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Comparative Antibacterial Activity and Molecular Docking of *Allium Sativum* Extract and a Novel Piperidinone Derivative Against Multidrug-Resistant Uropathogens

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Abstract

The growing burden of multidrug-resistant (MDR) uropathogens undermines antibiotic efficacy in urinary tract infections (UTIs), especially in resource-limited settings. Garlic (*Allium sativum*) possesses antimicrobial potential, yet its comparative efficacy and molecular interactions remain underexplored. This study evaluated the *in vitro* activity and docking interactions of garlic-derived compounds alongside Levofloxacin. MDR isolates including *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Citrobacterfreundii*, and *K. oxytoca* were obtained from the University of Ilorin Teaching Hospital. Garlic extract (200 mg/mL) produced inhibition zones of 14.6 ± 0.5 mm (*P. aeruginosa*) to 19.3 ± 0.7 mm (*E. coli*), while Levofloxacin produced 18.2 ± 0.4 – 22.3 ± 0.4 mm. Garlic extract showed significantly lower zones of inhibition than Levofloxacin ($p=0.031$). Docking against DNA gyrase and topoisomerase IV revealed garlic-derived 1-(4-Bromobutyl)-2-piperidinone binds at the same active-site residues as levofloxacin, suggesting it inhibits bacterial DNA replication through interference with the DNA cleavage-religation step and support 1-(4-Bromobutyl)-2-piperidinone as a candidate phytochemical for adjunctive UTI treatment.

Keywords: *Allium sativum*; urinary tract infection; multidrug resistance; phytochemicals; GC-MS; molecular docking; Nigeria.

Introduction

Urinary Tract Infections (UTIs) represent one of the most prevalent bacterial infections worldwide, affecting millions annually, particularly women, the elderly, and immune-compromised individuals¹. The increasing incidence of UTIs, coupled with the rise in antibiotic resistance, presents a significant public health concern². *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* are among the most frequently implicated uropathogens in UTIs³. However, the overuse and misuse of conventional antibiotics have led to the emergence of multidrug-resistant (MDR)

strains, significantly complicating treatment efforts and increasing healthcare costs, morbidity, and mortality⁴.

Amid this global health threat, the search for novel and natural alternatives to synthetic antibiotics has intensified. Medicinal plants such as *Allium sativum* (garlic) have garnered attention for their broad-spectrum antimicrobial, antioxidant, and anti-inflammatory properties⁵. Garlic contains a diverse range of bioactive phytochemicals, including flavonoids, alkaloids, saponins, terpenoids, and organosulfur compounds, which have shown promising antibacterial activity against various pathogens⁶. Its potential application in the management of MDR bacterial infections presents a sustainable, accessible, and cost-effective therapeutic avenue, particularly in low-resource settings⁷. The increasing failure of standard antibiotics due to the emergence of MDR uropathogens poses a dire threat to public health systems globally and more critically in developing countries like Nigeria, where antibiotic stewardship is poorly regulated⁸. Traditional treatment options are becoming increasingly ineffective, leading to prolonged infections, increased hospital stays, and a greater risk of complications⁹.

Despite Nigeria's rich biodiversity and ethnomedicinal heritage, there is limited empirical evidence validating the antimicrobial efficacy of indigenous plant-based therapies such as garlic extract against clinical MDR pathogens¹⁰. While previous studies have examined garlic's general antimicrobial activity¹¹. Few have specifically addressed its effectiveness against clinically isolated MDR uropathogens in Nigeria hospitals. Moreover, comprehensive evaluations that integrate phytochemical screening, antimicrobial sensitivity testing, and molecular docking remain scarce, highlighting the need for context-specific scientific validation of *Allium Sativum* as a potential therapy for MDR urinary tract infections in Nigeria.

At the University of Ilorin Teaching Hospital in Nigeria, a significant proportion of patients with UTIs present with infections caused by MDR organisms, complicating standard treatment protocols¹². The local healthcare infrastructure struggles to manage these infections due to limited access to advanced antibiotics and poor diagnostic turnaround time¹³. In this context, locally available and culturally acceptable remedies like garlic offer a potentially valuable complement or alternative to existing treatments.

This study aimed to evaluate the antibacterial efficacy and phytochemical properties of aqueous garlic (*Allium sativum*) extract against multidrug-resistant uropathogens isolated from UTI patients at the University of Ilorin Teaching Hospital, Nigeria through phytochemical screening, antimicrobial assays (MIC and MBC), GC-MS profiling, and molecular docking analysis of bioactive compounds with bacterial DNA gyrase and Topoisomerase IV.

Materials and Methods

Study Area

This study was carried out at the University of Ilorin Teaching Hospital (GPS: 8.4797° N, 4.5322° E), Ilorin, Kwara State, Nigeria. UITH serves as a major healthcare provider in the region, catering to a diverse patient population, and is a suitable site for

investigating urinary tract infections (UTIs) and associated antimicrobial resistance patterns.

Collection of Multidrug-Resistant Uropathogens

Urine samples from patients with clinically suspected UTIs were routinely processed at the Urine bench of the UITH microbiology laboratory. For this study, previously cultured and confirmed multidrug-resistant (MDR) bacterial isolates were obtained from archived urine culture. These isolates had been preserved in glycerol broth and were taken to the laboratory for further antimicrobial susceptibility testing. The use of archived isolates, rather than direct patient sampling was adopted in order to ensure access to well characterized MDR strains without requiring new patient recruitment.

Identification of Multidrug-Resistant Isolates

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following the 0.5 McFarland turbidity standard. The antibiotic discs used included ciprofloxacin (5 µg), levofloxacin (5 µg), gentamycin (10 µg), cefotaxime (30 µg), nitrofurantoin (300 µg), tetracycline (30 µg), and ampicillin (10 µg). After incubation at 37°C for 18–24 hours, inhibition zones were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates resistant to three or more antibiotic classes were classified as MDR.

Molecular Identification via 16S rDNA Sequencing

Genomic DNA was extracted using the Zymo Research Quick-DNA Miniprep Kit (USA) from bacterial cultures grown overnight in Luria-Bertani broth. DNA quality and concentration were measured using a Nanodrop spectrophotometer. PCR amplification of the 16S rDNA gene was performed using primers 27F and 1492R in a 25 µL reaction mixture. PCR products were visualized via agarose gel electrophoresis and sequenced using the Sanger method. Sequences were analyzed with BLASTn against the NCBI GenBank database and aligned phylogenetically using MEGA 7 software.

Identification and Authentication of Garlic

Fresh garlic (*Allium sativum*) was sourced from Ojatuntun market in Ilorin and authenticated at the herbarium of the Department of Plant Biology, University of Ilorin with voucher number: UILH/004/1565/2024

Preparation of Aqueous Garlic Extract

Fresh garlic (*Allium sativum*) cloves were peeled, crushed, and weighed using a digital analytical balance. The cloves were homogenized into paste using sterile laboratory blender. The homogenate was macerated in sterile distilled water for 24 hours. The mixture was filtered through Whatman No. 1 paper, and the filtrate was stored at 4°C for subsequent analysis.

Phytochemical Screening

The qualitative screening of the garlic for various phytochemical constituents such as flavonoids, saponin, tannin, alkaloids, phenols and terpenoids was conducted using standard methods, different methods were used to evaluate the quantity of phytochemical constituents of the garlic used. Spectrophotometric method was used to determine terpenoids, tannins, steroids, anthraquinone, and glycosides. Folin-ciocateu procedure was used to determine phenol content. (Deepa and Sivakumar, 2023).

