Evaluation Of Antibacterial & Antimicrobial Activity of Nerium Oleander Leaf Extract

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***Abstract*—The study explores the antibacterial and antimicrobial potential of Nerium oleander leaf extract, a traditionally recognized medicinal plant. Phytochemicals were extracted using ethanol, with preliminary analysis confirming the presence of bioactive compounds. Antimicrobial activity was tested against bacteria's presents on floor using the agar well diffusion method, showing significant zones of inhibition. The extract demonstrated moderate efficacy compared to standard antibiotics like Azithromycin, with a measured zone of inhibition of 4.52 cm².**

Despite its potential as a natural antimicrobial agent for herbal product development (e.g., sprays, disinfectants), the plant's known toxicity due to cardiac glycosides (e.g., oleandrin, oleandrigenin) necessitates further research on dosage standardization and safety. The study highlights Nerium oleander's promise as an alternative antimicrobial source, especially in regions with limited access to synthetic antibiotics, while emphasizing the need for toxicity and cytotoxicity evaluations.

***Index Terms*—Nerium oleander, antimicrobial activity, antibacterial, phytochemicals, herbal medicine**

I. INTRODUCTION

1) Nerium Oleander

The Latin name Nerium comes from the Greek nerion meaning "wet", indicating the predilection of this plant for wetlands. The specific name oleander comes from the Italian 'oleandro' which comes from the Latin "olea" which refers to the olive tree The plant is a small evergreen tree with 2–5 m in height and distributed in different geographical and ecological places. (Ayouaz. et al. 2023) (Evans 2025)

2) Synonyms

Plant is called with various names across the globe such as follows
[(flowersofindia.net, "Nerium Oleander - Oleander" 2025), (Stuartxchange 2023), (plants.ces.ncsu.edu)]

1) Afrikaans: selonsroos;
2) Arabic: Difl a, Ward AlHemar, Sim Al-Hemar;
3) English: oleander, rose bay, rose-laurel
4) German: Oleander
5) Japanese: kyōchiku-tō;
6) Portuguese: espirradeira, oleandro
7) Bengali - 'Karabi'

3) Characteristics of Nerium Oleander

Table 1 (Gilman and Watson 1994), (Missouribotanicalgarden)

Plant Type	Shrub
Exposure	Full Sun, Partial Sun, Partial Shade
Water Needs	Low, Average
Hardiness Zone	Zone 10 (30°F) Zone 8 (10° F) Zone 9 (20° F)
Flower Color	Red, White, Pink
Flower Season	Spring, Summer
Growth Rate	Moderate
Height	8-20ft
Spread	6-10ft
Characteristics	Evergreen, Fragrant, Great Foliage, Showy
Attracts	Bees, Birds, Butterflies, Hummingbirds, Pollinators, Wildlife
Maintenance	Low
Garden Uses	Arbors, Pergolas, Trellises, Beds and Borders, Hedges and Screens
Soil Type	Loam, Sand
Soil PH	Acid, Alkaline, Neutral
Soil Drainage	Well-Drained

4) Geographical Aspect

Considering geographically Nerium oleander is distributed across globe in their respective continent and their sub countries such as Asia, Europe, South Africa, etc. The plant was originated from sub-continent of Indo Pak and Mediterranean region. The plant leaves usually range from 5 to 20 cm long, it also consists of dark green leaves. The bark seems light green to light-gray bark that exudes milky juice when cut. (HERRERA), (Sankar Narayan Sinha and Karabi Biswas)

5) Use:

The plant is used and grown as an ornamental plant for hedging along highways, planting along beaches and decorating the surrounding having range of varying colored flowers such as Pink, White & Yellow. Besides all the use mentioned above Nerium oleander also exhibit following activities: (Sankar Narayan Sinha and Karabi Biswas 2016) (Khare 2007)

Antimicrobial

Antifungal

Anticancer

Anti inflammatory

Despite its well-recognized toxic potential, oleander has been used for centuries in traditional medicine for numerous ailments such as heart conditions, asthma, corns, epilepsy, cancer, painful menstrual periods, leprosy, malaria, ringworm, indigestion, and venereal disease; and to cause abortions. Roots of Nerium oleander shows healing properties for hemorrhoids and ulcers. Tincture of flowers used as cardiotonic and roots acts as a CNS- depressant and spasmolytic.

