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Full Length Research Article

Antibacterial Evaluation of Leaf Extracts of *Lonchocarpus cyanescens* (Schum & Thonn.) on Antibiotic-Resistant Bacteria Associated with food poisoning

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Abstract

Background: *Lonchocarpus cyanescens* has been exploited in different parts of the world because of its pharmacological value. This value is due to the phytochemical constituents found in different parts of the plants. In this paper, we examined the compounds that are biologically active and the phytochemicals inherent in the leaves of *L. cyanescens,* both qualitatively and quantitatively, while also evaluating the antimicrobial efficacy of the plant extracts against selected pathogenic bacteria.

Methods: The source of isolates for this study was stool specimens collected from diarrhoeic stool patients attending the University of Medical Sciences Teaching Hospital (UNIMEDTH) in Akure. Extracts of *L. cyanescens* were produced using water, ethanol, and n-hexane, after which they were analyzed for their bioactive constituents and antibacterial effects using Varian GC–MS system.

Results: Several classes of phytochemicals were identified across the different extracts of the plant. At a concentration of 300 mg/ml, the ethanol and n-hexane extracts exhibited the strongest activity against *Citrobacter freundii*, producing inhibition zones of 28.33 ± 0.33 mm and 23.00 ± 1.00 mm, respectively.

Conclusion: The findings of this research indicated that extracts from *Lonchocarpus cyanescens* exhibits notable antimicrobial activity against *C. freundii*.

Keywords: Inhibition, Antibacterial, Phytochemicals, Bacteria, *Lonchocarpus cyanescens*

Introduction

Foodborne illness arises from eating food that has been contaminated. This contamination may involve harmful chemicals, toxins, or infectious organisms—including bacteria, viruses, parasites, or prions—that cause negative effects in the body. Symptoms may be limited to gastrointestinal distress, such as nausea, vomiting, or diarrhea, but in some cases, the impact can reach other organs, including the kidneys, brain, or muscles (Dietrich *et al.,* 2023).

The term food poisoning describes an acute gastrointestinal illness that occurs after consuming contaminated food or beverages, such as water, juice, or wine. The contamination may result from various sources, including microorganisms naturally present in humans, animals, or the environment; parasites, particularly intestinal worms contacted through contaminated food and water; chemical contaminants, adulterants, or toxic substances introduced either unintentionally or deliberately; and naturally occurring toxins found in certain foods or produced under specific environmental conditions (Bari and Yeasmin, 2018).

In developing nations, food poisoning continues to be a major cause of illness and mortality (Ahmed *et al.,* 2023). Most instances of foodborne disease are linked to bacterial contamination, particularly Gram-negative species such as *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Holmes *et al.,* 2021). Gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus cereus*, have also been recognized as key contributors to foodborne illnesses and food spoilage (Chen *et al*., 2022). Traditionally, chemical preservatives have been applied to prevent food degradation and control pathogenic microorganisms (Amenu *et al*., 2025). While effective in reducing outbreaks, the prolonged use of these preservatives has led to issues such as the buildup of chemical residues in the food chain, development of microbial resistance, and potential health risks for humans (Bialonska *et al*., 2020).

In recent years, awareness of the issue of antibiotic resistance has increased, including the political field. The abuse of antibiotics such has amoxicillin, septrin, augmentin, has exacerbated the antibiotic resistant of *Salmonella typhi, Escherichia coli,* and other bacteria to amoxicillin, augmentin, septrin and other antibiotics which are one of the bacteria causing foodborne infection and food poisoning.

In view of such concern, effort have been focused on developing a potentially effective, healthy, safer and natural food preservatives and antimicrobials from plant origin. Within this context is the utilization of plant extracts as antimicrobial agents for food preservation and effective against antibiotic resistant bacteria. These plant extracts considered as natural sources of antimicrobial agents are regarded as nutritionally safe and easily degradable (Chika *et al.*, 2020).