Antimicrobial Activity Testing

The antimicrobial activity of the garlic extract was evaluated using the agar well diffusion method. Standardized MDR uropathogenic isolates were inoculated onto Mueller-Hinton agar. Wells (6 mm diameter) were filled with various concentrations of garlic extract (25-200 mg/mL). To achieve these concentrations, the aqueous filtrate obtained after maceration was concentrated by rotary evaporation at 40°C under reduced pressure and subsequently lyophilized to dryness. The dried extract was weighed and reconstituted in sterile distilled water to prepare stock solutions of 200mg/mL, from which lower concentrations were serially prepared. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters.

Determination of MIC and MBC

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth microdilution method. Garlic extract was serially diluted in Mueller-Hinton broth and incubated with standardized bacterial inocula (1×10^6 CFU/mL) in 96-well plates. MIC was defined as the lowest concentration without visible turbidity, while MBC was identified by subculturing from each well and assessing bacterial growth on agar after 24 hours.

GC-MS Analysis of Garlic Extract

The aqueous garlic extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Agilent 7890B GC system coupled with an Agilent 5977A mass selective detector. Separation was achieved using HP-5MS capillary column (30m x 0.25 mm i.d., 0.25 μ m film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0mL/min. the oven temperature was programmed as follows: initial temperature of 60°C held for 2 min, increased at 10°C/min to 280°C, and held for 10min. the injector temperature was set at 250°C, and injection volume was 1 μ L. The MS detector operated in electron impact mode, with ion source temperature maintained at 230°C and transfer line at 280°C. Mass spectra were acquired in the range of 50-600m/z. Major compounds were identified based on their retention times and comparison with the NIST mass spectral library.

Molecular Docking and Energy Minimization

Ligand Preparation

The 3D structures of 1-(4-Bromobutyl)-2-piperidinone and Levofloxacin were retrieved from PubChem and optimized using the LigPrep module of Maestro 12.8, employing the OPLS-4 force field for energy minimization and protonation state adjustment (Ayipoet *et al.*, 2021; Jangidet *et al.*, 2024).

Protein Preparation

Crystal structures of DNA gyrase and Topoisomerase IV from *E. coli* (PDB ID: 6KZV), *K. pneumoniae* (PDB ID: 4EM7), and *P. aeruginosa* (PDB ID: 7PTF) were obtained from the RCSB Protein Data Bank. Protein structures were preprocessed using Maestro's Protein Preparation Wizard by assigning bond orders, optimizing geometry, and identifying active sites (Ibrahim *et al.*, 2025).

Docking Protocol

Ligands and receptor grid files were subjected to Glide XP molecular docking (Maestro 12.8). Docking scores and binding affinities were recorded for each ligand-receptor complex.

2.12 Statistical Analysis

Experimental data, including inhibition zones and MIC/MBC values, were expressed as mean $\pm$ standard deviation from triplicate experiments. The non parametric Wilcoxon signed-rank test was applied to compare paired differences between garlic extract (200mg/mL) and Levofloxacin across test organisms

Results

For the purpose of this study, multidrug-resistant uropathogenic bacterial isolates were obtained with the support of a medical laboratory scientist assigned to the Urine Bench at the University of Ilorin Teaching Hospital (UITH). These isolates had been previously cultured from urine specimens submitted for routine diagnostic evaluation of urinary tract infections. While the isolates were not collected directly by the researcher through prospective sampling, they reflect bacterial species commonly identified in UTI cases within the institution.

The isolates made available for laboratory analysis included *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, *Citrobacterfreundii*, *Pseudomonas aeruginosa*, and *Klebsiellaoxytoca*. These organisms were subsequently utilized for antimicrobial susceptibility testing, as outlined in **Table 1**.

Table 1: Multidrug-Resistant Uropathogenic Isolates Obtained from the Urine Bench at the University of Ilorin Teaching Hospital

Bacterial Species	Gram Reaction	Cell Morphology	Resistance Profile
<i>Escherichia coli</i>	Gram-negative	Rod-shaped	Multidrug-resistant
<i>Klebsiellapneumoniae</i>	Gram-negative	Rod-shaped	Multidrug-resistant
<i>Proteus mirabilis</i>	Gram-negative	Rod-shaped (swarming)	Multidrug-resistant
<i>Citrobacterfreundii</i>	Gram-negative	Rod-shaped	Multidrug-resistant
<i>Pseudomonas aeruginosa</i>	Gram-negative	Rod-shaped (motile)	Multidrug-resistant
<i>Klebsiellaoxytoca</i>	Gram-negative	Rod-shaped	Multidrug-resistant

Note: All isolates were provided by a medical laboratory scientist from archived urine culture plates processed at the Urine Bench, UITH. These isolates were not directly obtained through patient sampling by the researcher.

The phylogenetic relationships among the multidrug-resistant (MDR) uropathogenic isolates obtained from urinary tract infection (UTI) patients at the University of Ilorin Teaching Hospital were established through partial 16S rDNA sequence analysis. As shown in **Fig. 1**, the clinical isolates (HU-001 to HU-007, accession numbers PV545031 to PV545038) clustered distinctly with reference strains from the NCBI GenBank database, confirming their species identities. HU-004 (PV545035) exhibited strong genetic relatedness to *Klebsiellaoxytoca* ATCC13182T, while HU-002 (PV545033) aligned with *Klebsiellapneumoniae* SG67. HU-003 (PV545034) grouped with *Proteus mirabilis* TQU 7, supported by a 100% bootstrap value. Two *Escherichia coli* isolate HU-003 and HU-007 (PV545034 and PV545038) clustered closely with ESBL-producing *E. coli* strains WP2-S18-ESBL-07 E57 (LCS99966.1) and OR134061.1. HU-006 (PV545037) formed a high-confidence clade with *Pseudomonas aeruginosa* MASJG5, while HU-005 (PV545036) aligned with *Citrobacterfreundii* HQ010168.1 with 100% bootstrap support. These findings confirm the accurate taxonomic classification of all isolates and highlight their genetic proximity to globally reported, clinically significant MDR strains, thereby emphasizing the urgent need for continued molecular epidemiological monitoring in local healthcare settings.

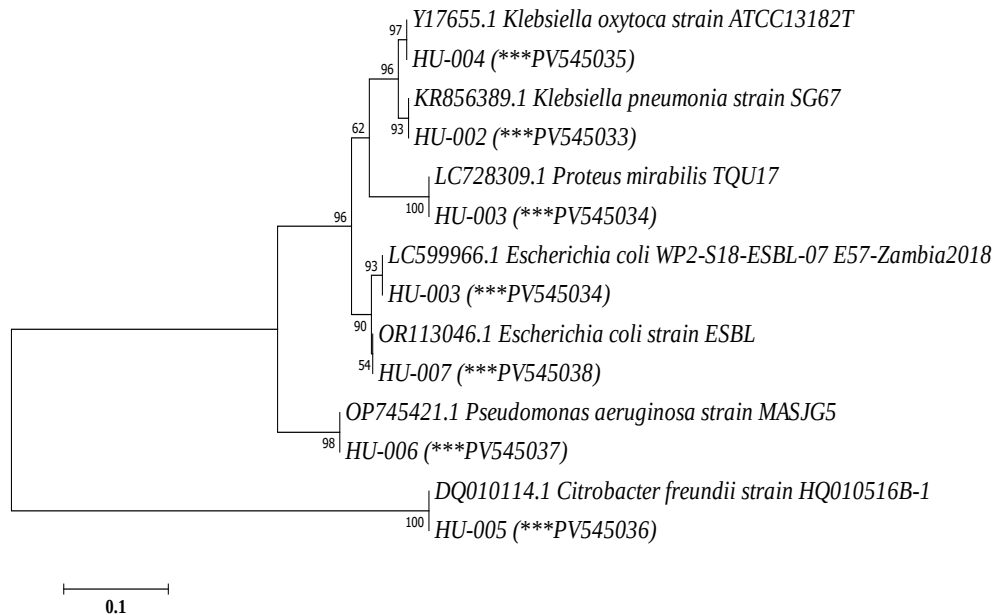


Fig. 1: Phylogenetic alignment of multidrug-resistant uropathogens isolated from UTI patients with reference strains from NCBI GenBank