Fig 1: - Nerium Oleander

6) Taxonomical Hierarchy of Nerium Oleander

Kingdom: Plantae

Division: Angiosperms

Class: Magnoliopsida

Subclass: Asteriidae

Order: Gentian ales

Family: Apocynaceae

Genus: Nerium L.

Species: Oleander L (Sankar Narayan Sinha and Karabi Biswas 2016)

7) Features

1. Leaves: Long, narrow, leathery, and dark green; arranged in pairs or whorls.
2. Flowers: Trumpet-shaped and appear in clusters; colors include pink, white, red, and yellow.
3. Blooming occurs from spring to fall.
4. Fragrance: Many varieties are fragrant, but not all.
5. Toxicity: All parts of the plant are highly toxic to humans and animals if ingested. The sap can also irritate skin and eyes.
6. Drought-Tolerance: Very drought-tolerant once established; ideal for hot, dry climates. (Rajasree et al. 2011)

8) Chemical Aspects

Nerium oleander leaves are rich in polyphenols, as evidenced by Siham et al. HPLC analysis identified cinnamic acid as the dominant phenolic compound, along with epicatechine, catechin, and chlorogenic acid. The leaves' aqueous extract contained 2.3% crude polysaccharide, mainly pectic polysaccharides consisting of arabinose, galacturonic acid, galactose, and rhamnose.

New compounds, neriucoumaric and isoneriucoumaric acids, were isolated from the leaves. Preliminary phytochemical screening indicated the presence of carbohydrates, flavonoids, alkaloids, steroids, cardiac glycosides, and tannins & also reported oleanderocinoic acid, flavonoid glycosides, and oleandigoside, which exhibit cytotoxic effects against MCF-7 breast cancer cells. Additionally, isolated two new compounds from the stems. A polysaccharide fraction from the flowers was obtained through various methods, revealing L-rhamnose, Lgalactose, and D-galacturonic acid. Siddiqui et al. discovered novel cytotoxic pentacyclic triterpenoids from the leaves. Other compounds include oleanderoic acid and oleanderen, along with cardiac glycosides kaneroside and neriumoside, characterized by spectral analysis. (Hase et al.) (Siddiqui et al.) (BINA SHAHEEN SIDDIQUI et al.) (Cao et al.)

9) Oleander Toxicity

Oleander, a poisonous plant, contains toxic compounds like oleandrin and oleandrigenin, which are harmful, especially in large quantities. These cardiac glycosides have a narrow therapeutic index and can cause various side effects, including weakness, diarrhea, nausea, vomiting, headaches, stomach pain, and potentially death. Toxicity studies indicate that while birds and rodents show relative insensitivity to oleander, mammals such as dogs and humans are more susceptible to glycoside intoxication. Additionally, grazing animals like sheep, horses, and cattle are at risk; for instance, consuming just 100 grams can be fatal for an adult horse. Plant clippings, particularly sweet-tasting leaves, pose significant dangers, as seen in a 2009 incident where several horses were poisoned. Symptoms in affected horses include severe diarrhea and irregular heartbeat. Despite these risks, research indicated that for humans, particularly children at higher risk, mortality from oleander ingestion is generally low, with a rare fatal case involving two toddlers in California attributed to malnutrition and pica. (Sacco et al.), (Gopalakrishnan et al.)

10)Effect of Toxicity

Ingesting oleander can affect the gastrointestinal system, heart, and central nervous system. The primary effect of its cardiotoxic glycosides is positive inotropy, where glycosides inhibit cardiac muscle cell ATPase by competing with K^+ ions, resulting in increased Na^+ and Ca^{2+} ion accumulation and stronger heart contractions. This may cause lethal hyperkalemia. Oleander poisoning symptoms mirror digoxin toxicity, including nausea, diarrhea, vomiting, neuropsychic disorders, and motor manifestations. Intoxication severity depends on the amount ingested and individual physiological responses, with symptoms manifesting quickly from teas or more slowly from unprepared plant parts. (Sacco et al.), (Gopalakrishnan et al.)

