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Original Article

Evaluating the Antimicrobial Activity of Crude Extracts from Leaves, Flowers and Roots of *Iris versicolor* and *Nerium oleander* Plants Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Bacterial infections are prevalent in most parts of Kenya and cause diseases like pneumonia, typhoid, cholera, and meningitis. This has contributed to unsustainable socio-economic development following the emergence of antimicrobial-resistant strains of bacteria and, hence, the need for alternative strategies that are effective against bacteria and environmentally safe. This study evaluated the antimicrobial activity of crude extracts from leaves, flowers and roots of *Iris versicolor* and *Nerium oleander* plants against *Escherichia coli* and *Staphylococcus aureus*. The plant parts were macerated and extracted to obtain phytochemicals that were then identified under different classes of compounds by treating them with varying reagents following standard laboratory procedures. To assess antimicrobial activity, discs were infused with an antimicrobial compound derived from the leaf, flower, and root extracts of *N. oleander* and *I. versicolor* at a concentration of 1000 µg/ml. Bacterial isolates (inoculum) were then introduced into plates containing Mueller-Hinton agar media. Infused discs were dispensed aseptically on the plates. The discs were pressed to ensure contact between the agar and the disc and incubated at 37 °C for 24 hrs. The Kirby-Bauer method was used to assess the antibacterial efficacy of the plant extracts against the bacterial strains *Staphylococcus aureus* and *Escherichia coli*. The crude plant extracts showed a greater zone of inhibition ranging from 2.5 mm to 3.2 mm in diameter, irrespective of the plant part and the test micro-organism. The methanolic crude extract of *N. oleander* leaves showed a greater zone of inhibition against *Staphylococcus aureus* at 3.1 mm and 2.9 mm against *E. coli*. The zone inhibited by the crude extract on *S. aureus* showed no growth of the micro-organisms; this was observed on all the crude extracts, irrespective of the extracting solvent, on both *E. coli* and *S. aureus*. The methanolic crude extract of *N. oleander* flowers showed a greater zone of inhibition against *S. aureus* at 2.9 mm. The hexane crude extract of *I. versicolor* leaves showed the least zone of inhibition against *E. coli* at 2.2 mm in diameter. This showed that the plant crude extracts exhibited much higher activity against *S. aureus*, followed by *E. coli*

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INTRODUCTION

Phytochemicals are non-nutritious plant chemicals with protective or disease-prophylactic properties (Muscolo et al., 2024). They are unessential nutrients in that they are not a strict requirement for sustaining life (Erdman, 2023). Although plants commonly make these compounds to defend themselves, new studies have shown that they can also shield people from illness. More than a thousand phytochemicals are known to exist. Among the well-known phytochemicals are isoflavones and lycopene (Oleszek et al., 2023). These phytochemicals play several roles in both plant and animal biological processes. For instance, some phytochemicals exhibit antioxidant activity, hormonal action, enzyme stimulation, DNA replication interference, antibacterial activity, and many other activities (Omojate et al., 2014).

Finding novel alternative antimicrobial agents is becoming increasingly necessary due to the increase in harmful bacteria resistant to antibiotics over the last few decades. The potential antimicrobial properties of *N. oleander* leaf and flower extracts and *I. versicolor* leaf and root extracts were tested on resistant microbial strains, such as *S. aureus* and *E. coli*. Limited research has been done to evaluate the antimicrobial qualities of these plants. Medicinal plants provide primary medical care for about 85 % of the world's population since they are easily accessible and reasonably priced. Over the past few decades, several bacterial pathogens have shown growing resistance to the current antimicrobial medications. Since ancient times, people have used herbal remedies made from medicinal plants to treat various ailments (Abdallah et al., 2023). They are a rich source of numerous powerful medications due to their antibacterial qualities.

The use of botanical medicinal herbs in the treatment and/or prevention of diabetes, heart issues, and other cancers has had beneficial results. Some medicinal plants have been used, alone or in combination, to create a range of pharmaceuticals and even as the main ingredients in other conventional medicines. Some of the components contained in *N. oleander* and *I. versicolor* extracts, such as tannins, phenols and alkaloids, have been investigated and proved to possess antibacterial properties that are effective against gram-positive and gram-negative bacteria. In ethno-medicine,

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these herbs are used as a laxative, an anti-emetic, and to lessen inflammation (redness, swelling, and discomfort) at low doses (Ugwu & Suru, 2023). It is also helpful in treating skin eruptions like acne, spots, and blemishes. Still, it is most beneficial with other treatments for more chronic skin conditions like psoriasis and eczema.

