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Comparative Study of Antimicrobial Efficacy and Phytochemical Profiles of Ethanol and n-Hexane Extracts from the Roots of *Ixora coccinea* Linn

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The root extracts of *Ixora coccinea* were evaluated for their phytochemical composition, antibacterial activity, and minimum inhibitory concentration (MIC) against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Quantitative phytochemical analysis was conducted to determine the levels of terpenoids, flavonoids, alkaloids, tannins, and saponins. Thin Layer Chromatography (TLC) was used to confirm the presence of bioactive components in the extracts under different solvent systems, followed by antibacterial susceptibility tests using the disc diffusion

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method. The MIC was determined through serial dilution to identify the lowest concentration that inhibited bacterial growth. Quantitative phytochemical analysis showed that the ethanol extract contained 14.80% flavonoids, 6.00% alkaloids, 1.20% saponins, 1.80% terpenoids, and 0.03% tannins. In contrast, the n-hexane extract had higher terpenoid content (3.40%) but lower flavonoids (5.20%) and alkaloids (2.10%). Thin Layer Chromatography (TLC) analysis under varying solvent systems showed different R_f values, with the ethanol extract displaying more diverse spots, indicating a richer variety of bioactive compounds. Antibacterial susceptibility tests revealed that the ethanol extract produced larger inhibition zones (10 ± 0.4–28 ± 1.0 mm) than the n-hexane extract (6 ± 0.2–26 ± 0.8 mm). MIC results indicated that ethanol extract inhibited *E. coli* and *S. aureus* at 50 mg/L and *B. subtilis* at 25 mg/L, while the n-hexane extract required 100 mg/L for effective inhibition. In conclusion, the findings indicate that the plant possesses significant antibacterial potential, warranting further research to isolate and characterize the specific phytoconstituents responsible for the observed antimicrobial activities.

Keywords: Ixora coccinea; *phytochemical analysis; antimicrobial activity; Escherichia coli; Staphylococcus aureus; bacillus subtilis; minimum inhibitory concentration.*

1. INTRODUCTION

For ages, plants have been employed for medicinal purposes, serving as remedies for various ailments in both humans and animals [1]. The therapeutic potential of these plants is attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and phenolics, which exhibit beneficial physiological or therapeutic effects, including antimicrobial activity in the human body [2,3]. Their use spans traditional remedies and modern pharmaceuticals, with many people in developing regions still relying on plant-based medicine for primary healthcare [4]. Even today, around 80% of the global population relies on herbal remedies for primary healthcare, particularly in regions with limited access to modern pharmaceuticals. Beyond traditional applications, plants have gained renewed scientific interest due to their bioactive compounds, especially amid concerns over antimicrobial resistance (AMR). Given the rising prevalence of antibiotic resistance and the associated health risks, there is an increasing interest in exploring natural alternatives to synthetic drugs, which are often expensive and may come with a range of side effects. This scenario emphasizes the need for accessible and effective natural remedies, particularly from plants that have a long history of safe use.

Ixora coccinea, commonly known as jungle flame or flame of the woods, is a perennial shrub belonging to the Rubiaceae family. This plant produces flowers almost year-round, with peak blooming during the rainy season. Native to the Western Peninsula, it is now widely cultivated across tropical regions. The plant is known to contain wide varieties of phytochemicals [5]. *Ixora coccinea* L. is traditionally utilized for managing various ailments and is also cultivated as an ornamental plant. The roots of *I. coccinea* serve multiple purposes, functioning as an astringent, antiseptic, stomachic, and sedative. They are also traditionally employed in treating conditions such as diarrhea, dysentery, gonorrhea, hiccups, fever, sores, loss of appetite, and chronic ulcers [6]. The plant's flowers are commonly used to address haemoptysis, leucorrhoea, dysentery, dysmenorrhea, and catarrhal bronchitis. Additionally, a decoction of the flowers or bark is applied as a lotion for eye-related issues [7]. Preclinical studies demonstrate that *I. coccinea* exhibits diverse pharmacological properties, including antimicrobial, anti-inflammatory, antinociceptive, antioxidant, anti-ulcerogenic, hepatoprotective, antidiarrheal, antimutagenic, and hypolipidemic activities [8]. Phytochemical analysis of *I. coccinea* identified several key compounds, including ursolic acid, lupeol, oleanolic acid, rutin, sitosterol, leucocyanidin, anthocyanins, proanthocyanidins, quercetin, and kaempferol glycosides [9], many of which exhibit antimicrobial properties [10]. However, while much of the research on *Ixora coccinea* has focused on its leaves and flowers, studies comparing the antimicrobial potential of n-hexane and ethanol extracts specifically from its roots are absent in the literature. This study is the first to conduct a comparative analysis of these two extracts, aiming to provide new insights into the bioactivity of *I. coccinea* root.

