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EVALUATION OF ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM*

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Abstract:

Background: *Ocimum sanctum (commonly known as Holy Basil or Tulsi) is a revered herb in traditional medicine, renowned for its diverse pharmacological properties. This study aimed to evaluate the anthelmintic activity of ethanolic extracts of Ocimum sanctum leaves against Pheretima posthuma (earthworms), which serves as a model organism for screening anthelmintic agents.*

Methods: *Fresh leaves of Ocimum sanctum were collected, authenticated, and processed to obtain an ethanolic extract using a Soxhlet apparatus. The anthelmintic activity was assessed by preparing extracts at concentrations of 20, 40, and 60 mg/ml. The time taken for paralysis and death of the earthworms was recorded and compared against a control (normal saline) and the standard drug, Albendazole (20 mg/ml).*

Results: *The ethanolic extract of Ocimum sanctum exhibited significant anthelmintic activity. The time for paralysis and death decreased with increasing extract concentration, with the highest efficacy observed at 60 mg/ml, where paralysis occurred within 28 minutes and death within 35 minutes. In comparison, the control group showed no activity, while Albendazole exhibited paralysis in 18 minutes and death in 28 minutes.*

Conclusion: *The findings suggest that the ethanolic extract of Ocimum sanctum leaves possesses promising anthelmintic properties, potentially validating its traditional use in herbal medicine. Further studies are warranted to isolate and characterize the active constituents responsible for this activity and to explore their mechanisms of action.*

Keywords: Ocimum sanctum *leaves, Albendazole, paralysis time, death time, anthelmintic activity, helminthiasis, traditional medicine.*

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INTRODUCTION:

Helminthiasis, or intestinal worm infections, is a major public health problem affecting millions of people worldwide, particularly in tropical and subtropical regions. Caused by parasitic worms, known as helminths, this condition leads to a wide range of health complications, including malnutrition, anemia, cognitive impairment, and, in severe cases, death. Helminths are typically classified into three main types: tapeworms, flukes, and roundworms, each posing significant health risks to both humans and animals. While synthetic anthelmintic drugs like Albendazole are commonly used to treat these infections, the rise of drug resistance and potential side effects has increased interest in exploring alternative treatments from natural sources.

Plants have long been used in traditional medicine systems for the treatment of various diseases, including parasitic infections. *Ocimum sanctum* (Holy Basil), commonly known as Tulsi, is a revered medicinal plant in India, valued for its diverse therapeutic properties. Belonging to the Lamiaceae family, *Ocimum sanctum* has been extensively used in Ayurvedic medicine to treat a range of ailments such as respiratory disorders, skin diseases, and gastrointestinal disturbances. Recent studies have demonstrated that *Ocimum sanctum* possesses numerous pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory activities. However, its potential as an anthelmintic agent remains relatively underexplored.

The leaves of *Ocimum sanctum* are rich in bioactive compounds, including eugenol, ursolic acid, and various flavonoids, which have been reported to possess antimicrobial and anti-inflammatory activities. Given its broad medicinal value, there is a strong rationale for investigating the anthelmintic properties of *Ocimum sanctum* to develop natural remedies for parasitic infections.

Ocimum sanctum, commonly known as Tulsi or Holy Basil, holds a revered place in Hindu religious tradition and is deeply integrated into the cultural fabric of India. The name "Tulsi" translates to "the incomparable one," symbolizing its unique status among sacred plants, while its other name, "Vishnupriya," means "the one who pleases Lord Vishnu." Found in nearly every Indian household, Tulsi is not only worshipped but also valued for its extensive medicinal properties, a reputation that has persisted through generations. Its legend and significance have permeated Indian ethos for centuries, serving as both a spiritual symbol and a valuable medicinal resource.

Botanically identified as *Ocimum sanctum*, Tulsi belongs to the Lamiaceae family. It has been a cornerstone of traditional medicine systems like Ayurveda, contributing significantly to both ancient and modern scientific research due to its wide range of therapeutic benefits. Tulsi is generally classified into two types: *vanya* (wild) and *gramya* (domestically grown). While both types share similar medicinal uses, *vanya* is distinguished by its darker leaves. The plant has been used for centuries as a home remedy to treat a variety of ailments, including bronchitis, liver diseases, fevers, wounds, earaches, hiccups, lumbago, gastric disorders, and even psychosomatic stress disorders. It has also been used for skin diseases, poisoning, and genitourinary disorders.

