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Evaluation of Antimicrobial Potential of *Vernonia travancorica* Hook. f. from the Southern Western Ghats

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KEYWORDS

Antimicrobial activity,Agar disc diffusion method ,Agar well method

ABSTRACT:

Introduction:*Vernonia travancorica* is a rare endemic tree of the Southern Western Ghats with ecological and medicinal importance. Traditionally used by indigenous communities, it shows therapeutic potential. This study evaluates its phytochemical composition and antimicrobial activity using agar disc diffusion and well diffusion, aiming to identify bioactive compounds effective against pathogenic microorganisms and antibiotic resistance.

Objectives: The present study aims to evaluate the antimicrobial potential of *Vernonia travancorica*, a rare endemic species of the Southern Western Ghats, by analyzing its phytochemical composition and testing its effectiveness against selected pathogens using the agar disc diffusion method and well diffusion method . The study also seeks to validate its traditional medicinal uses and explore its potential as a natural source for developing plant-based antimicrobial agents to combat antibiotic resistance.

Methods:

Plant materials are collected, dried, powdered, and extracted using suitable solvents. The extracts are tested for antimicrobial activity using agar disc and agar well diffusion methods. In both techniques, inhibition zones around discs or wells indicate effectiveness, with larger zones reflecting stronger antimicrobial properties of the plant extracts.

Results: The agar disc and well diffusion experiment demonstrated that plant extracts exhibited measurable antimicrobial activity against the tested microorganisms. Clear zones of inhibition were observed around discs containing the extracts, indicating the ability of bioactive compounds to suppress microbial growth. The extent of inhibition varied depending on the concentration of the extract and the type of microorganism, suggesting differential sensitivity. Compared to controls, the extracts showed significant activity, confirming their potential as natural antimicrobial agents. These findings highlight the effectiveness of the tested plant in inhibiting pathogenic microbes and support further investigation for isolation and development of novel therapeutic compounds applications

Conclusions: This study evaluates antimicrobial activity using the agar disc and well diffusion method against *Pseudomonas aeruginosa* and *Streptococcus mutans*. Zones of inhibition indicate effectiveness, with streptomycin as control. Varying concentrations reveal dose-dependent responses. The method is simple, reliable, and essential for screening potential antimicrobial agents and supporting development of new treatments.

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1. Introduction

Vernonia travancorica Hook.f., commonly known as Travancore ironweed, is a rare and unique tree species that is endemic to the Southern Western Ghats of India. This region, known for its incredible biodiversity and rich ecological heritage, is home to a variety of flora and fauna that are found nowhere else in the world. *V. travancorica* stands out not only because of its limited geographic distribution but also due to its ecological significance in this biodiversity hotspot. The tree is primarily found in the montane forests of the Western Ghats, which are a UNESCO World Heritage Site recognized for their outstanding biodiversity and ecological value. As an endemic species, *V. travancorica* plays an important role in the local ecosystem, contributing to the region's natural balance and biological richness.In addition to its ecological importance, *V. travancorica* has long been recognized by indigenous communities in the Western Ghats for its therapeutic properties. For generations, local tribes and healers have used various plant species from this region, including V. travancorica, in traditional medicine to treat a wide range of ailments. These indigenous practices are rooted in a deep knowledge of the natural world and have been passed down through centuries. The therapeutic potential of *V. travancorica* in treating infections, inflammation, and other health conditions makes it an intriguing subject for modern scientific research. While traditional knowledge of its medicinal uses exists, there is still much to learn about its bioactive compounds and the specific mechanisms by which it may exert therapeutic effects.The primary focus of this study is to investigate the antimicrobial properties of *V. travancorica* by analyzing its phytochemical composition and evaluating its ability to inhibit the growth of harmful microorganisms. Antimicrobial agents, both synthetic and natural, are of immense importance in the fight against infectious diseases. With the increasing resistance of bacteria to conventional antibiotics, the need for alternative antimicrobial agents has never been more urgent. Phytochemicals, which are naturally occurring compounds found in plants, have gained significant attention for their potential antimicrobial properties. Through this study, we aim to identify the bioactive compounds in *V. travancorica* and assess their efficacy in combating microbial infections. One of the most common methods used to evaluate the antimicrobial activity of plant extracts is the agar disc and well diffusion method. This simple yet effective technique involves placing small discs that have been impregnated with antimicrobial agents onto an agar plate that has been inoculated with the target microorganisms. As the antimicrobial agents diffuse outwards from the discs, they interact with the microorganisms present on the agar, inhibiting their growth. This creates a clear area around the disc, known as the zone of inhibition. The size of this zone is measured in millimeters, with a larger zone indicating a stronger antimicrobial effect. The agar disc diffusion method is a widely used and reliable tool for screening the antimicrobial potential of plant extracts and other substances. It provides a straightforward way to assess the effectiveness of natural products like *V. travancorica* in the fight against bacterial infections. By conducting this research, we hope to contribute to the growing body of knowledge surrounding natural antimicrobial agents and their potential role in modern medicine