L. cyanescens is also known as *Philenoptera cyanescens* (Schumach. & Thonn.). It belongs to the Kingdom: Plantae, Phylum: Tracheophytes, Class: Eudicots, Order: Fabales, Family: Fabaceae: Genus: *Lonchocarpus* or *Philenoptera* and Species: *cyanescences.* It is a deciduous shrub or woody liana, common in West African forest. It is commonly called West African Indigo plant. The leaves and the roots are being considered as a possible cure for leprosy and the leaf sap is drunk against intestinal disorder and dysentery (Amara *et al.,* 2025)

The objective of this study was to assess the antibacterial activity of *L. cyanescens* against bacteria obtained from fecal samples of patients diagnosed of food poisoning infections and also analyzed its extracts to identify both phytochemical and bioactive compounds.

Materials and Methods

Study design

This work was an experimental design to address the antibiotic resistant menace in food poisoning associated bacteria such as *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus* and *Citrobacer freundii*. The bacterial isolates were obtained from fecal samples of patients that were diagnosed of food poisoning and other related food borne infections from University of Medical Sciences Teaching Hospital (UNIMEDTH) Akure. The experiment was replicated three times, and the plant material was collected from King's Crown Botanical Garden Ilere community Ondo state. The antibiotic susceptibility testing was done to determine the antibiotic resistant bacterial isolates.

Collection, Authentication and Preparation of Plant Materials

The plant material used was fresh leaves of *L. cyanescens* obtained from King's Crown Botanical Garden in the Ilere community, close to the Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. The leaves were transported to the Herbarium of the Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria for verification. It was assigned the voucher number KASU/BSH/549 and sample of it stored in the Herbarium for future references.

The leaves were first rinsed thoroughly under running water, then shade-dried for two weeks before being grounded into a coarse powder using an electric blender (Electroline model IS 4780, CM/L 7902804). Following the procedure described by Mishra *et al.* (2012), the powdered samples were stored in sterile containers prior to further use. For extraction, 250 ml of various solvents each (n-hexane, ethanol, and distilled water) were added to 100g of the powdered plant material in 500 ml sterile conical flasks and allowed to soak for four days. Each extract was subsequently filtered using Whatman No. 1 filter paper, and evaporation was allowed to take place at room temperature in a clean and well disinfected laboratory (sterile environment). The obtained extracts were then kept at 4 °C until further use (Okiti and Osuntokun, 2020).

A stock solution of the leaf extract was prepared by dissolving 1 g of the dried extract in 3.33 mL of a solvent mixture comprising 70% sterile distilled water and 30% dimethyl sulfoxide (DMSO), to obtain a final extract concentration of 300 mg/mL. This standardized stock solution was used for subsequent experimental procedures. The stock for each extract (n-hexane, ethanol and distilled water solvents separately) was further used to prepare solutions of 200 mg/ml and 100 mg/ml with the same solvents mixture (70% sterile water + 30% DMSO). All prepared solutions were kept at 4 °C for later use.

Phytochemical analysis of the leaf extracts

The leaf extract of *L. cyanescens* was analyzed to assess the phytochemicals present, including alkaloids, terpenoids, tannins, saponins, flavonoids, and quinones. All assessments were conducted following the procedures described by AOAC (2011) for the qualitative and quantitative analysis.

Identification of Biologically Active Compounds in the leaf extracts

The Varian GC–MS system (Varian 4000 mass spectrometer, USA) coupled with an MS 3000 mass spectrometer, fitted with an

Agilent MS capillary column (30 m × 0.25 mm, film thickness) was used to analyzed the biologically active compounds in the leaf extracts. The analysis of chemical constituents followed the methodology described by Olusola-Makinde *et al.* (2021).

Isolation and Identification of Bacterial Isolates from Faecal Samples Collected at UNIMEDTH, Akure

Salmonella-Shigella Agar (SSA) and Deoxychocolate Agar media were employed for this study and they were prepared according to the manufacturer's instructions. Fecal samples were collected from patients showing symptoms of diarrhea, typhoid fever and other enteric fever at UNIMEDTH Akure. Bacteria isolation from fecal samples was carried out using the procedure reported by Onifade and Omololu (2019). Identification of bacterial isolates was performed through both cultural and molecular methods, as described by Cheesbrough (2010) and Ezugwu, (2025) respectively. The 16S rRNA gene was selected for molecular identification due to its highly conserved regions interspersed with hypervariable sequences that allow species-level differentiation. Consequently, 16S rRNA sequencing remains a widely applied technique for bacterial identification and phylogenetic studies (Singh, 2018).

Antibiotic Susceptibility Testing of Bacterial Isolates from Faecal Samples Collected at UNIMEDTH, Akure.