11)Treatment:

Poisoning from oleander plants necessitates immediate medical attention due to rapid onset of symptoms in humans and animals. Critical first-aid steps include induced vomiting and gastric lavage to minimize toxic absorption, alongside administering activated carbon to absorb residual toxins. Severity of symptoms may require further medical interventions, often leading to the need for temporary cardiac pacing. In severe cases, digoxin immune fab is the preferred treatment if initial measures are ineffective, although it is typically reserved for life-threatening situations due to potential side effects. Importantly, drying oleander materials does not remove toxins, and a variety of harmful compounds are present in the plant. Caution is essential around oleander, which is recognized by various names worldwide, as all parts, including sap and dried components, are toxic. (“Gastric Lavage and Activated Charcoal Application”)

12)Antibacterial & Antimicrobial Activity of Nerium Oleander

1) Definition

Anti-Microbial

An antimicrobial is an agent that kills microorganisms or stops their growth. These agents can be natural, synthetic, or semi-synthetic substances used to treat infections caused by bacteria, fungi, viruses, or parasites. Antimicrobials are classified into different categories such as antibiotics (against bacteria), antifungals (against fungi), antivirals (against viruses), and antiparasitic (against parasites). (“WHO”)

Anti-Bacterial:

An antibacterial is a type of antimicrobial agent specifically designed to kill or inhibit the growth of bacteria. Antibacterials are used to prevent and treat bacterial infections and are commonly found in antibiotics, disinfectants, and antiseptics. (“CDC”)

2) Role of Oleander in Antibacterial and Antimicrobial Activity:

Oleander (*Nerium oleander*), a widely known ornamental plant, has shown potential antibacterial and antimicrobial activity due to its rich phytochemical profile, including compounds like flavonoids, tannins, glycosides, and alkaloids. Despite its known toxicity, extracts from different parts of the plant (leaves, flowers, and bark) have demonstrated effectiveness against various bacterial and fungal strains. (“Rajasree, P. H., et al”)

a) In Antibacterial Activity: Oleander extracts, particularly methanolic and ethanolic extracts of the

leaves and flowers, have shown inhibition against Gram-positive bacteria like
Staphylococcus aureus and Gram-negative bacteria like Escherichia coli. The mechanism is primarily due to the presence of oleandrin, a cardiac glycoside, along with flavonoids and phenolic compounds, which damage bacterial cell membranes and interfere with protein synthesis. ("Rajasree, P. H., et al")

b) In Antimicrobial Activity: Extracts of oleander have been tested against various fungal pathogens such as Candida albicans and Aspergillus niger. The antifungal effect is attributed to the disruption of fungal cell walls by bioactive components, especially tannins and saponins.
(Ibrahim, T. A., et al.)

c) Synergistic Effect with Antibiotics: Some studies suggest that oleander extracts may enhance the efficacy of standard antibiotics, showing synergistic effects when combine with drugs like ampicillin or ciprofloxacin. ("Sharma, R., et al")

d) Presence of Triterpenoids
Triterpenoids have been reported to be effective against both Gram-positive and Gram-negative bacteria. Examples of Triterpenoids with Antibacterial Activity Present in *Nerium Oleander:*
1) Ursolic acid ("Al-Snafi et al")
2) Olenolic acid ("Al-Snafi et al")

e) Presence of Saponins
Saponins are natural compounds found in many plants, and they can kill or inhibit the growth of bacteria. (Manjunatha, M et al)