Furthermore, it has been used to aid in weight loss and stimulate the movement of fat. It is crucial for the management of glandular diseases and endocrine disorders, including hypothyroidism with an enlarged thyroid, splenomegaly, lymphadenopathy, irregular menstruation, uterine fibroids, and disorders of the sebaceous glands. While *N. oleander* contains glycosides, which have health benefits with tremendous immune boosting action, it also has natural support for asthma, epilepsy and paralysis. It inhibits angiogenesis and causes programmed cell death in cancerous cells, which is natural cell death (Harikrishnan & Balasundaram, 2020). In this regard, the current study sought to fill a gap in knowledge on evaluating the antimicrobial activity of crude extracts from leaves, flowers and roots of *Iris versicolor* and *Nerium oleander* plants against *Escherichia coli* and *Staphylococcus aureus*. It sought to scientifically validate the antimicrobial potential of the two ornamental plants, *Iris versicolor* and *Nerium oleander*, crude extracts against *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Study Area

The study was done at the Kabianga location in Kericho County, and the plants *Nerium oleander* and *Iris versicolor* were collected within the University of Kabianga and Kapmaso village, respectively. The university is situated in the lush tea-growing highlands of Kericho at the southwestern end of the Rift Valley province of Kenya, and it is within the proximity of the multinational tea-growing companies Unilever, James Finlay, and George Williamson. The vegetation of Kabianga is an evergreen land with moderate rainfalls of 2,125 mm per annum, the highest and 1400 mm per annum in the lower parts, while the temperature range is between 10ºC-29ºC. The agricultural activities involved are mainly tea, dairy, and poultry farming. Kabianga is situated at 0.4339 º S, 5.1324ºE coordinates.

Sampling Design

The study area was chosen based on the natural distribution of *Nerium oleander* and *Iris versicolor,* which were determined in advance by an ecological survey and information from local people. The sampling area of both locations was identified using Google Maps. The site was selected to cover a wide range of microhabitats, in which the target plant species were abundant. Each area was separated into 10 plots to have a representative sampling. Wooden stakes were used to define the demarcation of plots. The plots were kept at an interval of 10m to lower the overlap in the plant populations and lower the chances of sampling the same plants.

Plant Distribution within Plots

Nerium oleander occurred primarily in open sunny places, usually in clusters of 5 to 15 plants per plot. The plants were shrubs, and the size was between 1 and 2 m in height, with an average density of 10 to 20 individual plants per plot obtained through visual count. *Iris versicolor* preferably occurs in semi-shady areas in the periphery of Kabianga stream, mostly in groups of 10-30 plants per plot. They are about 30 to 40 cm tall, with an average density of 15 to 25 plants per plot.

Plant Collection Procedure

Simple random sampling was utilised to select the plants for collection. Five healthy mature plants for each species were randomly chosen in every plot. Plants with healthy development of leaves, flower structures, and roots were regarded as mature. The collection was done between 7:00 AM and 10:00 AM to guarantee the optimum content of

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phytochemicals. 100 g of fully developed, undamaged leaves were collected on each selected upper and middle canopy plant. The leaves were collected using sterile gloves and pruning cutters, disinfecting them using 70% ethanol to avoid cross-contamination. The leaves were placed in labelled sampling bags.

For *Nerium oleander,* 50g of fully bloomed flowers were picked and weighed. Sterile forceps were used to obtain the flowers. The collected flowers were placed in labelled sampling bags. The secondary and tertiary roots of *Iris versicolor* were collected by excavating the soil around the base of each plant to a depth of 15 to 20 cm using a trowel. Their roots were carefully washed with distilled water to remove soil particles, dried with sterile paper towels, and cut into 5 to 10-cm pieces using sterile scissors. The roots were placed in appropriately labelled sampling bags. All the sampling bags were closed, stored in a cooler box, and transported to the Department of Biological Sciences of the University of Kabianga Laboratory within 4 hours of collection.