Therefore, this study aims to evaluate and compare the phytochemical composition and antimicrobial efficacy of ethanol and n-hexane extracts from the roots of *I. coccinea*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh roots of *I. coccinea* were harvested from Ikot Ekpene, Akwa Ibom State, Nigeria. The plants were identified and authenticated at the Department of Microbiology, Rivers State University, Port-Harcourt. The harvested fresh roots of *I. coccinea* were washed thoroughly with distilled water to remove any dust and impurities. The roots were then chopped into small pieces and allowed to air dry at room temperature for three weeks. After drying, the roots were ground into a fine powder using a blender, sieved, and stored in an airtight container for further analysis.

2.2 Extraction of Plant Materials

About 120 g of the powdered samples were separately soaked in 300 mL of ethanol and 300 mL of n-hexane, yielding two sets of extracts for each plant (ethanol extract and n-hexane extract). The mixtures were macerated for 72 hours at room temperature with occasional shaking to enhance extraction. After the extraction period, the solutions were filtered using Whatman No. 1 filter paper to remove plant debris. The filtrates were then concentrated to dryness using a water bath maintained at 50℃, allowing for solvent evaporation and yielding the crude extracts. The crude ethanol and n-hexane extracts were transferred to sterile containers and stored in a refrigerator for further analysis.

2.3 Quantitative Phytochemical Screening

2.3.1 Total flavonoid content

The flavonoid content of the root extracts were determined using the method of Evuen & Kpomah, [11]. Each sample (2.50 g) was added to a 250 cm^3 beaker containing 50 cm^3 of 80% aqueous methanol and left to stand at room temperature for 24 hours. The supernatant was discarded, and the residue was subjected to three additional extractions with the same volume of ethanol. The combined solution from each sample was filtered through Whatman No. 42 filter paper (125 mm), then transferred to a crucible and dried over a water bath. The crucible and its contents were cooled in a desiccator and weighed repeatedly until a constant weight was achieved. The flavonoid content was calculated as follows:

$$\% \text{ flavonoid} = \text{weight of flavonoid} / \text{weight of sample} \times 100$$

2.3.2 Total alkaloid content

The alkaloid content of the root extracts were determined following the method by Biradar & Rachetti, [12]. Five grams (5 g) of the sample was added to 200 mL of 10% acetic acid in methanol in a 250 mL beaker, covered, and left to stand for 4 hours. After filtration, the filtrate was concentrated in a water bath to approximately 25% of its initial volume. Concentrated NH_4OH was then added dropwise until precipitation ceased. The mixture was allowed to settle, and the precipitate was collected, washed with dilute NH_4OH, and filtered. The residual material was dried and weighed, with the alkaloid content calculated as a percentage as follows:

$$\% \text{ alkaloid} = \text{weight of alkaloid} / \text{weight of sample} \times 100$$

2.3.3 Saponin content

The method of Obadoni & Ochuko, [13], with slight modifications, was used to quantify the saponin content in the root extracts. Ten grams (10 g) of each extract was placed in a conical flask with 50 mL of 20% aqueous methanol, then heated to 55 °C with continuous stirring for 4 hours. After filtration, the residue was re-extracted with 100 mL of 20% aqueous methanol. The combined extracts were reduced to 40 mL in a water bath at 90 °C. The concentrate was transferred to a separating funnel, where 10 mL of diethyl ether was added and gently mixed. The aqueous layer was collected, and the ether layer discarded. This purification step was repeated once. Next, 30 mL of n-butanol was added to the aqueous layer, followed by two washes with 10 mL of 5% aqueous NaCl. The remaining solution was evaporated, and the samples were oven-dried to a constant weight. The saponin content was then calculated as a percentage as follows:

$$\% \text{ saponin} = \text{weight of saponin} / \text{weight of sample} \times 100$$