II. LITERATURE SURVEY ON NERIUM OLEANDER AS AN ANTIBACTERIAL AND ANTIMICROBIAL AGENT

Table 2

Sr No	Author	Title	Year	Journal	Conclusion
1	1) Kamboj, A., & Saluja 2) Kumar, S., Kumar, D., Deshmukh, R. R., & Naidu, J.	1) Phytopharmacological review of *Nerium oleander* Linn. 2) Phytochemical and pharmacological profile of *Nerium oleander* Linn	1) 2011 2) 2012	1) Pharmacognosy Reviews 2) Asian Journal of Pharmaceutical and Clinical Research	Phytochemical composition and bioactivity of *Nerium oleander* contains a variety of bioactive compounds such as flavonoids, alkaloids, saponins, tannins, terpenoids, and cardiac glycosides. These secondary metabolites are believed to be responsible for its antibacterial and antifungal activities.
2	1) El-Baky, H. A., ElBaroty, G. S., & Ibrahim, S. A. 2) Dhalalakshmi and Manavalan	1) Antibacterial activity of plant extracts from marine algae and *Nerium oleander* 2) Evaluation of antibacterial activity of *Nerium oleander* against human pathogens	1) 2003 2) 2014	1) Journal of Food Technology 2) International Journal of Current Pharmaceutical Research	Studies have shown that ethanol and methanol extracts of *N. oleander* have significant antibacterial effects against both Gram-positive and Gram-negative bacteria bacteria, such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa.

Sr No	Author	Title	Year	Journal	Conclusion
3	El-Baky, H. H. A., El-Baroty, G. S., & Ibrahim, S. A.	Antibacterial activity of plant extracts from marine algae and Nerium oleander	2003	Journal of Food Technology	Although most studies focus on antibacterial effects, some evidence suggests Nerium oleander also shows antifungal and broad-spectrum antimicrobial properties.
4	Al-Yahya, M. A., Mossa, J. S., Ageel, A. M., & Rafatullah, S.	Pharmacological studies on Nerium oleander	2010	Planta Medica	The antimicrobial action of Nerium oleander extracts may involve: 1) Disruption of bacterial cell wall integrity 2) Leakage of cellular contents 3) Inhibition of nucleic acid synthesis 4) Generation of reactive oxygen species (ROS)
5	Langford, S. D., & Boor, P. J.	Oleander toxicity: An examination of human and animal toxic exposures	1996	Toxicology	Although the antimicrobial potential is promising, toxicity is a major limitation in the therapeutic use of *Nerium oleander. 1) The presence of toxic cardiac glycosides such as oleandrin and neriine can pose severe health risks if not properly dosed. 2) Safe extraction methods and formulation strategies are essential before clinical applications.*

III. AIM AND OBJECTIVES

1. To extract Phytochemicals from Nerium oleander leaves Using suitable solvents (ethanol/methanol) to obtain bioactive compounds.
2. To perform Phytochemical screening Identify the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and glycosides.
3. To evaluate the antibacterial and antimicrobial activity Test the extract against selected bacterial strains using standard microbiological methods like agar well diffusion.
4. To compare the effectiveness with standard antibiotics Measure and analyze the zone of inhibition and determine the relative potency.
5. To assess the potential of Nerium oleander in herbal antimicrobial product development Explore safe applications for use in sprays, disinfectants, or topical formulations.

IV. HYPOTHESIS

1. Presence of Bioactive Compounds

Hypothesis: Nerium oleander exhibits antibacterial activity due to the presence of secondary metabolites such as flavonoids, alkaloids, tannins, glycosides, and saponins, which disrupt bacterial cell walls or inhibit essential bacterial enzymes.

2. Disruption of Bacterial Cell Membranes

Hypothesis: Oleander extracts may damage bacterial cell membranes, leading to leakage of cellular contents and cell death, particularly in Gram-positive bacteria.

3. Inhibition of Nucleic Acid Synthesis
Hypothesis: Certain compounds in Nerium oleander, like oleandrin and neriine, may inhibit DNA or RNA synthesis in bacteria, thereby preventing replication and transcription.