Research Design

An experimental research design was used in the study. The collected leaves, flowers and roots were dried in the laboratory, and a few samples were pressed. The pressed samples were taken for identification at the Botany Department of the National Museums of Kenya. The leaves, flowers and roots were then dried at room temperature, crushed and milled into fine particles. The fine particles were then extracted separately using hexane, dichloromethane, methanol (DCM) (1:1) and methanol. Extracts were then exposed to different bacterial strains to evaluate antimicrobial sensitivity testing.

Solvent Extraction of Secondary Metabolites from Leaves, Flowers and Roots.

The grounded leaves, flowers and roots were separately solvent extracted as shown in Figure 1 below: 500g of the powder was weighed individually and put into a 2.5L reagent bottle. Then, 1.5L of the solvent was added, and the bottle was corked. The corked bottles were swirled to ensure that the whole powder was submerged. It was left to stand for 72 hours before filtration. The mixture was then filtered using Whatman filter paper No. 1, and the filtrate was collected using a conical flask. The filtrate was then evaporated under vacuum and dried to a constant weight (W1). The yield of the extract is evaluated as a percentage (%) of the initial weight of the grounded leaf, flower and root powder. The dry extract is stored in a 10ml beaker covered with parafilm and kept at 4°C, awaiting use. The residue was dried for the subsequent extraction using solvents of different polarities. The extracted mass appeared greenish for the leaf extract and brownish for the flower and root extract. The concentrated gummy semi-solid mass of the extract was coded and labelled for identification purposes and then stored aseptically in a refrigerator awaiting use. The procedure was repeated for ground flowers and roots. Extraction followed the schematic diagram.

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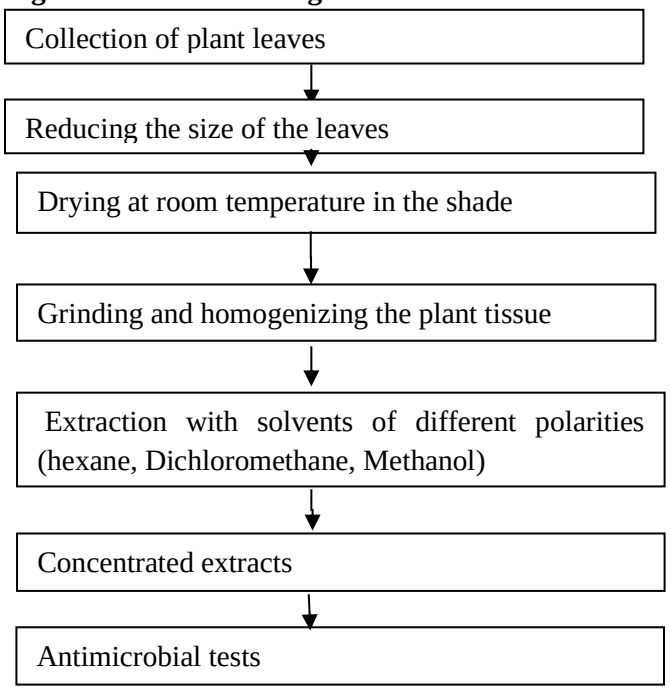
Figure 1: Schematic Diagram



Source: *Author 2024*

Antimicrobial Testing

Preparation of Susceptibility Test Discs

The 6 mm discs were prepared from Whitman No.1 filter paper. This was done by punching the filter papers with a paper punch. The discs prepared filled four McCartney bottles. The discs were then sterilised by autoclaving at 121°C for 15 minutes, after which the autoclave was left to cool before removing the McCartney bottles containing the discs. The discs were dried in a hot air oven at 50 °C to eliminate moisture.

To assess antimicrobial activity, the discs were infused with an antimicrobial compound derived from the leaf, flower, and root extracts of *N. oleander* and *I. versicolor* at a concentration of 1000 µg/ml. This process involved placing the sterile discs and the stock solutions within a laminar flow hood. Forceps designated for the retrieval of discs were initially sterilised with heat, utilising a spirit lamp and allowed to cool. Subsequently, the forceps were used to select the sterilised discs and transfer them into the stock solutions of plant leaves, flowers, and root extracts.

After each retrieval, the forceps underwent sterilisation, and the discs were permitted to remain in the stock solutions for two hours. Following this period, the discs were extracted and positioned in a sterile petri dish within a laminar flow hood, where they were left to dry for thirty minutes; thus, the discs were ready for the antimicrobial susceptibility test. Discs were positioned between the control and test zones. After an incubation period at 37 °C for 24 hours, a zone of growth inhibition was noted around the disc, depending on the sensitivity of the specific organism to the agent in question. The disk diffusion method utilises Mueller-Hinton agar (MHA), recognised as the optimal medium for standard susceptibility testing due to its excellent reproducibility and ability to support satisfactory growth of most bacterial pathogens.