2.3.4 Tannin content

The tannin content of the samples was determined following the method described by

Evuen and Kpomah, [11]. Five grams (5.0 g) of each sample were mixed with distilled water in a 1:10 (w/v) ratio, shaken for 30 minutes at room temperature, and filtered to obtain the extract. A standard tannic acid solution was prepared, with 2 mL of this solution and 2 mL of distilled water added separately to 50 mL volumetric flasks as the standard and reagent blank, respectively. Two milliliters (2 mL) of each sample extract were placed in labeled flasks, and 1 mL of Folin-Denis reagent was added to each after mixing with 35 mL of distilled water. Next, 2.5 mL of saturated Na_2CO_3 solution was added, and each flask was topped up with distilled water to the 50 mL mark, then left to stand for 90 minutes at room temperature. The absorbance was measured at 760 nm with the spectrophotometer, using the reagent blank as the reference. The formula for calculating tannin content is shown below:

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{au}{as} \times c \times \frac{vt}{va}$$

Where:

W= weight of the sample
au = absorbance of the test sample
as = absorbance of the standard tannin solution
C = concentration of standard tannin solution
vt = total volume of the ass mixture
va = volume of extract analyzed

2.3.5 Test for terpenoids

The total terpenoid content in the root extracts were determined using Evuen and Kpomah, [11]. Two grams (2 g) of powdered leaf sample were soaked in 50 mL of 95% ethanol for 24 hours. The mixture was then filtered using Whatman No. 42 filter paper. The resulting filtrate was further extracted with petroleum ether at 60–80 °C, and the solution was then dried in a water bath at 65 °C. The percentage of terpenoid content was calculated using the following formula:

% total terpenoid = weight of residue / weight of sample × 100

2.4 Antimicrobial Activity

The antimicrobial activity of ethanol and n-hexane extracts from Ixora coccinea roots was tested against three bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The strains were obtained from the Department of Microbiology, Rivers State University, Port-Harcourt, where they had been identified and stored under appropriate laboratory conditions. These organisms were chosen due to their clinical relevance and prevalence in bacterial infections.

2.5 Preparation of Culture Media

Mueller Hinton Agar (MHA) was used as the growth medium for bacteria. Mueller Hinton Agar was selected for bacterial growth and antimicrobial susceptibility testing because it is non-selective, non-differential, and provides reproducible results for most bacterial pathogens. A total of 39 g of MHA powder was dissolved in 1000 mL of distilled water, and the mixture was sterilized at 121°C for 15 minutes in an autoclave. The sterilized medium was allowed to cool before being poured into sterile petri dishes.

2.6 Preparation of Extract Concentrations

Ethanol and n-hexane extracts were prepared in serial concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/L. For each concentration, 0.5 g of extract was dissolved in dimethyl sulfoxide (DMSO) as the solvent.

2.6.1 Antibacterial susceptibility test

The disc diffusion method as described by Khalid et al., [14] was employed to determine the antibacterial activity. For this purpose, 10 mL of sterilized media was poured into sterilized 15 ml Petri dishes. Bacterial cultures were seeded on agar media in Petri plates by streaking method. Sterile filter paper discs (6 mm diameter) were impregnated with 20 µl of each extract concentration extracts (200 mg/L, 100 mg/L, 50 mg/L, 25 mg/L, 12.5 mg/L, 6.25 mg/L, and 3.125 mg/L) and placed on the agar surface. These concentrations were prepared using serial dilution from a stock solution of the crude extracts. Gentacycline (10 µg/disc) was used as a positive control to compare the antibacterial potency of the extracts. Prepared antibiotic disks were applied on each plate and the plates were then incubated upside down at 37°C for 24 h. The inhibition zones were measured and recorded in millimeters to assess the antibacterial activity of the extracts.

2.6.2 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is the concentration with the lowest inhibition of microorganisms. The Minimum Inhibitory

Concentration (MIC) of the extracts was determined using a broth microdilution method. Extracts were serially diluted in nutrient broth in a 96-well microtiter plate, starting from the highest concentration used in the disk diffusion test. Each well was inoculated with a standardized suspension of the test organisms (*E. coli, S. aureus, and B. subtilis*) prepared at a 0.5 McFarland standard to ensure consistent microbial load. The plates were incubated at 37°C for 24 hours. The presence or absence of growth was then observed. The MIC was recorded as the lowest concentration of the extract that inhibited visible growth of the microorganisms.