V. MATERIAL PROCESSING ("PATIL, R. H ET AL")

1) Collection of Plant Leaves – Nerium oleander:
Fresh and healthy leaves of *Nerium oleander* were collected from Botanical Garden of Nava Sahyadri institute of pharmacy Pune. during the January of Summer. The plant was identified based on morphological characteristics such as leaf shape, arrangement, and flower type, with confirmation from botanical references or a subject expert. The leaves were carefully plucked using sterilized scissors to avoid contamination and were placed in clean, dry, and labeled plastic bags or paper covers. The samples were immediately transported to the laboratory for further processing such as washing, drying, and extraction to preserve the phytochemical content.

Fig 2 - Collection of plant leaves Nerium oleander

2) Washing and Drying of Nerium oleander Leaves:
After collection, the Nerium oleander leaves were washed thoroughly under running tap water to remove dust, dirt, and other surface impurities. They were then rinsed with distilled water to eliminate any remaining contaminants and ensure cleanliness for further processing.
The cleaned leaves were spread evenly on blotting paper to remove excess water and then transferred to a hot air oven. Drying was carried out at 40–50°C for 24–48 hours, or until the leaves became crisp and completely moisture-free. This low-temperature drying helps preserve the active phytochemicals by preventing thermal degradation.
The dried leaves were then stored in airtight containers or zip-lock bags at room temperature until further use for extraction and testing.

Fig 3 - Washing and Drying of Nerium oleander Plant Leaves

3) Grinding of Dried Nerium oleander Leaves:
Once the leaves were completely dried in the hot air oven, they were allowed to cool at room temperature to avoid moisture absorption from the environment. The dried leaves were then coarsely crushed by hand and subjected to grinding using a clean, dry mechanical grinder or laboratory blender.
The grinding process was continued until a fine, uniform powder was obtained. The powdered leaves were then sieved using a standard mesh (e.g., 60 mesh) to ensure consistent particle size. The fine leaf powder was collected and stored in clean, dry, airtight containers, away from light and moisture, until further use for extraction and phytochemical analysis.

Procedure for grinding of Nerium oleander leaves
1. Collect mature Nerium oleander leaves.
2. Wash thoroughly with clean water to remove dirt.
3. Air dry the leaves in shade for 3–5 days until completely dry.
4. Crush the leaves roughly by hand or using a mortar and pestle.
5. Use a grinder or blender to grind them into a fine powder.
6. Store the powder in a labeled, airtight container.

Fig 4 - Grinding of Nerium oleander Dried leaves

4) Weighing of Powdered Nerium oleander Leaves:
The finely ground Nerium oleander leaf powder was accurately weighed using a digital analytical balance. An appropriate quantity of powder (e.g., 10 g, 25 g, or 50 g, depending on the requirement of the extraction process) was measured and recorded. The weighed powder was then transferred to a clean, dry container and used immediately for solvent extraction to ensure the stability of the bioactive compounds.

5) Procedure for Ethanolic Extract of Nerium oleander:
a) Collection and Authentication: Fresh leaves of *Nerium oleander* are collected, washed with distilled water, and shade-dried for 7–10 days.

b) Powdering:
The dried plant material is ground into a coarse powder using a mechanical grinder. c) Extraction:
50 grams of powdered material is soaked in ethanol (95%) in a Soxhlet extractor for 6–8 hours or until the solvent runs clear.

Fig 5 - Soxhlet Extraction of Nerium oleander Powder

6) Filtration of Nerium oleander Extract:
After the extraction process (e.g., by soaking or refluxing the leaf powder in a suitable solvent such as ethanol, methanol, or distilled water), the mixture was filtered to separate the liquid extract from the solid plant residue.

Filtration was carried out using Whatman No.1 filter paper or clean cotton cloth placed in a funnel. The extract was poured slowly through the filter to collect the clear filtrate. This process removed undissolved particles and ensured a clean extract suitable for further concentration and testing.
The filtrate (liquid portion) was collected in a clean, dry container and either stored in a refrigerator for short-term use or concentrated further using evaporation methods.