About 50 mg of the crude extract was weighed with a precision balance and dissolved in 10 mL of the solvent, hexane, DCM, and methanol. It was then

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stirred in the glass plate using a glass rod to ensure the homogeneity of the crude extract. The prepared discs (6 mm) were dipped in the crude extract and allowed to stand for 5 minutes to absorb the phytochemicals present in the sample. The discs were then removed aseptically using a pair of forceps onto the already sterilised Mueller-Hinton agar.

Inoculation of Bacteria into the Plates

Using a wax pencil, each of the plate lids was labelled. The bacterial isolates (inoculum) were then introduced into replica plates containing Mueller-Hinton agar media. This was done by picking a colony of the bacteria aseptically using a wet swab and spreading it on the Mueller-Hinton plate. Finally, the swab was run around the edge of the plate to ensure that the entire plate surface was seeded. The plates were then allowed to dry for 10 minutes at room temperature before mounting the paper discs with the leaf, flower and root extract onto them. The discs were dispensed aseptically on the plate using sterilised pointed forceps. The discs were pressed to ensure contact between the agar and the disc; thus, the plates were incubated at 37 °C for 24 hrs.

Evaluation of Antimicrobial Activity

The Kirby-Bauer method was used to assess the antibacterial efficacy of the plant extracts against the bacterial strains *Staphylococcus aureus* and *Escherichia coli*. Mueller Hinton Agar (MHA) was used to evaluate antimicrobial activity. The inhibition of bacterial growth was measured using the Kirby-Bauer technique, which is recognised as the standard procedure for antimicrobial sensitivity testing. This test involved streaking a bacterial inoculum onto the surface of a 90 mm diameter Mueller-Hinton agar plate. 50 mg of each plant extract was accurately weighed using a precision balance and dissolved in 10 mL of the extracting solvent. The paper discs were immersed in the solution and rested for 20 minutes. Following this, the treated paper discs were aseptically positioned on the surface of the inoculated plates. The plates were subsequently incubated at 37 °C for intervals of 24, 36 and 48 hours.

A positive control was established using paper discs (6 mm) containing the standard antibiotic amoxicillin at a concentration of 500 mg, while methanol served as the negative control. Using a clear ruler, the inhibition zones were measured in millimetres, representing the diameter of the areas devoid of growth surrounding the discs. The inhibited zone's size indicates the isolate's susceptibility to the plant extract. Each extract and the standard antibiotic were evaluated independently in triplicate. The disc diffusion technique was employed as the standard method for antimicrobial activity (Mehmood et al., 2024). Antimicrobial activity was determined using the disc diffusion method against one Gram-positive bacteria (*Staphylococcus aureus*) and one harmful bacteria, *Escherichia coli* (clinical isolates), obtained from Kericho Referral District Hospital.

RESULTS

Antimicrobial Activity of Crude Phytochemicals

The crude plant extracts showed a greater zone of inhibition ranging from 2.5 mm to 3.2mm in diameter, irrespective of the plant part and the test micro-organism. The methanolic crude extract of *N. oleander* leaves showed a greater zone of inhibition against *Staphylococcus aureus* at 3.1 mm and 2.9 mm against *E. coli*. However, after 36 hours of incubation, the zone inhibited by the crude extract on *S. aureus* showed no growth of the micro-organisms, meaning the bacteria were fully cleared; this was observed on all the crude extracts irrespective of the extracting solvent on both *E. coli* and *S. aureus*, since the zone of inhibition increased. The methanolic crude extract of *N. oleander* flowers showed a greater zone of inhibition against *S. aureus* with a 2.9 mm inhibition zone. The hexane crude extract of *I. versicolor* leaves showed the least zone of inhibition against *E. coli* at 2.2 mm diameter, whereas the commercial antibiotic

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(amoxicillin), which was tested against the two bacteria, showed a greater zone of inhibition of 4.0 mm irrespective of the micro-organism. This showed that the plant crude extracts exhibited much higher activity against *S. aureus*, followed by *E. coli*. The tables below present the zones of inhibition for antimicrobial testing.