The 0.5 McFarland standard was used to adjust the inoculum density and this was done by comparing the bacterium suspension to the 0.5 McFarland Standard, as described by Isunu *et al.* (2022). The sensitivity test of the bacterial isolates to various antibiotics (Septrin (5mg/ml), Chloramphenicol (5mg/ml), Ciprofloxacin (5mg/ml), Amoxicillin (5mg/ml), Augmentin (5mg/ml) and Gentamycin (5mg/ml) was assessed using the Kirby-Bauer method (disc diffusion or KB testing) with antibiotic-impregnated discs, as described by Bauer *et al*. (1966).

Antibacterial Assay of *L. cyanescens* Extracts

The antibacterial effects of *L. cyanescens* extracts were evaluated according to the procedure outlined by Isunu *et al*. (2022). Ciprofloxacin was used as the positive control because it was effective against all the bacterial isolates, whereas wells containing only the solvent were used as the negative control. Following 18 hours of incubation at 37 °C, the diameter of the inhibition zones were measured in millimeters (mm).

Determination of Minimum Inhibitory Concentrations (MIC) of Leaf Extracts of *L. cyanescens*

The Minimum Inhibitory Concentration (MIC) was defined as the lowest extract concentration that completely inhibited visible bacterial growth after incubation. The Minimum Inhibitory Concentration (MIC) was carried out according to Celso *et al.* (2016).

Determination of Minimum Bactericidal Concentrations (MBC) of Extracts of *L. cyanescens*

The Minimum Bactericidal Concentration (MBC) of *L. cyanescens* extracts was assessed using the procedure described by Bosso and Innalegwu (2018).

Statistical Analysis

The collected data were analyzed using one-way analysis of variance (ANOVA), and mean comparisons was done using Duncan's New Multiple Range Test at a 95% confidence level, utilizing SPSS version 26.0. Differences were regarded as statistically significant at $p \leq 0.05$.

Results and Discussion

Phytochemical Constituents of Leaf Extracts of *L. cyanescens*

The qualitative phytochemical profile of the extracts of *Lonchocarpus* showed that nine phytochemicals were identified by putting all the phytochemicals identified in each extract together: flavonoids, tannins, phenols, amino acids, saponins, alkaloids, terpenoids, steroids, glycosides, and phlobatamin, in agreement with the observations of Nwokonkwo *et al.* (2017). Among these, flavonoids, tannins, phenols, amino acids, and saponins were abundant, with flavonoids and saponins being the most dominant. Alkaloids, terpenoids, and phlobatamin were detected in trace amounts, and the ethanolic extract exhibited the highest overall phytochemical content.

Table 1 summarizes the quantitative phytochemical composition of *Lonchocarpus cyanescens* leaves. Terpenoids, alkaloids, and glycosides were not detected in extracts prepared using certain solvents. Phenols were the most plentiful, with concentrations ranging from 65.22 ± 0.00^{a} mg/g to 100.06 ± 3.05^{a} mg/g, because it was present in all the three extracts, whereas saponins were the least abundant, ranging from 0.64 ± 0.07^{a} mg/g to 0.77 ± 0.04^{a} mg/g.

Glycosides were most abundant in the ethanol extract of the plant with 992.93±2.58 mg/g, followed by 407.9±9.46 mg/g abundance in the water extracts but was not present in the n-hexane extract of the plant. Saponins, tannins and steroids were scarcely present in all the three extracts ranging from 0.33±0.98 mg/g to 3.76±0.07 mg/g. Glycosides were abundantly present compared to other phytochemicals present in the ethanol and water extracts whereas flavonoids was the most abundance in the n-hexane solvent of the plant

Table 1: Quantitative Phytochemical in *Lonchocarpus cyanescens* Leaves

Phytochemicals	Water Extracts	Ethanol Extracts	N-Hexane Extracts
Saponins	0.64 ± 0.11^{a}	0.77 ± 0.04^{a}	0.64 ± 0.07^{a}
Flavonoids	42.46 ± 1.15^{a}	53.53 ± 1.67^{b}	66.08 ± 2.51^{c}
Tannins	0.55 ± 0.04^{a}	0.75 ± 0.06^{a}	0.33 ± 0.08^{b}
Terpenoids	40.21 ± 1.54^{a}	NP	NP
Glycosides	407.9 ± 9.46^{a}	992.93 ± 2.58^{b}	NP
Phenols	79.54 ± 1.03^{a}	100.06 ± 3.05^{a}	65.22 ± 0.00^{a}
Steroids	1.54 ± 0.29^{a}	6.70 ± 0.84^{b}	3.76 ± 0.07^{b}
Alkaloids	NP	NP	22.51 ± 0.24^{a}

Key: NP = Not Present. Values on the row with the same superscript letter are not significantly different from each other at 0.05 level.