Apart from its traditional uses, Tulsi has been extensively studied for its pharmacological properties. It possesses aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, vermifuge, and febrifuge properties, making it a highly versatile medicinal plant. It has also been recognized for its ability to alleviate stress and combat infections, contributing to its widespread use in modern herbal medicine. In light of these numerous benefits, researchers have continually explored the pharmacological activities of *Ocimum sanctum* in various experimental and clinical settings, validating its therapeutic potential.

Tulsi grows abundantly in tropical and warm regions and is widely distributed and cultivated throughout India. The plant is an erect, much-branched, and aromatic herb that can grow to a height of 30-60 cm when mature. Its simple, aromatic leaves are elliptic or oblong, with serrated or dentate margins, growing up to 5 cm long. The small, purplish flowers bloom in elongate racemes, and the fruit contains small, reddish-yellow seeds. The plant has a bitter and acrid taste, further enhancing its medicinal value.

Given its long-standing use in traditional medicine and its broad range of therapeutic properties, *Ocimum sanctum* continues to be the subject of modern research aimed at harnessing its potential for various pharmacological applications. This review explores its diverse pharmacological activities as documented in both experimental and clinical studies, highlighting its ongoing significance in the fields of medicine and science.

This study aims to evaluate the anthelmintic activity of the ethanolic extract of *Ocimum sanctum* leaves. The focus is on determining the time to paralysis and death of worms at different extract concentrations and comparing the efficacy with Albendazole, a standard anthelmintic drug. By exploring the anthelmintic potential of *Ocimum sanctum*, this research seeks to provide scientific validation for its traditional use in parasitic infections and contribute to the development of safer, plant-based alternatives for managing helminthiasis.

MATERIALS AND METHODS:

MATERIALS:

The standard drug Albendazole, utilized for comparative analysis in this study, was generously provided by GlaxoSmithkline. All solvents (organic) and chemicals of analytical grade are obtained from SD Fine Chemical Limited, Mumbai to ensure the accuracy and reliability of the experimental procedures.

METHODOLOGY:

Experimental Earthworms:

Owing to anatomical and physiological resemblance to earthworms have been used extensively for basic evaluation for newer anthelmintic agents by researchers. Earthworms measuring between 6 to 12 cm in length were collected from damp and cool areas in Latur (Maharashtra, India), which provided an ideal habitat. To maintain their natural environment, the earthworms were carefully transferred into a glass container along with a portion of the soil from which they were gathered. This helped to minimize stress and ensure their viability for the study. The worms were then identified and authenticated by a certified zoologist to confirm their species and appropriateness for the anthelmintic tests.

Collection of Plant Material:

The plant material, specifically the red-leafed variety of *Ocimum sanctum* (Holy Basil), was collected from the herbal garden of Dinesh Bembade college of pharmacy, college campus in Chakur, Latur, Maharashtra, during the month of April. This seasonal harvesting ensured the collection of leaves at their peak medicinal potential. After collection, the plant material was authenticated by a qualified botanist to verify its species. The authenticated leaves were then thoroughly cleaned to remove dust and other impurities before being shade-dried. This drying method was chosen to preserve the delicate phytochemicals and bioactive compounds that may degrade under direct sunlight or high heat.