2.6.3 Thin Layer Chromatography (TLC) analysis

The *I. coccinea* root extracts were subjected to Thin Layer Chromatography (TLC) to identify their phytochemical composition. Pre-prepared silica gel TLC plates were used and 10 µL of ethanol and n-hexane extracts were spotted onto the plates. The plates were then developed using solvent systems of varying polarities, including hexane-ethyl acetate in the ratios 90:10, 80:20, 70:30, 50:50, and ethyl acetate-DCM (95:05), as well as hexane-ethanol (90:10). After placing the spotted plates in a developing chamber, the solvent front was allowed to ascend by capillary action. Upon completion of development, the plates were removed, air-dried, and visualized under ultraviolet (UV) light at 254 nm, revealing various spots with distinct colors. The retention factor (Rf) of the different components was computed using the following formula:

Retention Factor (Rf) = Distance moved by the spot (cm) / Solvent Front (cm)

2.7 Statistical Analysis

Statistical analysis of the research work was carried out using SPSS version 21.0. Results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Composition of *Ixora coccinea* Root

The phytochemical analysis of *I. coccinea* roots revealed significant variations in the content of bioactive compounds across the ethanol and n-hexane extracts (Table 1). Terpenoids, saponins, flavonoids, alkaloids, and tannins were identified in the both extracts. Flavonoids (14.8%) and alkaloids (6.0%) were more abundant in the ethanol extract, while terpenoids were higher in the n-hexane extract (3.4%). Saponins and tannins were present in lower concentrations in both extracts.

These phytochemicals have a wide range of biological activities. Flavonoids, abundant in the ethanol extract, are known for their antioxidant, antimicrobial, and anti-inflammatory effects by scavenging free radicals and disrupting bacterial membranes [15,16]. Alkaloids possess antimicrobial, anti-inflammatory, and analgesic properties and can inhibit bacterial DNA synthesis, making them useful in disease control [16,17]. Terpenoids, which were more abundant in the n-hexane extract, are recognized for their antimicrobial, antifungal, anticancer and antitumor properties due to their ability to disrupt cell membranes and interfere with signaling pathways [15,18]. Saponins, though present in lower concentrations, exhibit antioxidant, cholesterol-lowering, and immune-stimulating effects [16]. Tannins, despite their minimal concentration, have been reported to possess antimicrobial, antioxidant, and anti-inflammatory and astringent properties, contributing to the plant's potential use i,n wound healing and treatment of gastrointestinal disorders [15,19].

3.2 Thin Layer Chromatography (TLC) Analysis of *Ixora coccinea* Root Extracts

TLC is a widely used technique for the separation and identification of phytochemicals, providing insights into the polarity, purity, and chemical composition of plant extracts [20,21]. Table 2 presents the results of Thin Layer Chromatography (TLC) analysis of n-hexane and ethanol extracts of *I. coccinea* roots using various solvent systems. The analysis revealed distinct phytochemical profiles for both extract types, characterized by the number of spots, their colors, and retention factor (R_f) values. The number of spots observed in both extracts suggests the presence of multiple bioactive compounds with varying polarities.

In the hexane-ethyl acetate systems (90:10, 80:20, 70:30), n-hexane consistently produced a single gray spot, indicating a singular compound, while the ethanol extracts showed a pale yellow spot, suggesting the presence of different polar compounds. The Rf values varied across solvent compositions, reflecting how solvent polarity influences compound mobility. In ethyl

Table 1. Quantitative phytochemical evaluation of ethanol and n-hexane extracts of *Ixora coccinea* roots

Phytochemical Component	Extract Type	*Ixora coccinea* roots (%)
Terpenoids	Ethanol	1.80
	n-Hexane	3.40
Flavonoids	Ethanol	14.80
	n-Hexane	5.20
Alkaloids	Ethanol	6.00
	n-Hexane	2.10
Saponins	Ethanol	1.20
	n-Hexane	0.80
Tannins	Ethanol	0.03
	n-Hexane	0.01

acetate-DCM systems, the presence of multiple spots in the ethanol extracts (including gray and pale yellow) indicates interactions that are more complex and a broader range of compounds. Notably, the n-hexane-ethanol system (90:10) exhibited three distinct spots (yellow, pale yellow, gray) in the n-hexane extract, highlighting a diverse array of compounds. This analysis demonstrates the potential for isolating bioactive constituents from *I. coccinea*, warranting further investigation into its therapeutic applications.