Fig 6 - Filtration of Nerium oleander Extract

7) Evaporation of Nerium oleander Extract:
The filtered extract was subjected to evaporation to remove the solvent and obtain a concentrated crude extract. Evaporation was carried out using a water bath or rotary evaporator at a controlled temperature of 40–60°C, depending on the solvent used, to avoid degradation of heat-sensitive phytochemicals. The process continued until most of the solvent had evaporated, leaving behind a semi-solid or dry crude

extract. The final extract was collected, weighed, and stored in airtight containers or vials under refrigeration (4°C) for further use in antibacterial testing and phytochemical analysis.

8) Phytochemical Screening:

Table 3

Sr. No.	Test	Observation	Result
1	Salkowski Test Procedure: 1) Dissolve a small amount of the extract in chloroform. 2) Add an equal volume of concentrated sulfuric acid carefully along the side of the test tube. 3) Allow the mixture to stand without shaking. 4) Observe the color change at the interface (Silva et al.).	Reddish Brown Colour	Triterpenoid Present
2	Foam Test Procedure: 1) Add extract in 5 ml distilled water. 2) Shake vigorously. 3) Observe foam (Demurari et al.).	Foam Formed	Presence of saponins

Phytochemical Screening Results

Fig 7 Phytochemical Screening

9) Storage:
The semi-solid extract is stored in an airtight container at 4°C for further use.

10) Microbial Assay:
Preparation of Agar. ("Kokare Chandrakant et al") a) Requirement:

Table 4

Sr. No.	Name Of Material	Weight
1	Nutrient Agar powder	2.3gm
2	Distilled water	100ml
3	Beaker or conical flask(250ml)	1
4	Measuring cylinder	1
5	Glass rod	1
6	Cotton plug or aluminum foil	1
7	Autoclave	1
8	Sterile Petri dishes	6

b) Procedure:

1. Weigh exactly 2.3 g of Nutrient Agar using a digital balance.
2. Measure 100 mL distilled water using a measuring cylinder. Transfer water to a 250 mL flask or beaker. Add the weighed nutrient agar powder into the water. Heat gently (on burner) while stirring until the media completely dissolves. The solution should be clear.
3. Once dissolved, cover the flask with a cotton plug or aluminum foil to prevent contamination.
4. Place the flask in an autoclave at 121°C, 15 psi for 15 minutes
5. Allow the sterilized medium to cool down to 45–50°C before pouring into Petri dishes
6. In a sterile environment (like near a flame or in a laminar airflow), pour about 15–20 mL into each Petri dish. Let it solidify at room temperature.
7. Label with media type and date.

VI. RESEARCH METHODOLOGY

1) Agar Dilution Model: - ("Hossain et al")
To determine the Minimum Inhibitory Concentration (MIC) of antibacterial agents by incorporating them into agar and assessing their ability to inhibit bacterial growth.
Procedure:
1) Prepare extract of the antimicrobial agent.
2) Prepare agar medium and cool to ~45–50°C.
3) Add extract of the antimicrobial agent to the sum of agar.
4) Pour the agar into sterile Petri dishes A, and allow to solidify.

5) Pour agar and Anti-Microbial solution in sterile Petri dish B.
6) Spot-inoculate each plate with a bacterial sample.
7) Incubate the plates at 35–37°C for 18–24 hours.
8) Compare Bacterial Growth in both dishes.

Fig 8 - Agar Dilution Method

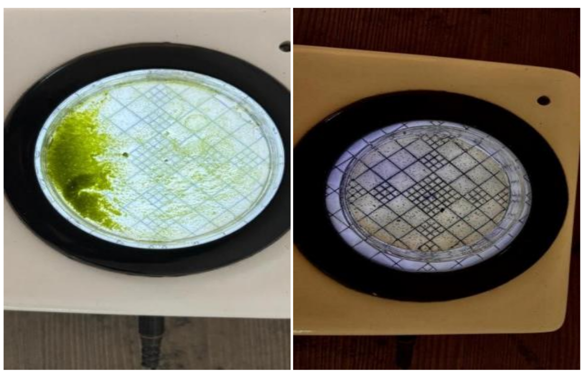

Fig 9: Comparative study of Bacterial growth in drug mixed agar and Non drug mixed agar

2) Agar Well Diffusion Method: – ("Balouiri et al")
This is a common technique used to evaluate the antimicrobial activity of plant extracts, chemical compounds, or antibiotics against microorganisms.