Antibiotic susceptibility testing was conducted on all recovered multidrug-resistant uropathogenic isolates using the disk diffusion method, and the resistance trends were visually represented using a spider chart (**Fig. 2**). The analysis focused on five frequently prescribed antibiotics: ciprofloxacin (CIP), levofloxacin (LEVO), gentamycin (GEN), cefotaxime (CTX), and nitrofurantoin (NIT). Among the tested pathogens, *Escherichia coli* exhibited the highest resistance rates, particularly against ciprofloxacin and cefotaxime. Similarly, substantial resistance was noted in *Klebsiella pneumoniae* and *Proteus mirabilis*. In contrast, *Pseudomonas aeruginosa* and *Klebsiella oxytoca* demonstrated relatively lower resistance to levofloxacin and nitrofurantoin, suggesting that these agents may still retain therapeutic efficacy against such strains.

Cumulatively, more than 80% of the bacterial isolates displayed resistance to three or more antibiotic classes, meeting the criteria for classification as multidrug-resistant (MDR) organisms. Among these, *P. aeruginosa* and *K. pneumoniae* were identified as having the most extensive MDR profiles, reinforcing concerns regarding their capacity to withstand conventional antimicrobial therapy. Additionally, widespread resistance to ciprofloxacin and ampicillin across all isolates highlights the declining effectiveness of these first-line antibiotics in the empirical treatment of urinary tract infections, especially in resource-limited clinical settings.

The radar plot in **Fig. 2** presents the antibiotic susceptibility patterns of six multidrug-resistant (MDR) uropathogens *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, *Citrobacterfreundii*, *Pseudomonas aeruginosa*, and *Klebsiellaoxytoca* tested against six antimicrobial agents: Ciprofloxacin (CIP), Levofloxacin (LEVO), Nitrofurantoin (NIT), Ampicillin (AMP), Cefotaxime (CTX), and Gentamicin (GEN).

All isolates demonstrated complete or near-complete susceptibility to Levofloxacin, as evidenced by uniformly high values aligned along the LEVO axis. This observation affirms Levofloxacin's preserved efficacy against the tested MDR strains. In contrast, the susceptibility profiles for the other antibiotics varied considerably among the organisms. Ampicillin and Ciprofloxacin exhibited the lowest efficacy, with most isolates showing high-level resistance to both agents. Gentamicin and Cefotaxime showed intermediate patterns, with moderate susceptibility across isolates. Nitrofurantoin retained partial activity, with *P. aeruginosa* displaying comparatively higher sensitivity to this agent than other organisms. Critically, over 80% of the isolates met the criterion for multidrug resistance, defined as resistance to at least three distinct antibiotic classes. Among these, *Pseudomonas aeruginosa* and *Klebsiellapneumoniae* displayed the most extensive resistance profiles, showing reduced susceptibility across nearly all tested antibiotics. Collectively, the chart underscores two key trends: the sustained efficacy of Levofloxacin across diverse MDR strains, and the diminishing clinical value of older, broad-spectrum antibiotics in treating uropathogenic infections.

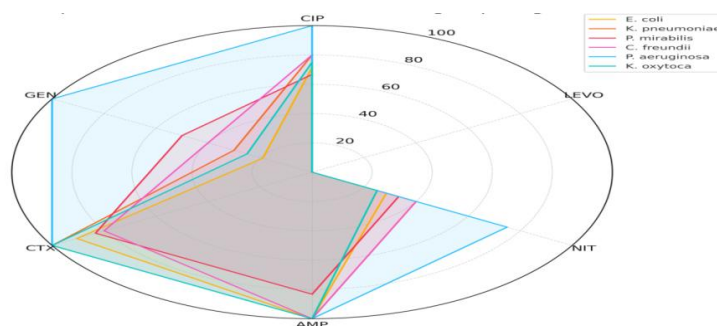


Figure 2: Spider Chart of Antibiotic Resistance patterns among Uropathogens

The Qualitative Phytochemical Screening of the Aqueous Garlic Extract, as shown in **Table 2**, revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, tannins, phenolics, steroids, glycosides, and terpenoids, with saponins and phenolics being present in high to moderate concentrations. These compounds suggest the extract has significant potential for antimicrobial, anti-inflammatory, and antioxidant activities. The presence of glycosides and terpenoids further supports the extract's therapeutic potential, particularly for their antimicrobial and anticancer properties. However, anthraquinones were absent, indicating that the extract

does not contain compounds typically associated with laxative effects. Overall, the results highlight the diverse medicinal properties of the aqueous garlic extract.

As shown in Fig. 3, saponins are the most abundant constituent in the Aqueous Garlic Extract, followed by phenolics and flavonoids. The presence of tannins, alkaloids, and glycosides suggests that the extract has a multi-target potential for antimicrobial activity. These bioactive constituents are known for their ability to disrupt bacterial cell walls, inhibit enzymes, and produce synergistic effects when combined with antibiotics.

Table 2: Qualitative Phytochemical Screening of Aqueous Garlic Extract

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Saponins	+++
Tannins	+
Phenolics	++
Steroids	+
Glycosides	+
Terpenoids	+
Anthraquinones	—

+ present, - Absent

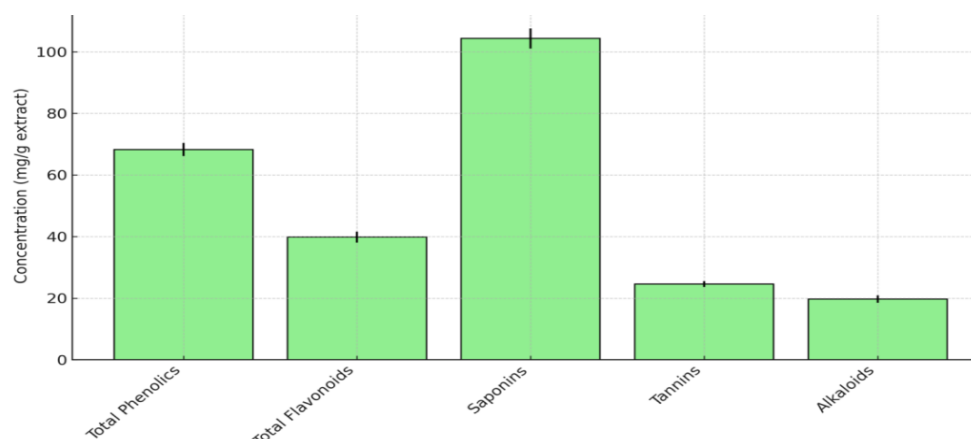


Fig. 3: Concentration of major phytochemicals in aqueous garlic extract (in mg/g extract).