Table 1: Methanolic Extracts Antimicrobial Testing Zone of Inhibition in mm

			Zone of inhibition in mm			
Micro-organism	**Incubation time**	**Plant**	**Leaves**	**Flowers/Roots**	**Solvent**	**Amoxicillin**
E. coli	24 hours	*N. oleander*	2.8	2.7	0.9	3.0
		I. versicolor	2.5	2.4	0.9	3.0
	36 hours	*N. oleander*	2.9	2.8	1.0	3.0
		I. versicolor	2.6	2.5	1.0	3.0
	48 hours	*N. oleander*	2.9	2.5	1.1	3.0
		I. versicolor	2.6	2.5	1.1	2.5
S. aureus	24 hours	*N. oleander*	3.1	2.5	0	3.4
		I. versicolor	2.9	2.9	0	3.4
	36 hours	*N. oleander*	3.1	2.6	0	3.4
		I. versicolor	3.0	2.9	0	3.4
	48 hours	*N. oleander*	1.1	1.2	0	3.4
		I. versicolor	2.3	1.4	0	3.5

Table 2: DCM Extracts Antimicrobial Sensitivity Testing Zone of Inhibition in mm

Micro-organisms	**Incubation period**	**Plants**	**Zone of inhibition in mm**			
Escherichia coli	24 hours	*Nerium oleander*	3.1	2.6	1.6	4.0
		Iris versicolor	3.0	2.6	1.6	4.0
	36 hours	*Nerium oleander*	3.1	2.6	1.6	4.0
		Iris versicolor	3.1	2.6	1.6	4.0
	48 hours	*Nerium oleander*	3.1	2.6	1.6	4.0
		Iris versicolor	3.2	2.9	1.6	4.0
S. aureus	24 hours	*Nerium oleander*	2.7	2.9	2.6	4.0
		Iris versicolor	2.9	3.0	2.6	4.0
	36 hours	*Nerium oleander*	2.8	3.0	2.6	4.0
		Iris versicolor	2.7	2.9	2.6	4.0
	48 hours	*Nerium oleander*	2.9	3.0	2.6	4.0
		Iris versicolor	2.8	3.0	2.6	4.0

Table 3: Hexanoic Extracts Antimicrobial Sensitivity Testing Zone of Inhibition in mm

Micro-organisms	**Incubation period**	**Plants**	**Zones of inhibition in mm**			
E. coli	24 hours	*N. oleander*	2.4	2.5	0	3.4
		I. versicolor	2.3	2.4	0	3.4
	36 hours	*N. oleander*	2.3	2.4	0	3.5
		I. versicolor	2.2	2.3	0	3.5
	48 hours	*N. oleander*	2.3	2.4	0	3.5
		I. versicolor	2.2	2.4	0	3.5
Staphylococcus aureus	24 hours	*N. oleander*	2.7	2.7	0	3.0

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Micro-organisms	Incubation period	Plants	Zones of inhibition in mm			
		I. versicolor	2.5	2.6	0	3.0
	36 hours	*N. oleander*	2.8	2.8	0	3.0
		I. versicolor	2.5	2.6	0	3.0
	48 hours	*N. oleander*	2.8	2.8	0	3.0
		I. versicolor	2.9	2.9	0	3.0

DISCUSSION OF RESULTS

The current study reported zones of inhibition ranging from 2.2 mm to 3.2 mm for crude extracts from leaves, flowers, and roots of *N. oleander* and *I. versicolor*, with methanolic extracts of *N. oleander,* leaves showing the highest activity of 3.1 mm against *S. aureus* and 2.9 mm against *E. coli*. According to the findings, the commercial antibiotic amoxicillin exhibited zones of 3.0–4.0 mm, suggesting limited efficacy. The findings indicate that *S. aureus*, a gram-positive bacterium, was more susceptible than *E. coli*. This gram-negative bacterium aligns with previous studies that plant-derived compounds may penetrate gram-positive cell walls more effectively due to their structural differences (Atanasov et al., 2015).