PREPARATION OF EXTRACT:

Once dried, the leaves of *Ocimum sanctum* were mechanically ground into a fine powder using a mechanical grinder. The ground material was then sieved through a 20# mesh to achieve uniform particle size, which is crucial for consistent extraction efficiency. A total of 500 grams of this powdered leaf material was used for the extraction process. The ethanolic extract was prepared using a Soxhlet apparatus, a method chosen for its ability to efficiently extract bioactive compounds over continuous cycles of solvent reflux and percolation. For comparison, an aqueous extract was prepared using the cold maceration method, where the plant material is soaked in water for an extended period at room temperature to allow the extraction of water-soluble components. Both extracts were subjected to filtration, initially through a cotton plug to remove coarse particulate matter and subsequently through Whatman filter paper (No. 1) for finer filtration. The filtered extracts were concentrated using a rotary evaporator under reduced pressure to ensure that the solvent was removed without exposing the extracts to high temperatures that could potentially degrade sensitive bioactive compounds. The temperature was strictly maintained between 40°C and 50°C during evaporation.

Once all solvents were evaporated, the final concentrated extracts were collected and the weight of each residue was accurately measured and recorded. These extracts were then stored in airtight containers for further pharmacological evaluation and analysis.

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

The ethanolic extract of *ocimum sanctum* was subjected to a series of qualitative tests to identify the presence of various phytoconstituents such as alkaloids, flavonoids, carbohydrates, glycosides, saponins, proteins, steroids, and phenols.

1. Detection of Alkaloids

Mayer's Test: A few milliliters of the extract were treated with 1-2 drops of Mayer's reagent (Potassium mercuric iodide). The appearance of a creamy white or yellow precipitate indicated the presence of alkaloids.

Wagner's Test: A few milliliters of the extract were treated with 1-2 drops of Wagner's reagent (Iodine in potassium iodide). The formation of a brown or reddish precipitate confirmed the presence of alkaloids.

Dragendorff's Test: A few milliliters of the extract were mixed with 1-2 milliliters of Dragendorff's reagent (Potassium bismuth iodide solution). A

reddish-brown precipitate indicated the presence of alkaloids.

Hager's Test: A few milliliters of the extract were treated with 1-2 milliliters of Hager's reagent (Saturated picric acid solution). The formation of a creamy white precipitate confirmed the presence of alkaloids.

2. Detection of Flavonoids

Alkaline Reagent Test: The extract was treated with 2 milliliters of 2% sodium hydroxide solution. The development of an intense yellow color indicated the presence of flavonoids.

Lead Acetate Test: The extract was treated with a few drops of 10% lead acetate solution. A yellow precipitate formed, suggesting the presence of flavonoids.

3. Detection of Carbohydrates

Molisch's Test: Two drops of alcoholic alpha-naphthol were added to 2 milliliters of the extract filtrate, followed by the addition of 1 milliliter of concentrated sulfuric acid along the sides of the test tube. A violet ring at the junction indicated the presence of carbohydrates.

Benedict's Test: The extract was treated with Benedict's reagent and boiled for 2 minutes. The formation of green, yellow, or red color indicated the presence of reducing sugars.

4. Detection of Glycosides

Modified Borntrager's Test: The extract was hydrolyzed with dilute hydrochloric acid and treated with ferric chloride solution. The mixture was boiled, cooled, and extracted with benzene. The benzene layer was separated and treated with ammonia solution. A rose-pink color in the ammoniacal layer indicated the presence of anthraquinone glycosides.

5. Detection of Saponins

Hemolytic Test: A drop of blood was placed on a glass slide and treated with the extract. The formation of a hemolytic zone indicated the presence of saponins.

Foam Test: Half a gram of the extract was shaken with 2 milliliters of distilled water for 15 minutes. The formation of stable foam suggested the presence of saponins.

6. Detection of Proteins

Biuret's Test: The extract was treated with 1 drop of copper sulfate, 1 milliliter of 95% ethanol, and potassium hydroxide pellets. A pink to violet color in the ethanolic layer indicated the presence of proteins.

Millon's Test: The extract was treated with a few drops of Millon's reagent and heated. The formation of a white precipitate indicated the presence of proteins.

Xanthoprotein Test: Concentrated nitric acid was added to the extract, resulting in yellow coloration, confirming the presence of proteins.

Ninhydrin Test: The extract was treated with 2 drops of ninhydrin solution. A purple color indicated the presence of amino acids.

7. Detection of Steroids

Salkowski Test: The extract was shaken with chloroform, and 2 milliliters of concentrated sulfuric acid were added along the sides of the test tube. A reddish-brown color indicated the presence of terpenoids.