The Aqueous Garlic Extract exhibited dose-dependent antimicrobial activity against various bacterial organisms, as shown in Table 3, with inhibition zones increasing as the concentration of the extract rose. *E. coli*, *K. pneumoniae*, *P. mirabilis*, *C. freundii*, and *K. oxytoca* demonstrated progressively larger inhibition zones, ranging from 9.2 ± 0.4 mm at 25 mg/mL to 19.3 ± 0.7 mm at 200 mg/mL. *P. aeruginosa* showed the smallest

inhibition zones, ranging from 6.3 ± 0.5 mm to 14.6 ± 0.5 mm at 200 mg/mL. While the garlic extract was effective against all tested organisms, its antimicrobial activity was somewhat less potent compared to Levofloxacin, which consistently produced larger zones of inhibition, ranging from 18.2 ± 0.4 mm to 22.3 ± 0.4 mm. Overall, the results in Table 4.2 indicate that the garlic extract has moderate antimicrobial potential, with its effectiveness increasing at higher concentrations, although Levofloxacin remains more effective at inhibiting bacterial growth.

Table 3: Zone of Inhibition (mm) of Aqueous Garlic Extract against MDR Uropathogens and Levofloxacin (Mean $\pm$ SD)

Organism	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	Levofloxacin
<i>E. coli</i>	9.2 ± 0.4	13.1 ± 0.6	16.4 ± 0.5	19.3 ± 0.7	21.7 ± 0.5
<i>K. pneumoniae</i>	8.6 ± 0.6	12.3 ± 0.4	15.7 ± 0.6	18.9 ± 0.6	22.3 ± 0.4
<i>P. mirabilis</i>	7.5 ± 0.3	10.8 ± 0.5	13.9 ± 0.6	16.5 ± 0.7	20.8 ± 0.5
<i>P. aeruginosa</i>	6.3 ± 0.5	8.9 ± 0.6	11.3 ± 0.4	14.6 ± 0.5	18.2 ± 0.4
<i>C. freundii</i>	8.1 ± 0.4	11.2 ± 0.3	14.8 ± 0.5	17.7 ± 0.6	21.0 ± 0.5
<i>K. oxytoca</i>	8.4 ± 0.5	11.5 ± 0.4	15.0 ± 0.6	18.0 ± 0.5	21.5 ± 0.6

Table Footnote

Note: The Wilcoxon signed-rank test comparing zones of inhibition at 200 mg/mL garlic extract and Levofloxacin across the six organisms yielded a test statistic $W = 0.00$ and $p = 0.031$ ($p < 0.05$), indicating a statistically significant difference in efficacy.

As shown in **Table 4**, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the Aqueous Garlic Extract were determined against selected multidrug-resistant (MDR) isolates. The results show that the extract exhibited bactericidal activity against most of the tested organisms, including *E. coli*, *K. pneumoniae*, *P. mirabilis*, *C. freundii*, and *K. oxytoca*, with an MBC/MIC ratio of 2.0. This suggests that the garlic extract not only inhibits bacterial growth but also kills the bacteria at a concentration twice that of the MIC. However, for *P. aeruginosa*, the garlic extract showed bacteriostatic activity, with an MBC/MIC ratio greater than 2.0 and an MBC value exceeding 200 mg/mL, indicating that while the extract inhibits the growth of *P. aeruginosa*, it does not kill the bacteria. These findings demonstrate the effectiveness of the garlic extract as a bactericidal agent for most MDR isolates, with a notably reduced effect against *P. aeruginosa*.

Table 4: MIC and MBC Values of Aqueous Garlic Extract Against Selected MDR Isolates

Organism	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio	Interpretation
E. coli	25.0	50.0	2.0	Bactericidal
K. pneumoniae	25.0	50.0	2.0	Bactericidal
P. mirabilis	50.0	100.0	2.0	Bactericidal
P. aeruginosa	100.0	>200.0	>2.0	Bacteriostatic
C. freundii	25.0	50.0	2.0	Bactericidal
K. oxytoca	25.0	50.0	2.0	Bactericidal

Key:

- **MIC:** Minimum Inhibitory Concentration – the lowest concentration of the garlic extract that inhibits the growth of the bacteria.
- **MBC:** Minimum Bactericidal Concentration – the lowest concentration of the garlic extract that kills the bacteria.
- **MBC/MIC Ratio:** This ratio indicates whether the garlic extract is bactericidal (MBC/MIC ratio ≥ 1) or bacteriostatic (MBC/MIC ratio < 1).
- **Interpretation:**
 - **Bactericidal:** The extract kills the bacteria.
 - **Bacteriostatic:** The extract inhibits the growth of bacteria but does not kill them.

The GC-MS chromatogram of the Aqueous Garlic Extract, shown in **Fig. 4**, displays the Total Ion Chromatogram (TIC) with peaks corresponding to various compounds present in the extract. The large peaks at 14.978 and 19.168 minutes represent the most abundant compounds, while smaller peaks at other retention times, such as 10.577, 11.527, 13.902, and 15.178, indicate the presence of compounds in lower concentrations. The blue labels mark the retention times of the major peaks, and the red lines highlight the areas associated with each peak, representing their relative abundance. This chromatogram provides valuable insight into the chemical composition of the garlic extract. The GC-MS analysis of the Aqueous Garlic Extract, as shown in the Table 4, identified several major compounds with varying concentrations. The most abundant compound was 9-Octadecenoic acid, methyl ester (Oleic acid methyl ester), making up 18.93% of the extract, followed by 8-Heptadecenoic acid 10.37% and Oxalic acid, cyclobutyl pentadecyl ester at 9.96%. Other notable compounds include 2-Piperidinone, N-[4-bromo-n-butyl]-, which appeared twice at 8.37% and 6.49%, and Nonadecane, accounting for 6.73%. Additionally, n-Hexadecanoic acid (Palmitic acid) contributed 5.77%, while 1-Bromodocosane made up 5.13%. Compounds like Octatriacontyl penta fluoropropionate and Tridecane were present in smaller amounts, 4.85% and 2.13%, respectively as shown in **Table 5**



Fig. 4: Total Ion Chromatogram (TIC) of Aqueous Garlic (*Allium sativum*) Extract.