2. Objectives

The present study is undertaken with the following objectives:

- To investigate the antimicrobial properties of *Vernonia travancorica* Hook. f., a rare endemic tree species of the Southern Western Ghats.
- To analyze the phytochemical composition of *V. travancorica* in order to identify the presence of bioactive compounds.
- To evaluate the efficacy of plant extracts of *V. travancorica* against selected pathogenic microorganisms using the agar disc and well diffusion method.
- To determine the potential of *V. travancorica* as a natural source of antimicrobial agents in response to increasing antibiotic resistance.

Methods

Plant material collection and extraction.

Vernonia travancorica Hook.f. plant material was collected from Agasthyamala Biosphere Reserve of Thiruvananthapuram (N08°40’34.0” E077°11‘34.8”,δ= 4.69yd from gps), Kerala, India were authenticated by Curator, Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India and a voucher

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specimen has been deposited in this centre with an accession number KUBH 11027.The plant part such as leaf, stem, and flower of *Vernonia travancorica* Hook. f. selected for the present study. The collected plant samples were shade dried at room temperature (26±2°C) and ground to powdered form using an electric grinder.30 g of powder of plant samples are subjected to successive extraction by the usage of a Soxhlet apparatus. The powder so obtained was extracted with solvents such as petroleum ether, ethanol, and water (250 mL each). The plant extract were filtered by using Whatman 1 filter paper and it was concentrated in vacuum by using the rotary evaporator naming Rotavapor R210; BUCHI, Flawil, Switzerland .The crude plant extract were stored at 4℃ for further analyses.

Antimicrobial Activity Evaluation Using the Agar Disc Diffusion Method

Principle: The agar disc diffusion method evaluates the antimicrobial activity of a sample by allowing its antimicrobial agents to diffuse out onto an agar plate that has been seeded with the target microorganisms. When antimicrobial agents are present, they will inhibit bacterial growth in the surrounding area, forming a **zone of inhibition**. This zone is measured in millimeters, with larger zones indicating stronger antimicrobial activity. The test measures the size of the inhibition zone to assess the effectiveness of the antimicrobial agents.

Materials Required:

1. **Muller Hinton Agar Medium (1L)**

o **Preparation:** 33.8g of Muller Hinton Agar powder is dissolved in 1000ml of distilled water. This solution is autoclaved at 15 lbs pressure at 121°C for 15 minutes to sterilize it. Once autoclaved, the medium is mixed well and poured into sterile 100mm Petri dishes, with 25-30ml of medium per plate. The plates are left to solidify.

2. **Nutrient Broth (1L)**

o **Preparation:** 13g of commercially available nutrient broth powder is dissolved in 1000ml of distilled water. The solution is heated until fully dissolved, then sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes.

3. **Sterile Whatman Paper Discs (10mm diameter)**

o These discs are used to hold the antimicrobial sample, which is placed on the agar plate to allow diffusion.

4. **Streptomycin (Standard Antibacterial Agent, 10mg/mL)**

o Streptomycin is used as a positive control to compare the antimicrobial effect of the test sample. The standard concentration of 10mg/mL is used.

5. **Culture of Test Organisms (Growth adjusted to Mc Farland Standard 0.5%)**

o The test organisms are prepared by growing them in nutrient broth and adjusting their concentration to match the McFarland 0.5% standard, which corresponds to a bacterial concentration of approximately 1-2 x 10^8 CFU/mL.

1. *Pseudomonas aeruginosa* (ATCC 27853)

2. *Streptococcus mutans* (ATCC 25175)

Procedure:

1. **Preparation of Agar Plates:**

o Muller Hinton agar medium (20ml) is poured into each sterile Petri dish and allowed to solidify. The surface of the agar must be smooth and even to ensure uniform growth of the bacterial cultures.