Liebermann-Burchard Test: The extract was shaken with chloroform and a few drops of acetic anhydride, followed by heating in a water bath and cooling. Concentrated sulfuric acid was added along the sides of the test tube. The appearance of a brown ring at the junction and green coloration in the upper layer indicated the presence of steroids.

8. Detection of Phenols

Ferric Chloride Test: The extract was treated with a few drops of 5% ferric chloride solution. The appearance of dark green or bluish-black coloration indicated the presence of phenols.

ANTHELMINTIC ACTIVITY:

The anthelmintic activity of the extracts was evaluated using earthworms as the model organism. Test solutions of the extracts were prepared at concentrations of 20 mg/ml, 40 mg/ml, and 60 mg/ml by dissolving the extracts in 25 ml of distilled water containing 2% Tween 80 to enhance the solubility of the extracts. Six earthworms of similar size, approximately 6-12 cm in length, were placed in separate Petri dishes (9 cm diameter) containing the extract solutions.

Albendazole, a well-known anthelmintic drug, was used as the standard reference at a concentration of 20 mg/ml. Distilled water with 2% Tween 80 served as the negative control. Albendazole exerts its anthelmintic action through hyperpolarization of parasite muscle cells, leading to muscle relaxation and flaccid paralysis, ultimately causing the death of the worms .

For each test, the time to induce paralysis and death of the worms was recorded. Paralysis was defined as the point when the earthworms ceased all movement, except when forcefully shaken. The time of death was noted when the worms did not respond to shaking or stimulation, such as immersion in warm water at 50°C . These parameters were used to assess the efficacy of the extracts at varying concentrations in comparison to Albendazole.

RESULT AND DISCUSSION:

Preliminary phytochemical analysis

The preliminary phytochemical analysis of the ethanolic leaves extract of *Ocimum sanctum* revealed the presence of various phytochemicals, as indicated by the results of several qualitative tests.

The analysis confirmed the presence of alkaloids, with all tests yielding positive results. Mayer's test, Wagner's test, Dragendorff's test, and Hager's test produced distinct precipitates, signifying that alkaloids are abundant in the extract. Similarly, the presence of flavonoids was established through positive results in both the alkaline reagent test, which exhibited an intense yellow color, and the lead acetate test, which produced a yellow precipitate.

Testing for carbohydrates also returned positive results, with the Molisch's test indicating the formation of a violet ring, while the Benedict's test showed a range of colors from green to red, confirming the presence of reducing sugars. For glycosides, the modified Borntrager's test indicated a rose pink color in the ammoniacal layer, confirming their presence in the extract.

The analysis further identified saponins, evident from the formation of a hemolytic zone in the hemolytic test and the stable foam produced in the foam test, suggesting notable saponin content.

Proteins were also detected, as shown by the positive outcomes in various tests: Biuret's test produced a pink to violet color, Millon's test resulted in a white precipitate, and both the Xanthoprotein and Ninhydrin tests indicated yellow and purple colors, respectively.

Steroids were confirmed through the Salkowski and Liebermann-Burchard tests, which produced reddish-brown and brown ring formations, along with a green coloration, respectively. Lastly, the presence of phenols was indicated by a dark green to bluish-black coloration in the Ferric Chloride test. Overall, the ethanolic leaves extract of *Ocimum sanctum* demonstrated a rich profile of phytochemicals.

Table 1: preliminary phytochemical analysis of the ethanolic leaves extract of *Ocimum sanctum*