The antibacterial efficacy of Levofloxacin and aqueous garlic extract (200 mg/mL) against multidrug-resistant uropathogens as shown in **Fig. 5**. The chart highlights that Levofloxacin consistently demonstrated a high level of antibacterial efficacy across all tested organisms. In comparison, the aqueous garlic extract showed a moderate yet promising antimicrobial effect, suggesting its potential as a natural alternative with a similar antibacterial impact. The consistent susceptibility to Levofloxacin further underscores the significance of evaluating garlic extract for its antimicrobial properties, as it could serve as a complementary or alternative treatment against multidrug-resistant infections

Table 5: Major Compounds Identified in Garlic Aqueous Extract (GC-MS)

RT (min)	Compound Name	CAS No.	Area %
14.978	9-Octadecenoic acid, methyl ester (Oleic acid methyl ester)	001937-62-8	18.93%
15.498	Oxalic acid, cyclobutyl pentadecylester	1000309-70-5	9.96%
19.612	8-Heptadecene	002579-04-6	10.37%
19.881	2-Piperidinone, N-[4-bromo-n-butyl]-	195194-80-0	8.37%

15.178	Nonadecane	000629-92-5	6.73%
15.876	2-Piperidinone, N-[4-bromo-n-butyl]- (repeat)	195194-80-0	6.49%
13.902	n-Hexadecanoic acid (Palmitic acid)	000057-10-3	5.77%
16.019	1-Bromodocosane	006938-66-5	5.13%
20.207	Octatriacontyl penta fluoropropionate	1000351-89-1	4.85%
11.527	Tridecane	000629-50-5	2.13%

Fig. 6 presents a flowchart categorizing the compounds identified in the Aqueous Garlic Extract based on their bioactivity and mechanisms of action. The flowchart classifies compounds into four main groups: DNA Gyrase Targeting, which includes 2-Piperidinone, N-[4-bromo-n-butyl]- and Levofloxacin, both of which target DNA gyrase and potentially Topoisomerase IV; Bioactive but Non-Antimicrobial, comprising compounds like 9-Octadecenoic acid, methyl ester, Oxalic acid, cyclobutyl pentadecylester, and n-Hexadecanoic acid, which possess antioxidant and anti-inflammatory properties but do not target DNA gyrase; Hydrocarbons, such as 8-Heptadecene, Nonadecane, and Tridecane, which primarily act through membrane disruption and show antimicrobial effects via different mechanisms; and Other Experimental Compounds, like 1-Bromodocosane and Octatriacontyl penta fluoropropionate, which exhibit potential bioactivity but do not directly interfere with DNA replication or gyrase inhibition. The flowchart highlights the diverse bioactive compounds in the garlic extract, each contributing to its potential therapeutic applications through various mechanisms.

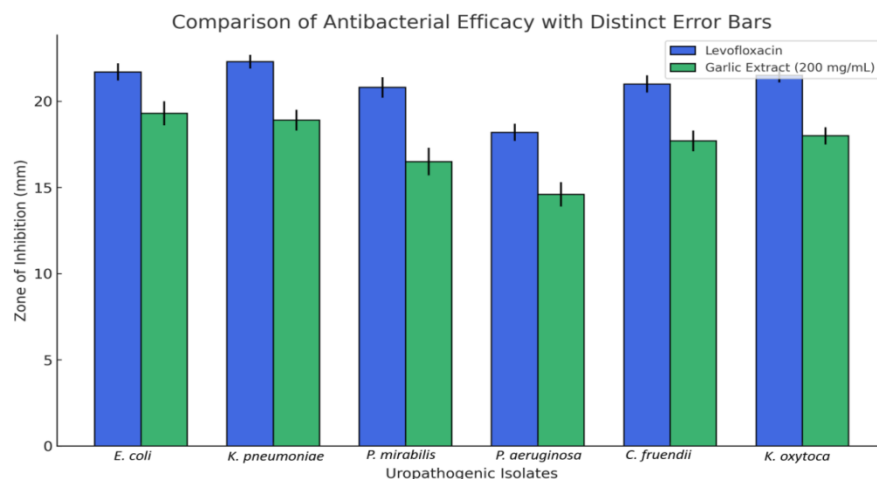


Fig. 5: Antibacterial activity comparison showing zone of inhibition (mm) with distinct error bars for Levofloxacin and garlic extract.

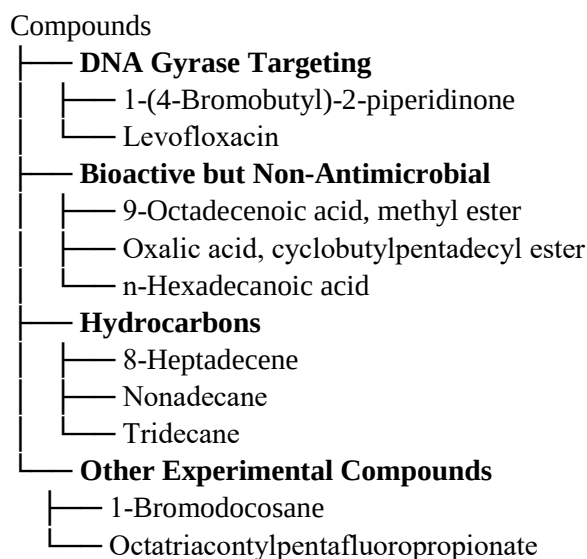


Figure 6: Hierarchical Classification of Identified Compounds Based on Functional Properties

The Protein–Ligand Docking Summary **Table 6** reveals that both Levofloxacin and 1-(4-Bromobutyl)-2-piperidinone exhibit strong binding interactions with DNA gyrase and Topoisomerase IV across different bacterial species, with Levofloxacin generally showing more favorable binding energies. For *E. coli*, Levofloxacin binds more effectively to DNA gyrase with a binding energy of -4.751 kcal/mol, compared to 1-(4-Bromobutyl)-2-piperidinone at -3.371kcal/mol. Similarly, Levofloxacin demonstrates stronger binding to Topoisomerase IV in *Streptococcus pneumoniae* (-5.680 kcal/mol) compared to 1-(4-Bromobutyl)-2-piperidinone (-4.271 kcal/mol). *S. pneumoniae* DNA gyrase and Topoisomerase IV crystal structures were included as docking targets because they represent the most well-characterized and structurally resolved bacterial enzymes available in the protein Data Bank. However, in *P. aeruginosa*, 1-(4-Bromobutyl)-2-piperidinone shows a slightly stronger binding energy (-4.228 kcal/mol) than Levofloxacin (-4.210 kcal/mol) for DNA gyrase. Both compounds interact with similar amino acid residues, such as ASP 73, ARG 76, GLU 50, and others, indicating that they share a comparable mode of action, particularly in stabilizing the Topo-DNA complex or interfering with DNA replication. These findings suggest that 1-(4-Bromobutyl)-2-piperidinone has the potential to serve as a natural alternative to Levofloxacin, exhibiting similar antimicrobial activity through a comparable mechanism of action.

Table 6: Protein–Ligand Docking of Levofloxacin and 1-(4-Bromobutyl)-2-piperidinone with DNA gyrase and Topoisomerase IV.

Organism	Protein Target	Ligand	Binding Energy (kcal/mol)	Interacting Amino Acid Residues
<i>E. coli</i>	DNA Gyrase	Levofloxacin	-4.751	ASP 73, ARG 76, GLU 50, GLY 75, GLY 77, THR 165, VAL 71, ASN 46, ALA 47, VAL 120, VAL 43, ILE 94
<i>E. coli</i>	DNA Gyrase	1-(4-Bromobutyl)-2-piperidinone	-3.371	ASP 73, ARG 76, GLU 50, GLY 75, GLY 77, THR 165, VAL 71, ASN 46, ALA 47, VAL 120, VAL 43, ILE 94
<i>Streptococcus pneumoniae</i>	Topoisomerase IV	Levofloxacin	-5.680	ASP 78, ARG 81, GLU 55, ASP 54, GLY 82, GLY 121, MET 83, PRO 84, VAL 76, THR 172, ILE 48, ASN 51, ALA 52, HIS 120, PHE 99
<i>Streptococcus pneumoniae</i>	Topoisomerase IV	1-(4-Bromobutyl)-2-piperidinone	-4.271	ASP 78, ARG 81, GLU 55, GLY 80, GLY 82, MET 83, PRO 84, THR 172, VAL 174, ILE 48, ASN 51, ALA 52, HIS 120, PHE 99, SER 124
<i>Pseudomonas aeruginosa</i>	DNA Gyrase	Levofloxacin	-4.210	ASP 87, ARG 91, GLY 85, THR 165, ILE 90, GLY 118, MET 88, PHE 88, ALA 90, GLU 58, ASN 84
<i>Pseudomonas aeruginosa</i>	DNA Gyrase	1-(4-Bromobutyl)-2-piperidinone	-4.228	GLU 58, ASP 87, ARG 91, GLY 85, THR 165, ILE 90, PHE 88, ASN 84, MET 88, GLY 118, ALA 90

Fig. 7a illustrates the 2D binding interaction of Levofloxacin with key amino acid residues at the active site of *Escherichia coli* DNA gyrase (PDB ID: 6KZV). The interaction map reveals that Levofloxacin forms multiple stabilizing contacts within the enzyme's active pocket, indicative of its strong binding affinity and mechanism of antibacterial action. Critical hydrogen bonding interactions are observed between Levofloxacin and ASP 73 and ARG 76.