3.3 Antibacterial Activity of n-Hexane and Ethanol Extracts of *Ixora coccinea* Roots

Table 3 presents the antibacterial activity of n-Hexane and Ethanol extracts of *I. coccinea* roots against three bacterial strains: *E. coli*, *S. aureus*, and *B. subtilis*. The results indicate varying levels of inhibitory activity across different concentrations.

From the findings, both extracts exhibited concentration-dependent inhibition, with higher concentrations producing larger inhibition zones. Ethanol extracts generally showed stronger antibacterial activity than n-hexane extracts. For *S. aureus*, the ethanol extract produced an inhibition zone of 28 ± 1.0 mm at 200 mg/L, surpassing the n-hexane extract's 26 ± 0.8 mm at the same concentration. This suggests that polar compounds in the ethanol extract may be more potent against this organism. *E. coli* exhibited comparable sensitivity to both extracts at 200 mg/L, with inhibition zones of 24 ± 0.5 mm for both ethanol and n-hexane extracts. However, the ethanol extract performed better at lower

Table 2. TLC analysis of n-hexane and ethanol extracts of *Ixora coccinea* roots under different solvent systems

Solvent System	Name of Extract	No of Spots	Colours	R_f Values
Hexane - ethylacetate 90:10	n-hexane	1	Gray	0.15
	Ethanol	1	Pale yellow	0.97
Hexane - ethylacetate 80:20	n-hexane	1	Gray	0.38
	Ethanol	1	Pale yellow	0.20
Hexane - ethylacetate 70:30	n-hexane	1	Gray	0.16
	Ethanol	1	Pale yellow	0.71
Ethylacetate - DCM 50:50	n-hexane	1	Gray	0.47
	Ethanol	1	Pale yellow	0.92
Ethylacetate -DCM 95:05	n-hexane	1	Gray	0.92
	Ethanol	2	Gray	0.75
			Pale yellow	0.21
Hexane - Ethanol 90:10	n-hexane	3	Yellow	1.03
			Pale yellow	0.20
			Gray	1.07
	Ethanol	1	Pale yellow	0.71

Table 3. Antibacterial susceptibility test of ethanol and n-hexane extracts of *Ixora coccinea* Root

Organism	Extract Type	200 mg/L (mm)	100 mg/L (mm)	50 mg/L (mm)	25 mg/L (mm)	12.5 mg/L (mm)	6.25 mg/L (mm)	3.125 mg/L (mm)	Gentamicin (10 µg) (mm)
E. coli	Ethanol	24 ± 0.5	20 ± 1.0	18 ± 1.2	14 ± 0.8	12 ± 0.6	10 ± 0.4	0 ± 0.0	24 ± 0.7
	n-Hexane	24 ± 0.5	18 ± 0.9	16 ± 1.1	14 ± 0.5	12 ± 0.7	8 ± 0.3	6 ± 0.2	24 ± 0.6
S. aureus	Ethanol	28 ± 1.0	22 ± 1.3	18 ± 1.2	16 ± 0.9	12 ± 0.8	10 ± 0.5	0 ± 0.0	22 ± 0.5
	n-Hexane	26 ± 0.8	20 ± 1.0	18 ± 0.9	16 ± 0.8	12 ± 0.6	10 ± 0.5	8 ± 0.4	22 ± 0.4
B. subtilis	Ethanol	22 ± 0.9	20 ± 1.1	18 ± 0.8	16 ± 0.7	14 ± 0.6	10 ± 0.4	0 ± 0.0	22 ± 0.6
	n-Hexane	22 ± 0.9	18 ± 0.9	16 ± 0.7	14 ± 0.6	10 ± 0.5	6 ± 0.3	0 ± 0.0	22 ± 0.5

Values represent mean ± standard deviation (n=3)

Table 4. Minimum inhibitory test of ethanol and n-hexane extracts of *Ixora coccinea* root

Organism	Extract Type	200 mg/L	100 mg/L	50 mg/L	25 mg/L	12.5 mg/L	6.25 mg/L	3.125 mg/L
E. coli	Ethanol	-	-	+	+	+	+	+
	n-Hexane	-	-	-	+	+	+	+
S. aureus	Ethanol	-	-	+	+	+	+	+
	n-Hexane	-	-	-	+	+	+	+
B. subtilis	Ethanol	-	-	-	+	+	+	+
	n-Hexane	-	-	-	+	+	+	+