Previous studies on *N. oleander* have shown significant antimicrobial activity. Shafiq et al. (2021) found ethanolic flower extracts at 30 mg/mL produced zones of 27.8 ± 6.92 mm for *E. coli* and 28.8 ± 5.74 mm for S. aureus. The findings of this study are consistent with the previous studies on the antimicrobial activity of *N. oleander*. In addition, Jamal et al. (2012) reported zones of 19 mm for E. coli using petroleum ether extracts, which is consistent with the current findings. *I. versicolor*, literature on antimicrobial activities is relatively sparse. However, a recent study by Jaegerova et al. (2024) confirmed its antimicrobial activity, consistent with the current research. However, specific zone sizes against *E. coli* and *S. aureus* were not detailed in the previous studies, as captured herein.

The current study used extracts at 1000 µg/mL (1 mg/mL), while other studies, including Shafiq et al. (2021), used 30 mg/mL, a 30-fold higher concentration, likely contributing to larger inhibition zones. Higher concentrations typically enhance diffusion and activity, as seen in the dose-dependent effects reported in the literature. The current study employed methanolic, DCM: methanol, and hexane extracts, whereas other studies used ethanolic or petroleum ether extracts Jamal et al., 2012). Solvents with different polarities extract varying phytochemicals. Ethanol may extract more polar compounds like flavonoids and tannins, which are known for antimicrobial activity, potentially explaining the larger inhibition zones. The current study included crude extracts of leaves, flowers, and roots, while some previous studies focused on specific parts, such as leaves or roots. The distribution of active compounds, such as cardiac glycosides in *N. oleander* or terpenoids in *I. versicolor*, varies by plant part, which could affect activity levels. In addition, differences in bacterial strains used in other studies could also influence the differences in results.

Numerous investigations have been conducted to determine the antimicrobial activity of phytochemicals found in plants that prevent disease and promote health, as well as to comprehend the fundamental mechanisms underlying their effects. The chemical constituents have been identified and isolated as part of these investigations, and their biological potency has been assessed through epidemiological and clinical case-control studies in humans. In addition to antimicrobial activity, various studies have also indicated that phytoconstituents may reduce the incidence of coronary heart disorder by lowering the production or absorption of cholesterol, reducing the oxidation of low-density lipoprotein (LDL) cholesterol, restoring arterial flexibility, and stabilising blood

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pressure and coagulation (Saxena et al., 2013). In addition, phytochemicals have the potential to detoxify carcinogens. They seem to prevent free radicals from forming, block the actions of enzymes that cause cancer, and activate enzymes that break down harmful substances (Lobo et al., 2010).

The findings contribute to the growing body of evidence supporting the antimicrobial potential of medicinal plants, aligning with their traditional use in ethnomedicine for treating bacterial infections. Phytochemicals like tannins, phenols, and alkaloids contribute to antimicrobial activity, reinforcing the role of secondary metabolites in plant defence and human health. The study also highlights the need for further research into *I. versicolor*, given the limited literature, potentially expanding the theoretical framework for plant-based antimicrobials. The findings open avenues for further exploration, such as isolating and purifying active compounds for enhanced efficacy or combining extracts with other agents for synergistic effects. For *N. oleander*, despite its activity, practical use is constrained by its toxicity, necessitating careful formulation to mitigate risks, such as in topical applications for skin infections for conditions like psoriasis and eczema. For *I. versicolor*, the results support its potential in traditional remedies, such as root teas, for emetic and detoxifying properties. Given its use in cosmetics and dietary products, it could inform the development of natural preservatives or supplements.

CONCLUSION

The current study reported zones of inhibition ranging from 2.2 mm to 3.2 mm for crude extracts from leaves, flowers, and roots of *N. oleander* and *I. versicolor*, with methanolic extracts of *N. oleander*, leaves showing the highest activity of 3.1 mm against *S. aureus* and 2.9 mm against *E. coli*. The findings indicate that *S. aureus*, a gram-positive bacterium, was more susceptible than *E. coli*, a gram-negative bacterium. The plant extracts showed antimicrobial activity against the bacterial pathogens *E. coli* and *S. aureus*. Antimicrobials with low MICs are more effective than those with high MICs, as only a low dosage is necessary to eradicate microbes. Therefore, bactericidal kills the bacteria while bacteriostatic inhibits bacteria, thus stopping bacteria from multiplying.

Recommendations

After scientifically validating their safety, the plant's leaves, flowers, and root extract can be used to formulate a drug to fight bacterial micro-organisms. There is a need to elucidate the extracts' phytochemical components that might be responsible for the antimicrobial activity.

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