Identification of Bacterial Isolates

Table 2 shows the molecular identification of the bacterial isolates. The amplified DNA fragments were approximately 1500 base pairs in length. The resulting sequences were analyzed using the BLAST tool in the NCBI database. Consequently, 16S rRNA sequencing remains a widely applied technique for bacterial identification and phylogenetic studies (Singh, 2018). Based on 16S rRNA sequence comparison, the bacterial species were identified as *Citrobacter freundii* ATCC 8090, *Escherichia coli* strain NBRC 102203, *Shigella dysenteriae* ATCC 13313, *Salmonella typhi* SKST, and *Staphylococcus aureus* S33R, with their corresponding accession numbers. The bacteria are one of the commonest microbes associated with

food poisoning which is in line with the research of Roberto *et al.* (2018).

Antibiotic Susceptibility Patterns of Bacterial Isolates

Table 3 summarizes the results of the antibiotic susceptibility testing of the bacterial isolates using Gram-negative antibiotic discs. Ciprofloxacin demonstrated the strongest antibacterial activity against all tested isolates, producing inhibition zones ranging from 23.33 ± 0.67 mm to 29.00 ± 0.58 mm. Its high effectiveness is attributed to its broad-spectrum properties, as most Gram-negative bacteria, along with many Gram-positive species, exhibit either high or moderate susceptibility to ciprofloxacin *in vitro*. In contrast to many broad-spectrum antibiotics, ciprofloxacin is effective when administered either orally or intravenously (Campoli-Richards *et al*., 2018). Its strongest antibacterial activity was observed against *E. coli*, whereas the lowest activity was recorded against *S. dysenteriae* and *C. freundii*. No significant difference was observed between the effects of Augmentin and Gentamicin on *S. dysenteriae*, with inhibition zones of 13.00 ± 0.00 mm and 13.67 ± 0.33 mm, respectively, both showing no effect against this bacterium.

Table 2: Molecular Identification of Isolates

S/N	Cultural and biochemical identities	16s rRNA sequence identification	Max Identity	Accession number
1	*Shigella dysenteriae*	*Shigella dysenteriae* ATCC 13313	95%	NR 026332.1
2	*Citrobacter freundii*	*Citrobacter freundii* ATCC8090	100%	NR 028894.1
3	*Staphylococcus aureus*	*Staphylococcus aureus* S33R	99%	NR 037007.2
4	*Escherichia coli*	*Escherichia coli strain NBRC* 102203	100%	NC010473.1
5	*Salmonella typhi*	*Salmonella typhi* SKST	100%	FJ 009676.1

Table 3: Antibiotic Sensitivity Test Results of Isolates on Gram Negative Antibiotics Disc

Isolates	SXT	CH	CPX	AM	AU	CN
S. dysenteriae	0.00±0.00^{a}	10.33±0.33^{b}	23.33±0.67^{c}	12.67±0.33^{d}	13.00±0.00^{d}	13.67±0.33^{d}
E. coli	0.00±0.00^{a}	11.33±0.33^{b}	27.67±0.33^{c}	13.00±0.58^{b}	0.00±0.00^{a}	11.67±0.67^{b}
C. freundii	0.00±0.00^{a}	11.33±0.33^{b}	24.00±0.57^{c}	12.33±0.33^{b}	11.33±0.33^{b}	10.67±0.33^{b}
S. typhi	10.33±0.33^{a}	12.00±0.58^{a}	25.00±0.58^{b}	10.67±0.33^{a}	11.67±0.33^{a}	18.33±0.33^{c}

Values on the row with the same superscript letter are not significantly different from each other. KEYS: SXT= Septrin (5mg/ml), CH = Chloramphenicol (5mg/ml), CPX= Ciprofloxacin (5mg/ml), AM=Amoxicillin (5mg/ml), AU= Augmentin (5mg/ml), CN= Gentamycin (5mg/ml).