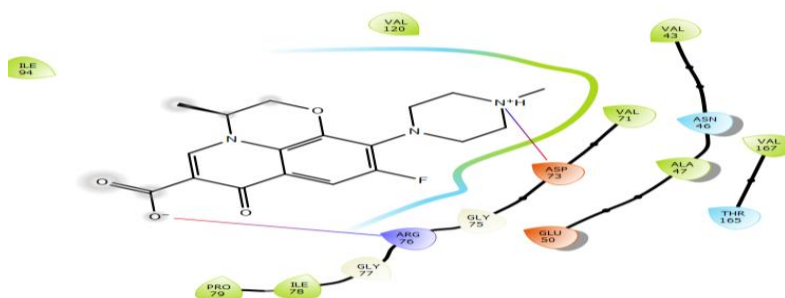


Fig. 7a: 2D Binding poses of interaction of *Levofloxacin* with amino acid residues of *E. coli* DNA gyrase

Fig. 7b shows the 2D binding interaction of 1-(4-Bromobutyl)-2-piperidinone with *E. coli* DNA gyrase (PDB ID: 6KZV). The compound forms key hydrogen bonds with ARG 76, GLU50, and THR 165, anchoring it within the enzyme's active site. Additional interactions with residues such as ASP 73, GLY 75, GLY 77, and ILE 78 support hydrophobic stabilization, while the bromobutylside chain is accommodated in a nonpolar pocket. Although it forms fewer polar interactions than Levofloxacin, the compound demonstrates favorable binding orientation, suggesting potential DNA gyrase inhibitory activity through a similar mechanism.

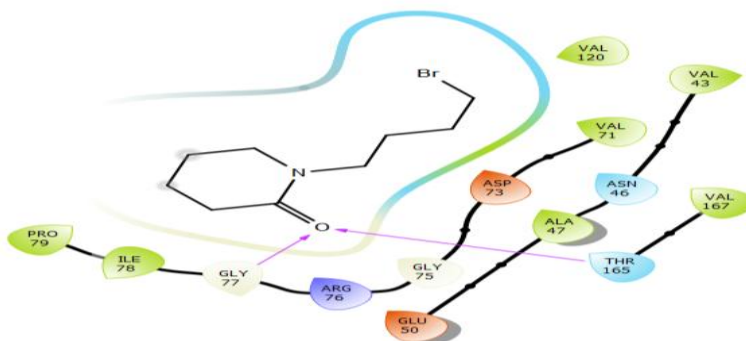


Fig. 7b: 2D Binding poses of interaction of 1-(4-Bromobutyl)-2-piperidinone with amino acid residues of *E. coli* DNA gyrase

Fig. 7c depicts the 2D binding pose of Levofloxacin with *Streptococcus pneumoniae* Topoisomerase IV (PDB ID: 4EM7), highlighting strong polar and electrostatic interactions with catalytic residues. Key hydrogen bonds are observed with ASP 78, ARG 81, and ASP 54, anchoring the ligand within the active site. Additional contacts with GLU 55, GLY 82, and MET 83 further stabilize the drug's orientation. The fluoroquinolone scaffold interacts favorably with surrounding residues, while

hydrophobic contacts with ILE 48, VAL 76, ALA 52, and PHE 99 support binding stability. These interactions affirm Levofloxacin's mechanism of action through the inhibition of DNA cleavage-religation activity in bacterial Topoisomerase IV, leading to effective antibacterial activity.

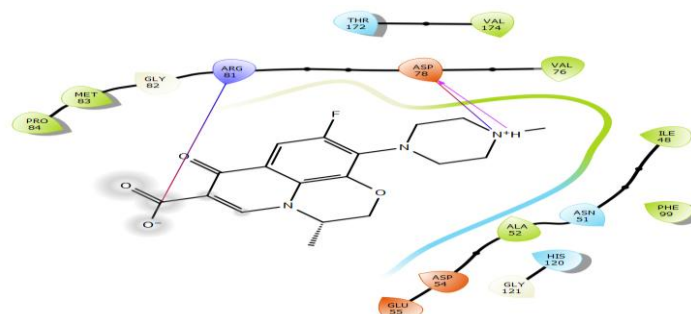


Fig. 7c: 2D Binding poses of interaction of *Levofloxacin* with amino acid residues of *Streptococcus pneumoniae* Topoisomerase IV

Fig. 7d illustrates the 2D binding interaction of 1-(4-Bromobutyl)-2-piperidinone with the active site of *Streptococcus pneumoniae* Topoisomerase IV (PDB ID: 4EM7). The compound engages in key hydrogen bonding interactions with ARG 81, GLY 82, and THR 172, indicating stable anchorage within the enzyme's catalytic pocket. Additional nonpolar interactions are formed with ILE 48, PHE 99, and MET 83, while residues such as ASP 78, GLU 55, and ASN 51 provide a supportive electrostatic environment. The bromobutyl moiety is accommodated within a hydrophobic cavity, potentially enhancing membrane permeability. Though fewer in hydrogen bonds than Levofloxacin, the binding profile suggests that this compound may interfere with the enzyme's function, possibly mimicking fluoroquinolone-like inhibitory mechanisms.

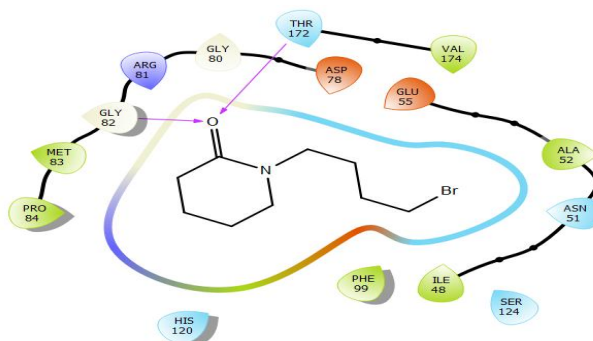


Fig. 7d: 2D Binding poses of interaction of 1-(4-Bromobutyl)-2-piperidinone with amino acid residues of *Streptococcus pneumoniae* Topoisomerase IV

Fig. 7e illustrates the 2D binding pose of Levofloxacin within the active site of *Pseudomonas aeruginosa* DNA gyrase (PDB ID: 7PTF). The interaction network highlights key hydrogen bonds between Levofloxacin and ASP 75, ARG 78, and GLY 77, stabilizing the ligand within the gyrase catalytic pocket. Additional polar contacts with residues such as ASN 48, THR 167, and SER 49 further enhance ligand binding. Hydrophobic interactions involving VAL 73, VAL 45, and ILE 96 contribute to nonpolar stabilization. The fluoroquinolone core is oriented favorably for inhibition of the DNA cleavage-religation step, affirming Levofloxacin's mechanism of action. These interactions underscore its maintained efficacy, although some variability in binding orientation compared to *E. coli* and *S. pneumoniae* targets may affect binding affinity.