2. **Inoculation of Test Organisms:**

o Using a sterile swab, *Pseudomonas aeruginosa and Streptococcus mutans* are separately inoculated onto the surface of the agar plates. The bacterial suspension should be adjusted according to the McFarland standard (0.5% suspension) to ensure that each plate has a consistent bacterial inoculum.

o The plates are then allowed to dry for a few minutes to ensure proper adherence of the bacterial culture to the agar surface.

3. **Placement of Antibiotic Discs:**

o After the agar surface is inoculated, sterile 10mm paper discs are placed on the agar plate.

Each disc is impregnated with different concentrations of the antimicrobial sample (e.g., 250µg, 500µg, 1000µg).

o In addition, a disc containing streptomycin (10mg/mL) is placed as a control to ensure the validity of the test.

4. **Incubation:**

o The inoculated plates are incubated at **37°C for 24 hours**. During this time, the antimicrobial agents diffuse radially from the discs into the agar, interacting with the bacterial culture.

5. **Measurement of Zones of Inhibition:**

o After incubation, the plates are observed for the presence of zones of inhibition, which are clear areas around the discs where bacterial growth has been prevented.

o The **diameter of each zone of inhibition** is measured in millimeters using a ruler or caliper.

o A larger zone of inhibition typically indicates a more potent antimicrobial effect.

Data Analysis and Interpretation:

1. **Zone of Inhibition Measurement:**

▪ Measure the diameter of the clear zone surrounding each paper disc. The measurements should be taken in millimeters. This is the zone where bacterial growth has been inhibited by the antimicrobial sample.

2. **Comparative Analysis:**

▪ Compare the zones of inhibition for the different concentrations of the antimicrobial sample. Larger zones of inhibition indicate greater antibacterial activity.

▪ The activity of the test sample is compared to the zone size produced by **streptomycin**. This helps establish whether the antimicrobial activity of the test sample is strong, weak, or comparable to the standard antimicrobial agent.

3. **Results Evaluation:**

- **No inhibition**: If there is no visible zone around the disc, it suggests that the test sample does not possess significant antimicrobial activity against the organism.
- **Moderate inhibition**: A smaller zone indicates some level of antimicrobial activity.
- **Strong inhibition**: Larger zones of inhibition show that the sample is highly effective against the microorganism.

4. **Antimicrobial Susceptibility Classification:**

o Based on the size of the inhibition zone, the antimicrobial activity of the sample can be classified as:

▪ **Resistant**: No or very small zone of inhibition

▪ **Intermediate**: Moderate zone of inhibition

▪ **Susceptible**: Large zone of inhibition

- The test should be performed in a controlled environment to minimize contamination and ensure consistent results.
- The concentration of antimicrobial agents used should cover a range of dilutions to help determine the effective concentration against the microorganisms.
- It is crucial to follow standard guidelines (e.g., NCCLS) for accurate interpretation of results and to ensure reproducability across different labs.

Antimicrobial Activity Evaluation Using the Agar Well Diffusion Method

An experimental procedure used to assess the antibacterial activity of a sample against two bacterial species, **Pseudomonas aeruginosa** and **Streptococcus mutans**. Here's an explanation of the key components of the procedure:

Principle

- The principle behind the experiment is based on the **diffusion method** (often referred to as the agar diffusion method or disk diffusion method). In this method, the antimicrobial agents present in the sample (which could be antibiotics or other antimicrobial substances) are allowed to diffuse into the surrounding agar medium. The agents interact with the test organisms, inhibiting their growth.

- The **zones of inhibition** (areas where bacteria cannot grow due to the antimicrobial agent) are observed as **clear circular areas** around the sample. The size of these zones is measured in **millimeters** to determine the antimicrobial effectiveness of the sample. Larger zones of inhibition indicate greater antimicrobial activity.

Materials Required

1. **Muller Hinton Agar Medium (1 L)**:

- This is the medium on which bacterial growth will occur. It is prepared by dissolving a specific amount of commercially available **Muller Hinton Agar** powder in distilled water, then sterilized by **autoclaving**. This ensures that the medium is free of any microorganisms.

2. **Nutrient Broth (1 L)**:

- This is a liquid medium used to grow bacterial cultures. It is made by dissolving a specific amount of **nutrient powder** in distilled water and autoclaving to sterilize it.