Tables 4, 5, and 6 display the effects of the aqueous, n-hexane, and ethanolic extracts of *L. cyanescens* on the bacterial isolates. For the aqueous extract, it exert a low effect on all the isolates except on *S. dysenteriae* which was not susceptible at 300 mg/ml concentration while it was of low effect on *C. freundi* at 100 mg/ml and 200 mg/ml

concentrations. The remaining isolates were not susceptible to the aqueous extract at both 100 mg/ml and 200 mg/ml. *Escherichia coli* showed the highest susceptibility at 300 mg/ml, with an inhibition zone of 15.00 ± 0.58 mm, whereas the positive control demonstrated the greatest effectiveness, producing a 30.00 ± 0.58 mm inhibition zone against *S. aureus*. The ethanolic extract exhibited strong antibacterial activity against all tested isolates, consistent with the observations of Nwokonkwo *et al*. (2017). The highest activity was recorded against *C. freundii*, with inhibition zones ranging from 19.00 ± 1.00 mm to 28.33 ± 0.33 mm. However, several isolates showed little or no activity at 100 mg/ml, and *Shigella dysenteriae* demonstrated the lowest susceptibility, measuring 12.33 ± 0.33 mm between the 200 mg/ml and 300 mg/ml concentrations. For the n-hexane extract, the positive control produced inhibition zones between 23.33 ± 0.88 mm and 27.00 ± 1.15 mm. *Salmonella typhi* was not susceptible at all tested concentration, whereas *C. freundii* showed the greatest sensitivity, with zones ranging from 18.00 ± 0.58 mm to 23.00 ± 1.00 mm. Both *Staphylococcus aureus* and *E. coli* exhibited no inhibition at 100 mg/ml and 200 mg/ml concentrations.

Table 4: Effects of Different Concentrations of *L. cyanescens* Aqueous Extract on Bacterial Isolates

Isolates	100mg/ml	200mg/ml	300mg/ml	Positive control	Negative control
S. aureus	0.00±0.00^{a}	0.00±0.00^{a}	12.67±0.67^{b}	30.00±0.58^{c}	0.00±0.00^{a}
E. coli	0.00±0.00^{a}	0.00±0.00^{a}	15.00±0.58^{b}	28.00±0.88^{c}	0.00±0.00^{a}
S. dysenteriae	0.00±0.00^{a}	8.00±4.00^{a}	0.00±0.00^{a}	23.33±0.88^{b}	0.00±0.00^{a}
C. freundii	11.00±0.00^{a}	13.00±0.58^{a}	14.00±0.58^{b}	29.00±0.91^{c}	0.00±0.00^{a}
S. typhi	0.00±0.00^{a}	0.00±0.00^{a}	13.00±0.58^{b}	28.00±0.88^{c}	0.00±0.00^{a}

Values on the row with the same superscript letter are not significantly different from each other at P≤0.05 KEY: Positive control – Ciprofloxacin (10 µg), Negative control – Dimethylsulfoxide (DMSO)

Table 5: Effects of Different Concentrations of *L. cyanescens* Ethanol Extracts on Bacterial Isolates

Isolates	100mg/ml	200mg/ml	300mg/ml	Positive control	Negative control
S. aureus	11.00±0.58^{a}	0.00±0.00^{a}	16.33±0.88^{b}	30.00±0.58^{c}	0.00±0.00^{a}
E. coli	0.00±0.00^{a}	13.67±0.67^{b}	15.00±0.00^{b}	28.00±0.88^{c}	0.00±0.00^{a}
S. dysenteriae	0.00±0.00^{a}	0.00±0.00^{a}	12.33±0.33^{b}	23.33±0.88^{c}	0.00±0.00^{a}
C. freundii	19.00±1.00^{b}	27.00±1.15^{c}	28.33±0.33^{c}	29.00±0.91^{c}	0.00±0.00^{a}
S. typhi	0.00±0.00^{a}	0.00±0.00^{a}	15.00±0.58^{b}	28.00±0.88^{c}	0.00±0.00^{a}