Phytochemical Test	Observation	Result
Alkaloids		
Mayer's Test	Creamy white/yellow precipitate	Positive
Wagner's Test	Brown/reddish precipitate	Positive
Dragendorff's Test	Reddish-brown precipitate	Positive
Hager's Test	Creamy white precipitate	Positive
Flavonoids		
Alkaline Reagent Test	Intense yellow color	Positive
Lead Acetate Test	Yellow precipitate	Positive
Carbohydrates		
Molisch's Test	Violet ring formation	Positive
Benedict's Test	Green/yellow/red color formation	Positive
Glycosides		
Modified Borntrager's Test	Rose pink color in ammoniacal layer	Positive
Saponins		
Hemolytic Test	Formation of hemolytic zone	Positive
Foam Test	Stable foam formation	Positive
Proteins		
Biuret's Test	Pink to violet color	Positive
Millon's Test	White precipitate	Positive
Xanthoprotein Test	Yellow coloration	Positive
Ninhydrin Test	Purple coloration	Positive
Steroids		
Salkowski Test	Reddish-brown color	Positive
Liebermann-Burchard Test	Brown ring formation; green coloration	Positive
Phenols		
Ferric Chloride Test	Dark green to bluish-black coloration	Positive

Anthelmintic Activity of *Ocimum Sanctum* Ethanolic Extract Leaves (OSE)

The ultimate aim of present research work is to evaluate anthelmintic activity of *ocimum sanctum* leaves ethanolic extract (OSE) against Indian earthworm. In this study the paralysis time and death time of the earthworms in different doses of the extracts (20mg/ml, 40mg/ml, and 60 mg/ml) were determined. Albendazole drug at concentration (20 mg/ml) were used as standard/reference drug to compare anthelmintic activity of the ethanolic extract *ocimum sanctum* leaves against Indian earthworm. The result suggests that ethanolic extract of *ocimum sanctum* leaves possess concentration dependent anthelmintic activity.

The table 2 shows the anthelmintic activity of the ethanolic extract of *Ocimum sanctum* leaves at varying concentrations (20 mg/ml, 40 mg/ml, and 60 mg/ml) in comparison to the standard drug, Albendazole, and a control group (normal saline). The time required for paralysis and death of earthworms was measured and recorded.

Albendazole exhibited the shortest time for paralysis (18 minutes) and death (28 minutes), indicating its strong anthelmintic activity. Among the ethanolic extracts, the highest concentration (60 mg/ml) showed relatively quick paralysis (28 minutes) and death (37 minutes), followed by 40 mg/ml and 20 mg/ml extracts, which required 33 minutes and 41 minutes for paralysis, and 42 minutes and 50 minutes for death, respectively. The control group did not induce any paralysis or death, confirming the effectiveness of the extracts at the tested concentrations. These results suggest that the ethanolic extract of *Ocimum sanctum* has significant anthelmintic properties, with effectiveness increasing with higher concentrations.

Table 2: Anthelmintic Activity of *Ocimum sanctum* Ethanolic Extract Leaves (OSE)

Sr. No.	Concentration (mg/ml)	Time for Paralysis (min)	Time for Death (min)
1	Control (normal saline)	---	---
2	Albendazole (20 mg/ml)	18	28
3	Ethanolic extract (20 mg/ml)	41	50
4	Ethanolic extract (40 mg/ml)	33	42
5	Ethanolic extract (60 mg/ml)	28	37

Figure 1: Standard: Albendazole

Figure 2: OSE- 60 mg/ml

CONCLUSION:

The evaluation of the anthelmintic activity of the ethanolic extract of *Ocimum sanctum* leaves demonstrated significant potential in inducing paralysis and death in earthworms, which serves as a model organism for assessing anthelmintic effects. The results revealed that higher concentrations of the ethanolic extract (60 mg/ml) resulted in reduced times for both paralysis (28 minutes) and death (35 minutes), indicating a dose-dependent response. In comparison to the standard drug, Albendazole, which exhibited the shortest times for both parameters, the ethanolic extract of *Ocimum sanctum* showed promising anthelmintic activity, particularly at elevated concentrations.

These findings suggest that *Ocimum sanctum* may serve as a valuable source of natural anthelmintic agents, supporting its traditional use in herbal medicine. Further studies are warranted to isolate and

identify the active phytochemicals responsible for this activity and to investigate their mechanisms of action, as well as to evaluate their efficacy in clinical settings. Overall, the ethanolic extract of *Ocimum sanctum* presents a promising avenue for the development of alternative anthelmintic therapies.

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All authors have contributed equally.

CONFLICTS OF INTERESTS:
All authors have declared no conflict of interest.

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