3. **Streptomycin**:

- A standard antibacterial agent used in the experiment as a **positive control**. This helps compare the antimicrobial effectiveness of the sample being tested.

4. **Culture of Test Organisms**:

- The test organisms are bacteria whose susceptibility to the antimicrobial sample is being tested. The cultures of **Pseudomonas aeruginosa (ATCC 27853)** and **Streptococcus mutans (MTCC 890)** are adjusted to a standard concentration of bacteria based on the **McFarland Standard (0.5%)**. This ensures consistent bacterial density in the experiment.

Procedure

1. **Preparation of Plates**:

- The **Muller Hinton Agar Medium** is poured into **petri plates** (20 ml per plate) and allowed to solidify.

2. **Seeding the Plates**:

- The bacterial cultures of **Pseudomonas aeruginosa** and **Streptococcus mutans** (which have been adjusted to the 0.5% McFarland standard for bacterial density) are spread evenly on the surface of the agar in each plate. This creates a **lawn of bacterial growth**, providing an area where the antimicrobial agent can act.

3. **Making Wells**:

- Wells of approximately **10mm** in diameter are made in the agar using a **well cutter**. These wells will be used to place the antimicrobial sample.

4. **Adding the Sample**:

- Different concentrations of the sample being tested (250µg, 500µg, and 1000µg) are added to the wells. This allows the experiment to assess how different amounts of the sample affect bacterial growth.

5. **Incubation**:

- The plates are incubated at **37°C** for **24 hours** to allow the bacteria to grow and the antimicrobial sample to diffuse and act on the bacteria.

6. **Measuring the Zone of Inhibition**:

- After 24 hours, the presence of an **inhibition zone** around the well indicates that the antimicrobial sample has effectively prevented bacterial growth in that area. The **diameter of the zone of inhibition** is measured in millimeters.

- The **Streptomycin** control is used to compare the antimicrobial activity of the test sample. If the test sample produces a zone of inhibition similar to or larger than Streptomycin, it suggests that the test sample has strong antimicrobial properties.

3. Results

Antimicrobial Activity Evaluation Using the Agar Disc Diffusion Method

Figure 1

Figure 2

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Figure 3

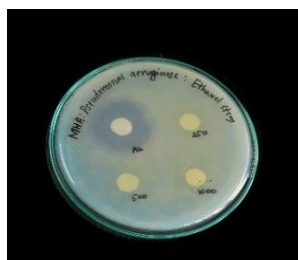

Figure 4

Figure 5

Figure 6

Figure 1*:* *Streptococcus mutans* Dic diffution method *in the ethanol extract of* Plant Stem
Figure 2*:* *Streptococcus mutans* Dic diffution method *in the ethanol extract of* Plant Leaf
Figure 3*:* *Streptococcus mutans* Dic diffution method *in the ethanol extract of* Plant Flower
Figure 4 *:* *Pseudomonas aeruginosa -* Dic diffution method in the ethanol extract of Plant Leaf
Figure 5 *: Pseudomonas aeruginosa* Dic diffution method *in the ethanol extract of* Plant Stem
Figure 6: *: Pseudomonas aeruginosa* Dic diffution method *in the ethanol extract of* Plant Stem

4. <u>GRAM NEGATIVE</u>
Organism: *Pseudomonas aeruginosa*

Sample	**Concentration(µg)**	**Zone of Inhibition(mm)**
Ethanol Leaf	Streptomycin(100µg)	29
	250	Nil
	500	Nil
	1000	11

Table 1

Sample	**Concentration(µg)**	**Zone of Inhibition(mm)**
Ethanol Stem	Streptomycin(100µg)	30
	250	Nil
	500	Nil
	1000	Nil

Table 2

Sample	**Concentration(µg)**	**Zone of Inhibition(mm)**
Ethanol Flower	Streptomycin(100µg)	30
	250	Nil
	500	Nil
	1000	Nil

Table 3

<u>GRAM POSITIVE</u>

Organism: *Streptococcus mutans*

Sample	**Concentration(µg)**	**Zone of Inhibition(mm)**
Ethanol Leaf	Streptomycin(100µg)	24
	250	Nil
	500	11
	1000	12

Table 4

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Sample	Concentration(µg)	Zone of Inhibition(mm)
Ethanol Stem	Streptomycin(100µg)	26
	250	Nil
	500	Nil
	1000	11