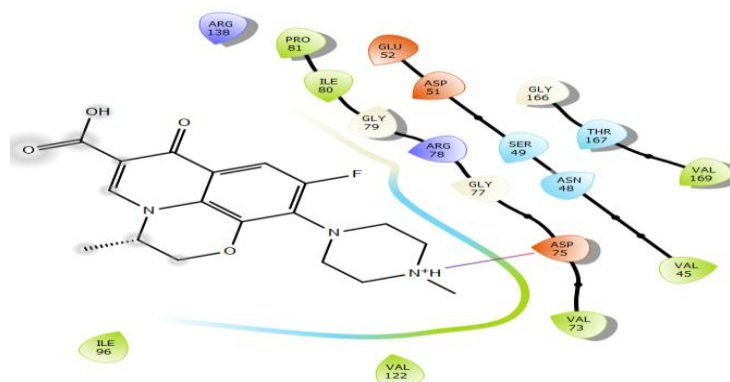


Fig. 7e: 2D Binding poses of interaction of *Levofloxacin* with amino acid residues of *Pseudomonas aeruginosa* DNA gyrase

Fig. 7f displays the 2D interaction profile of 1-(4-Bromobutyl)-2-piperidinone with *Pseudomonas aeruginosa* DNA gyrase (PDB ID: 7PTF). The compound establishes hydrogen bonding interactions with GLY 77, GLY 79, and THR 167, anchoring the ligand within the active site. Additional surrounding residues, including ARG 78, ASP 75, and GLU 52, contribute to polar stabilization, while hydrophobic interactions with VAL 45, VAL 122, ILE 80, and PRO 81 provide nonpolar support. The bromobutyl side chain fits snugly into the hydrophobic groove, enhancing lipophilic contacts. Although the ligand forms fewer interactions than Levofloxacin, the observed binding orientation suggests a potential inhibitory role by interfering with the DNA supercoiling mechanism of the enzyme.

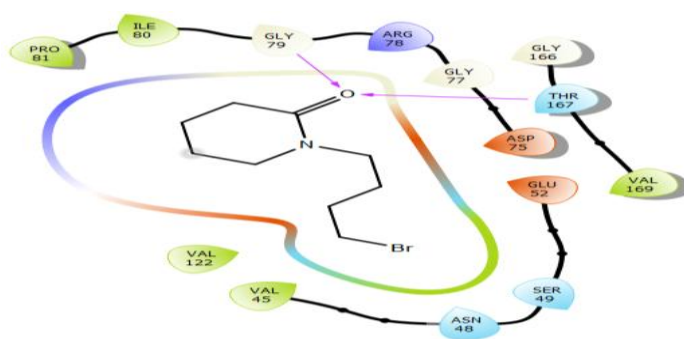


Fig. 7f: 2D Binding poses of interaction of 1-(4-Bromobutyl)-2-piperidinone with amino acid residues of *Pseudomonas aeruginosa* DNA gyrase

Discussion

This study assessed the antimicrobial efficacy of aqueous garlic (*Allium sativum*) extract against multidrug-resistant (MDR) uropathogens isolated from patients at the University of Ilorin Teaching Hospital, while also exploring its phytochemical constituents, GC-MS profile, and the molecular interactions of key bioactive compounds. The microbiological analysis confirmed *Escherichia coli* as the most prevalent uropathogen, followed by *Klebsiellapneumoniae*, *Proteus mirabilis*, *Citrobacterfreundii*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca*¹⁴. This pattern aligns with previous studies in Nigeria, where *E. coli* consistently emerges as the leading cause of UTIs¹⁵. The antibiotic resistance profile revealed extensive resistance, particularly to ciprofloxacin and ampicillin, which is reflective of the growing trend in fluoroquinolone and beta-lactam resistance across Nigerian healthcare facilities¹⁶. Over 80% of the isolates were confirmed as multidrug-resistant, with *P. aeruginosa* and *K. pneumoniae* showing the most aggressive resistance patterns¹⁷.

Despite the worrisome resistance patterns, the aqueous garlic extract demonstrated dose-dependent inhibitory activity against all tested organisms¹⁸. Its performance, though slightly less potent than Levofloxacin, was consistent and reproducible, particularly against *E. coli*, *K. pneumoniae*, and *K. oxytoca*¹⁹. These findings support earlier reports affirming garlic's antimicrobial efficacy against Gram-negative bacteria²⁰, and further extend the literature by determining the MIC and MBC values, which confirmed a bactericidal effect against most isolates, with the exception of *P. aeruginosa*, where the activity was primarily bacteriostatic.

The phytochemical screening revealed the presence of a broad range of bioactive compounds including saponins, flavonoids, phenolics, alkaloids, tannins, glycosides, steroids, and terpenoids²¹. These classes of phytochemicals are widely recognized for their roles in membrane disruption, enzyme inhibition, and synergistic interactions with

conventional drugs²². However, the novelty of this study lies not only in its confirmation of these constituents but in its in-depth GC-MS analysis and subsequent molecular docking evaluations.

Unlike prior GC-MS-based studies on garlic that mostly identified sulfur-containing volatiles such as allicin, diallyl disulfide, and trisulfide derivatives²³, this study identified a distinct spectrum of compounds including 9-octadecenoic acid methyl ester, 8-heptadecene, oxalic acid cyclobutylpentadecyl ester, and particularly 2-Piperidinone, N-[4-bromo-n-butyl]-. The identification of this brominated piperidinone is particularly significant due to its structural and functional resemblance to Levofloxacin. Both compounds target DNA gyrase (Topo II) and potentially Topoisomerase IV, key enzymes involved in bacterial DNA replication²⁴. While Levofloxacin is a well-established fluoroquinolone that inhibits DNA replication by binding to DNA gyrase and stabilizing the cleavage complex, 2-Piperidinone, N-[4-bromo-n-butyl]- appears to mimic this mechanism. It possesses a brominated lactam ring and a bromoalkyl side chain, features that likely enable it to stabilize the Topo-DNA complex or interfere with replication machinery in a manner similar to Levofloxacin. This structural similarity was supported by molecular docking simulations, which showed both compounds interacting with conserved amino acid residues within the active sites of DNA gyrase and Topoisomerase IV across *E. coli*, *P. aeruginosa*, and *Streptococcus pneumoniae*.

Furthermore, both molecules exhibit high cell permeability; 2-Piperidinone, N-[4-bromo-n-butyl]- likely benefits from its lipophilic bromoalkyl chain, while Levofloxacin is optimized through fluorine substitution and pharmacokinetic refinement.

However, unlike Levofloxacin which is FDA-approved and widely used clinically, 2-Piperidinone, N-[4-bromo-n-butyl]- remains an experimental compound. Yet, its structural and mechanistic resemblance to Levofloxacin suggests it could serve as a natural, plant-derived alternative, particularly in cases involving fluoroquinolone-resistant pathogens. This mechanistic overlap further supports the hypothesis that garlic-derived bioactives, when appropriately extracted and characterized, can serve as functional mimetics of synthetic antibiotics and potentially contribute to novel drug discovery²⁵.