Key: - = Clear tubes (indicates no bacterial growth); + = Turbidity in tubes (indicates bacterial growth)

concentrations, as indicated by the absence of inhibition in the n-hexane extract at 3.125 mg/L, compared to 10 mm inhibition at 6.25 mg/L in the ethanol extract. Similarly, *B. subtilis* demonstrated maximum inhibition of 22 ± 0.9 mm at 200 mg/L for both extracts. However, the ethanol extract maintained superior inhibitory effects at intermediate concentrations (e.g., 14 ± 0.6 mm at 12.5 mg/L vs. 10 ± 0.5 mm for the n-hexane extract), indicating that ethanol-soluble compounds possess stronger antibacterial properties.

Compared to gentamicin (10 µg), which served as the control, both extracts showed promising antibacterial potential, especially at higher concentrations. Notably, the ethanol extract's inhibition of 28 ± 1.0 mm against *S. aureus* exceeded the inhibition zone of 22 ± 0.5 mm produced by gentamicin, suggesting that *I. coccinea* extract may contain potent antibacterial agents.

These findings are consistent with Muhammad et al. [22], who reported significant antibacterial activity for *I. coccinea* root extracts using various solvents. Their study found that methanol, petroleum ether, chloroform, and ethyl acetate extracts were effective against most bacterial strains but not against *Escherichia coli*. In contrast, this study demonstrated that both ethanol and n-hexane extracts exhibited antibacterial activity against *E. coli*. This suggests that ethanol and n-hexane may extract distinct or additional bioactive compounds effective against *E. coli*. Furthermore, our findings are consistent with Selvaraj et al. [23], who also observed antibacterial activity in ethanolic extracts of *I. coccinea* root.

3.4 Minimum Inhibitory Concentration (MIC) of *Ixora coccinea* Root Extracts

Table 4 presents the Minimum Inhibitory Concentration (MIC) results for of n-Hexane and Ethanol extracts of *I. coccinea* root against *E. coli*, *S. aureus*, and *B. subtilis*. The MIC is the lowest concentration of the extract at which visible bacterial growth is inhibited. The ethanol extract demonstrated stronger antibacterial activity at lower concentrations compared to the n-hexane extract. For both *E. coli* and *S. aureus*, the ethanol extract inhibited bacterial growth up to 50 mg/L, with turbidity indicating microbial growth from 25 mg/L downward. In contrast, the n-hexane extract exhibited inhibitory activity up to 100 mg/L, with bacterial growth observed starting from 50 mg/L. These findings suggest that ethanol may extract more potent bioactive compounds from the plant root than n-hexane, likely due to the polarity of ethanol, which can dissolve a broader range of antibacterial compounds.

Bacillus subtilis was equally sensitive to both extracts at 50 mg/L and higher concentrations, with growth resuming in both extracts from 25 mg/L downward. This consistent sensitivity suggests that the antibacterial compounds in the extracts have a broad-spectrum effect.

These results highlight the potential of *I. coccinea* root extracts, particularly the ethanol extract, as a natural antibacterial agent. The observed antibacterial activities may be attributed to the presence of various phytochemicals, including saponins, flavonoids, tannins, alkaloids, and terpenoids, which have been recognized for their antimicrobial properties [16,24].

4. CONCLUSION

This study demonstrates that the ethanol and n-hexane extracts of *Ixora coccinea* roots possess significant antimicrobial activity, likely attributed to the presence of bioactive phytochemicals such as flavonoids, alkaloids, terpenoids, and saponins. The ethanol extract, with higher concentrations of these compounds, exhibited stronger antibacterial effects, showing larger inhibition zones and lower MIC values against *E. coli, S. aureus*, and *B. subtilis*. In comparison, the n-hexane extract showed less potency, indicating that solvent type plays a crucial role in the extraction efficiency of antimicrobial constituents. These results suggest that *I. coccinea* roots have potential as a natural source of antimicrobial agents. Further studies are recommended to isolate, characterize, and evaluate the specific bioactive compounds responsible for the observed antibacterial activity, and to explore their potential applications in pharmaceutical formulations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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