Table 5

Sample	Concentration(µg)	Zone of Inhibition(mm)
Ethanol Flower	Streptomycin(100µg)	26
	250	Nil
	500	Nil
	1000	Nil

Table 6

Note: Concentration of Stock 10mg/mL DMSO

Antimicrobial Activity Evaluation Using the Agar Well Diffusion Method

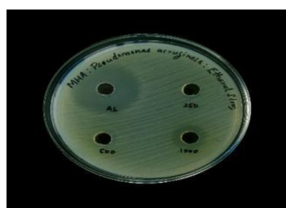

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 7: *Pseudomonas aeruginosa* Well diffusion method *in the ethanol extract of* Plant Stem

Figure 8: *Pseudomonas aeruginosa* Well diffution method *in the ethanol extract of* Plant Leaf

Figure 9: *Pseudomonas aeruginosa* Well diffution method *in the ethanol extract of* Plant Flower

Figure 10 : *Streptococcus mutans* Well diffution method in the ethanol extract of Plant Leaf

Figure 11: *Streptococcus mutans* Well diffution method *in the ethanol extract of* Plant Stem

Figure 12: *Streptococcus mutans* Well diffution method *in the ethanol extract of* Plant Stem

GRAM NEGATIVE

Organism: *Pseudomonas aeruginosa*

Sample	Concentration(µg)	Zone of inhibition (mm)

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Ethanol leaf	Streptomycin (100µg)	32
	250	Nil
	500	Nil
	1000	16

Table 7

Sample	Concentration(µg)	Zone of inhibition (mm)
Ethanol stem	Streptomycin (100µg)	32
	250	Nil
	500	Nil

Table 8

Sample	Concentration(µg)	Zone of inhibition (mm)
Ethanol flower	Streptomycin (100µg)	32
	250	Nil
	500	Nil
	1000	15

Table 9

GRAM POSITIVE

Organism: *Streptococcus mutans*

Sample	Concentration(µg)	Zone of inhibition (mm)
Ethanol leaf	Streptomycin (100µg)	26
	250	Nil
	500	Nil
	1000	12

Table 10

Sample	Concentration(µg)	Zone of inhibition (mm)
Ethanol stem	Streptomycin (100µg)	26
	250	Nil
	500	Nil
	1000	Nil

Table 11

Sample	Concentration(µg)	Zone of inhibition (mm)
Ethanol flower	Streptomycin (100µg)	26
	250	Nil
	500	Nil
	1000	11

Table 12

NOTE: Concentration of Stock 10mg/mL in DMSO

5. Discussion

The agar disc diffusion method is a widely used technique for assessing antimicrobial activity. By measuring the zones of inhibition, the effectiveness of various antimicrobial agents can be quantified and compared. This method provides a clear, quantitative way to test the susceptibility of microorganisms to different agents, making it essential in the study of antimicrobial resistance and in the development of new antimicrobial treatments.The experiment is designed to assess the effectiveness of antimicrobial agents by measuring their ability to inhibit the growth of *Pseudomonas aeruginosa and Streptococcus mutans*. The size of the inhibition zones is used to determine the relative potency of different concentrations of the sample, with Streptomycin serving as a benchmark for comparison.The method of measuring antimicrobial activity through the size of the zone of inhibition is a reliable and effective approach for evaluating the antimicrobial properties of various substances. By comparing the results to a positive control such as streptomycin, which serves as a standard, we can assess the strength of the antimicrobial activity of the sample.

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Larger zones of inhibition indicate more potent antimicrobial effects, offering valuable insight into the sample's efficacy. The use of different concentrations, such as 250µg, 500µg, and 1000µg, enables a comprehensive, dose-dependent analysis, providing a better understanding of how the concentration of the tested sample influences its antimicrobial effectiveness. This method is widely utilized in both research and clinical microbiology to screen and identify potential antimicrobial agents, helping in the development of new treatments for bacterial infections. Its simplicity and reproducibility make it a valuable tool for both laboratory experiments and clinical applications, ensuring consistent and accurate results when assessing antimicrobial agents against specific bacterial strains. Ultimately, this technique remains an essential part of antimicrobial research, guiding further studies and therapeutic developments.

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6. The method follows the guidelines outlined by the National Committee for Clinical Laboratory Standards (NCCLS), specifically their M2-A5 standard from 1993, which provides standardized procedures for antimicrobial disk susceptibility tests.