Procedure:
1. Preparation of Media:
Prepare a suitable agar medium (commonly Mueller-Hinton Agar or Nutrient Agar).
Pour the molten agar into sterile Petri dishes and allow it to solidify.

2. Inoculation of Microorganisms:
Using a sterile cotton swab, evenly spread a bacterial swab which is taken from floor, over the entire surface of the agar plate to form a uniform lawn.

3. Well Formation:
Use a sterile cork borer or pipette tip to punch small wells (holes) in the agar (usually 6–8 mm in diameter).

4. Sample Addition:
Fill each well with a specific volume of the test sample or plant extract.

5. Incubation:
Incubate the plates at 37°C for 18–24 hours.

6. Observation:
After incubation, observe and measure the zone of inhibition (clear area around the well where bacteria did not grow). Larger zones usually indicate higher antimicrobial activity.

7. Calculation:
Zone Of Inhibition = Area of circle which is formed
So, Area of Circle = $A=\pi r^2$ ("Kokare Chandrakant et al")

For *Nerium Oleander* Leaf extract:
r = Radius of circle = 1.2 π = Standard value = 3.14
Here,
According to formula
3.14 x (1.2 x1.2)
= 3.14 x 1.44
= 4.52 cm²

For Azithromycin Standard drug
r = Radius of circle = 2.1cm π = Standard value = 3.14
Here,
According to formula
3.14 x (2.1 x 2.1)
= 3.14 x 4.41
= 13.84 cm²

Calculation Table:

Table 5

Name Of Drug	Radius	Zone Of Inhibition
Azithromycin	2.1 cm	4.52 cm²
Nerium Oleander Extract	1.2cm	13.84 cm²

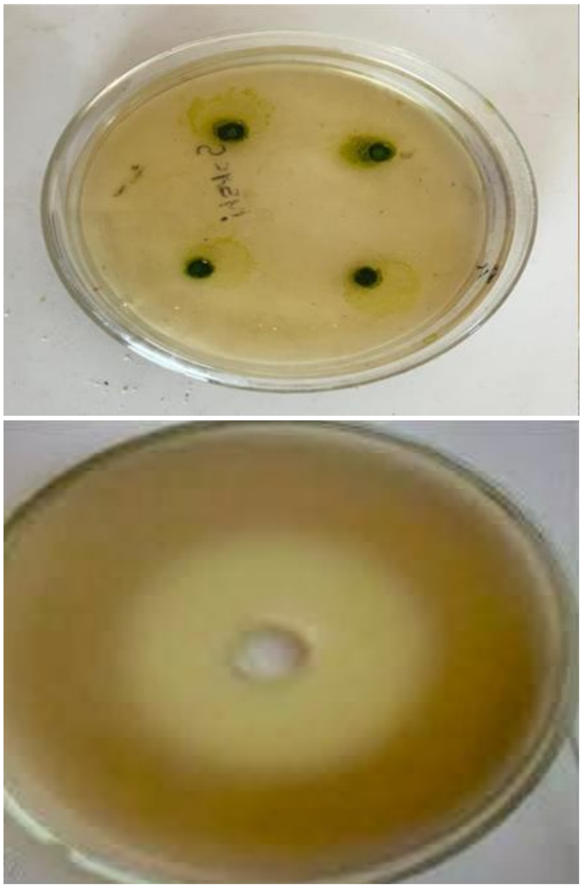

Fig 10 - Zone of inhibition A) Nerium Oleander extractB) Azithromycin

VII. NOVELTY IN RESEARCH

1) Exploration of Floor Bacterial Strains:
The study uniquely tests Nerium oleander extract against unknown bacterial strains collected from the floor, expanding the scope beyond commonly studied pathogens like E. coli and S. aureus, offering insights into its efficacy against environmental microbes.

2) Synergistic Potential with Ethanol:
The research investigates the synergistic effects of oleander extracts with ethanol, a novel approach that could enhance existing antimicrobial Activity.

