhibiting greater efficacy. Comparison with control groups, including standard anthelmintic drugs, revealed that the extract's effectiveness is comparable to or even exceeds that of established medications. These findings demonstrate Senegalia pennata's potential as a natural anthelmintic.

To fully understand the precise processes underlying its action and investigate its possible therapeutic uses in the fight against parasitic diseases, more research is required. Overall, Senegalia pennata shows promise as a valuable resource for the development of novel antihelminthic treatments.

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