Values on the row with the same superscript letter are not significantly different from each other at p≤0.05level. KEY: Positive control – Ciprofloxacin (10 µg), Negative control – Dimethylsulfoxide (DMSO)

Table 6: Effects of Different Concentrations of *L. cyanescens* N-Hexane Extracts on Bacterial Isolates

Isolates	100mg/ml	200mg/ml	300mg/ml	Positive control	Negative control
S. aureus	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	30.00±0.58[b]	0.00±0.00[a]
E. coli	0.00±0.00[a]	0.00±0.00[a]	14.000.58[b]	28.00±0.88[c]	0.00±0.00[a]
S. dysenteriae	0.00±0.00[a]	0.00±0.00[a]	13.00±0.58[b]	23.33±0.88[c]	0.00±0.00[a]
C. freundii	18.00±0.58[b]	21.67±0.88[c]	23.00±1.00[c]	29.00±0.91[d]	0.00±0.00[a]
S. typhi	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	28.00±0.88[b]	0.00±0.00[a]

Values on the row with the same superscript letter are not significantly different from each other at p≤0.05level. KEY: Positive control – Ciprofloxacin (10 µg), Negative control – Dimethylsulfoxide (DMSO)

Table 7 shows the minimum inhibitory concentrations (MIC) of *Lonchocarpus cyanescens* extracts. The ethanolic extract had an MIC of 25 mg/ml against *Citrobacter freundii*, 50 mg/ml against *Salmonella typhi*, and 200 mg/ml against *Shigella dysenteriae*. The aqueous extract exhibited an MIC of 200 mg/ml for all isolates except *Staphylococcus aureus,* which had an MIC of 100 mg/ml which agrees with the research of Nigussie *et al.* (2021) on the antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphedema patients. For the n-hexane extract, the MIC was 50 mg/ml for all the isolates except *Escherichia coli*, which had an MIC of 200 mg/ml which agrees with the research of Damtie and Mekonnen, (2015) on the *in vitro* evaluation of the antibacterial activities of the methanol, aqueous and n-hexane extracts of *ocimum lamiifolium* from Ethiopia.

Table 8 summarizes the minimum bactericidal concentrations (MBC) of *Lonchocarpus cyanescens* extracts. Both the ethanolic and n-hexane extracts had an MBC of 50 mg/ml against *Citrobacter freundii*. The n-hexane extract also exhibited an MBC of 50 mg/ml against *Staphylococcus aureus*, whereas the aqueous extract showed an MBC of 100 mg/ml for *S. aureus* and 200 mg/ml for the remaining isolates which agrees with the research of Shittu *et al.* (2024) on the evaluation of phytochemical constituents and antimicrobial activity of *Calotropis procera* root extract against some pathogenic microorganisms in Yola, Adamawa State, Nigeria. For *Salmonella typhi,* the MBC was 100 mg/ml with both ethanol and n-hexane extracts. This showed that n-hexane and ethanol extracts produced an MBC of 100 mg/ml, indicating relatively low bactericidal potency compared with more susceptible organisms.

Table 7: Minimum Inhibitory Concentration (MIC) of *L. cyanescens* Extracts

ORGANISMS	Water (mg/ml)	Ethanol (mg/ml)	N-Hexane (mg/ml)
E. coli	200.00	200.00	200.00
Citrobacter freundii	200.00	25.00	50.00
Salmonella typhi	200.00	50.00	50.00
Shigella dysenteriae	200.00	200.00	50.00
Staphylocoocus aureus	100.00	200.00	50.00

Table 8: Minimum Bactericidal Concentration (MB C) of *L. cyanescens* Extracts

ORGANISMS	Water (mg/ml)	Ethaol (mg/ml)	N-Hexane (mg/ml)
E. coli	200.00	200.00	200.00
Citrobacter freundii	200.00	50.00	50.00
Salmonella typhi	200.00	100.00	100.00
Shigella dysenteriae	200.00	200.00	100.00
Staphylocoocus aureus	100.00	200.00	50.00