3) Methodological Innovation:
The use of both agar dilution and well diffusion methods, combined with precise extraction and drying techniques (e.g., Soxhlet extraction), provides a robust framework for evaluating antimicrobial activity, enhancing reproducibility.

4) Focus on Practical Applications:
The study proposes specific herbal product applications (e.g., topical formulations, disinfectants), bridging traditional use with modern therapeutic development, while addressing the challenge of oleander's toxicity through a call for safety studies.

5) Comparative Efficacy Analysis:
Comparing oleander extract's performance with Azithromycin using a calculated zone of inhibition (4.52 cm^2) introduces a quantitative benchmark, offering a novel perspective on its relative potency in a controlled experimental setting.

VIII. RESULT AND DISCUSSION

1. Introduction to Results
The antibacterial activity of Nerium oleander leaf extract was evaluated using the agar plate zone of inhibition method. This method is widely accepted for its simplicity, reproducibility, and visual clarity in detecting antimicrobial activity. In this study, Unknown floor bacterial strains were subjected to treatment with various concentrations of the plant extract to assess its effectiveness in inhibiting bacterial growth.

2. Observation of Agar Dilution Method:
No bacterial growth is seen on Agar which is mixed with Leaf extract of Nerium oleander.

3. Observation of Zones of Inhibition
The results were recorded in the form of clear zones around the wells containing the Nerium oleander extract, measured in centimeters (cm) and Zone of Inhibition found to be 4.52 cm^2. The diameter of the zone of inhibition reflects the antibacterial efficacy of the extract. It was observed that the extract exhibited varying degrees of antibacterial activity against the floor microorganisms. When compared to the standard antibiotic Azithromycin, the Nerium oleander extract showed moderate antibacterial activity. While it did not surpass the standards in most cases, its performance was noteworthy.

4. Phytochemical Relevance:
The antimicrobial properties observed can be attributed to the presence of secondary metabolites such as Triterpenoids, Saponins in Nerium oleander.

These compounds are known for their ability to interfere with microbial cell membranes, disrupt enzymatic activity, or inhibit nucleic acid synthesis. The presence of these bioactive constituents in Nerium oleander thus supports the observed antibacterial action in this study

5. Literature Support:
Previous studies on Nerium oleander have also confirmed its antimicrobial potential. Many of them reported inhibition zones against E. coli and S. aureus, supporting the results obtained in this study. Moreover, other studies have highlighted the role of Phytochemicals, present in Nerium oleander, which may have direct or indirect antimicrobial effects. The consistency of the current findings with existing research suggests the reliability and reproducibility of Nerium oleander's antibacterial potential. (Ibrahim et al. 2010)

IX. LIMITATIONS OF THE STUDY

The extract used was crude; therefore, the exact compounds responsible for the activity are unknown. No toxicity or cytotoxicity tests were conducted, which are essential for determining the therapeutic safety of the extract. Only the in vitro zone of inhibition method was used. Other methods like broth dilution or in vivo models can further validate the findings.

X. FUTURE SCOPE

This study opens multiple avenues for further research:

1. Isolation and characterization of individual bioactive compounds from Nerium oleander
2. Conducting cytotoxicity and safety studies for potential therapeutic use.
3. Exploring synergistic effects of the extract with existing antibiotics.
4. Testing against multi-drug resistant strains to assess its efficacy in addressing antibiotic resistance.

Overall Discussion:
The findings of this study demonstrate that Nerium oleander has considerable potential as a natural antibacterial agent. The zone of inhibition method effectively showcased the plant's antimicrobial capacity. While the extract does not outperform modern antibiotics, its efficacy is sufficient to consider it for supplementary or alternative medicinal use, especially in rural or under-resourced regions where synthetic antibiotics may be unavailable or unaffordable. Given the global concern of rising antibiotic resistance, natural sources such as *Nerium oleander* may play a role in future antibacterial drug discovery. However, caution must be exercised due to the known toxicity of the plant, especially the cardiac glycosides, and proper dose standardization and safety evaluation must be ensured before any clinical application.

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