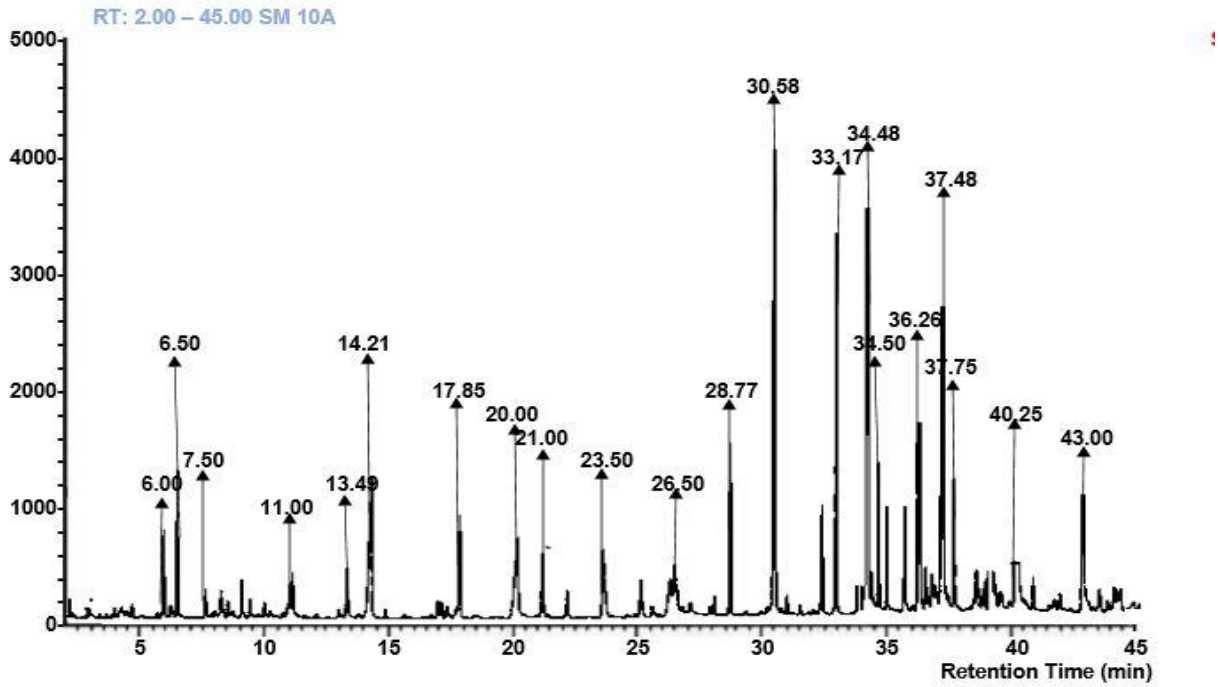


Figure 1: GC-MS Spectra of Ethanol Extract of *Lonchocarpus cyanescens* Leaf

Figure 1 showed the Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the *Lonchocarpus cyanescens* leaf extract revealed the presence of 21 organic compounds, with their corresponding peak areas and structures identified. The detected compounds included Hexenal, Octanoic acid, Resorcinol, 2-Hydroxy-5-methylbenzaldehyde, 4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-R, Hexadecanal,

2,4-Dimethoxybenzaldehyde phenylhydrazone, Methyl tetradecanoate, 9-Hexadecenoic acid, Mono(2-ethylhexyl) phthalate, 9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z), Octadecanoic acid methyl ester, Isopropyl stearate, 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene, Stigmastan-3,5-diene, 1,2-Benzenedicarboxylic acid diheptyl ester, Cholesterol, Squalene, β-Sitosterol, Campesterol, and Stigmasta-5,22-dien-3-ol (Ajayi *et al.,* 2020).

Conclusion

The leaf extract of *Lonchocarpus cyanescens* showed considerable antibacterial activity against *Citrobacter freundii*. The results of this study reinforce the ethnomedicinal application of the plant and indicated that both the ethanolic and n-hexane extracts contain bioactive compounds with significant antimicrobial effects that merit further exploration. The observed antibacterial activity suggests that traditional herbal remedies could be as effective as conventional drugs and cost-effective and alternative for the management of infectious diseases.

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Authors' Contributions

Anthony Kayode Onifade conceptualized and designed the study, while Williams Adesina developed the methodology. Data was collated and analyzed by Ikhiboya Friday I. The first draft of the manuscript was prepared by Williams Adesina, Adebayo Jumoke Oluwafisayo, and Ayoola Ayorinde Joshua. Anthony Kayode Onifade supervised the research and reviewed the manuscript. All authors have read and approved the final version of the manuscript.

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