This study not only reaffirms the ethnopharmacological value of garlic as an antimicrobial agent but also provides compelling evidence for the potential of its constituents, particularly 2-Piperidinone, N-[4-bromo-n-butyl]-, to act as natural analogues to established antibiotics such as Levofloxacin. The integration of phytochemical screening, GC-MS profiling, and in silico molecular docking offers a comprehensive framework for the rational evaluation of plant-derived antimicrobials²⁶. These findings underscore the need for further in vivo and clinical evaluations of garlic extract and its lead compounds, with the goal of developing alternative treatments for multidrug-resistant infections, particularly in resource-limited settings like Nigeria.

Conclusion

This study confirmed the antimicrobial efficacy of aqueous garlic (*Allium sativum*) extract against multidrug-resistant (MDR) uropathogens isolated from patients at the University of Ilorin Teaching Hospital, with *Escherichia coli* emerging as the most prevalent pathogen. The high resistance levels observed across isolates to fluoroquinolones and beta-lactams highlight the urgency for alternative therapies. The garlic extract exhibited dose-dependent bactericidal activity against most organisms and contained bioactive phytochemicals such as saponins, phenolics, flavonoids, and terpenoids. A key contribution of this study is the GC-MS identification of 2-Piperidinone, N-[4-bromo-n-butyl]-, and its demonstrated structural and functional similarity to Levofloxacin through molecular docking. These findings position garlic and its compounds as promising potential lead for drug design.

Recommendations

Based on the findings of this study, the following recommendations are made:

1. In vivo and clinical evaluations of the garlic extract and its key compound, 2-Piperidinone, N-[4-bromo-n-butyl]-, should be prioritized to determine their antimicrobial efficacy, toxicity, and pharmacokinetic properties, establishing their potential for therapeutic use.

Acknowledgement

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Competing Interests

The author declares no competing interests. There are no financial, personal, or professional relationships that could be perceived to have influenced the outcome or interpretation of this research.

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Exploring Climate-Resilient Endophytic Fungi for Sustainable Drug Discovery: Antioxidant Potential of *Lasiodiplodia pseudotheobromae* in a Changing Climate

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Abstract

Background: The increasing impacts of climate change have prompted a shift towards sustainable drug production, focusing on natural and resilient biological sources. Endophytic fungi and microorganisms living symbiotically within plant tissues, have demonstrated remarkable potential in this regard. Endophytic fungi, such as *Lasiodiplodia pseudotheobromae* from the *Botryosphaeriaceae* family, produce bioactive secondary metabolites with antimicrobial, antioxidant, and anticancer properties. Identifying climate-resilient strains of such fungi could advance pharmaceutical development. This study explores the antioxidant potential of *L. pseudotheobromae*, leveraging its resistance to environmental stressors and therapeutic potential.

Methodology: *Lasiodiplodia pseudotheobromae* was cultured and the secondary metabolites were extracted using ethyl acetate (EA) and methanol (MeOH). The crude extract was fractionated using diethyl ether (DE), dichloromethane (DCM), and n-hexane (NH). These fractions were evaluated for their antioxidant properties and then characterized via UV and IR spectroscopy.

Results: All fractions demonstrated significant antioxidant activity. Ultraviolet (UV) spectroscopy indicated electronic transitions linked to chromophores associated with antioxidant activity. Infrared (IR) spectra confirmed the presence of bioactive functional groups, including conjugated hydroxyl (O-H), carbonyl (C=O), and hydrocarbon (C-H) stretching bands.

Conclusion: This study has indicated that *L. pseudotheobromae* possess strong antioxidant properties, positioning it as a promising candidate for sustainable drug production. Further research into its bioactive compounds could expand its therapeutic applications.

Keywords: Characterization, Antioxidant Activities, Endophytic Fungi, *Lasiodiplodia pseudotheobromae*, Fractionation.

Introduction

Endophytes are organisms that live in active, disease-free plant tissues without harming their host plants. Endophytic fungi protect plants from diseases by colonizing infection sites, competitive exclusion for nutrients and ecological niches, biosynthesis of antimicrobial metabolites, and activation of host systemic resistance pathways. They can also increase the host plant's resistance to various stresses or create phytohormones to stimulate plant growth or manufacture insecticides to shield plants from herbivores. More than a million fungal species are thought to make up the significant and highly diversified group of endophytes known as endophytic fungi. Recently, endophytic fungus isolation and the study of their natural products have drawn a lot of interest from the general public (Caruso *et al.*, 2022).

Comparing endophytic fungi to soil fungi connected to cyanobacteria, endophytic fungi are found to be more metabolically trending, considered to be a reliable source of novel bioactive chemicals are entophytic fungus. Due to the interaction between endophytes and their hosts, several biologically active substances are created and classified into various groups. Steroids, alkaloids, phenols, isocoumarins, xanthonenes, quinones, and terpenoids are examples of the secondary metabolites produced by endophytic fungi (Hashem *et al.*, 2023).

Recent studies have underscored the remarkable antioxidant capabilities of metabolites produced by endophytic fungi. These natural antioxidants play a vital role in alleviating diseases associated with oxidative stress and are increasingly being pursued in the realm of drug development (Asomadu *et al.*, 2024). The ability of endophytic fungi, such as *L. pseudotheobromae*, to withstand environmental stressors renders them essential in the face of climate change. Their inherent adaptability indicates a reliable and sustainable source of bioactive compounds, even amid changing environmental conditions. This trait is particularly beneficial for the ongoing processes of drug discovery and production (Fite *et al.*, 2023). Hashem *et al.*, (2023) examine the contributions of fungal endophytes in addressing both biotic and abiotic stresses, emphasizing their potential applications in sustainable agriculture and pharmaceuticals. Additionally, some reports suggest that some endophytic fungi produce more than twelve metabolites similar to those produced by the host plants, such as alkaloids, flavonoids, saponins, peptides, phenolic acids, terpenes, and other active substances and steroids and it is thought that this is a promising source for new compounds (Chandra *et al.*, 2024). This study aims to characterize the bioactive compound of the endophytic fungus *Lasiodiplodia pseudotheobromae* extract using ultraviolet (UV) and infrared (IR) spectroscopic technique and antioxidant analysis.

Materials and Methods

Collection of the Endophytic Fungus

The pure culture of *Lasiodiplodia pseudotheobromae* isolated from *Ocimum gratissimum* L. was obtained from the Mycology Laboratory of the Department of Plant Biology, University of Ilorin, Ilorin.

Fungal Cultivation

The pour plate method was employed for the fungal cultivation. In this method a solution of Potato Dextrose Agar (PDA) was prepared by measuring 24 g of the potato dextrose agar on a weighing balance and dissolving it in 1000 ml of distilled water. The solution was then heated while shaking gently until a clear solution was observed. The solution was sterilized in an autoclave at 121 °C for 15 minutes. The sterilized PDA solution was then allowed to cool a bit and 20 mg/mL of streptomycin was added to the cooling solution in order to prevent and/or suppress the growth of bacteria. After a while, 50 ml of the cooling PDA solution was transferred to a Petri dish for complete cooling and solidification. The *Lasiodiplodia* fungus was then swab at the center of the Petri dish containing the solidified PDA solution and then covered and wrapped up immediately to prevent contamination; it was then left in a sterile airtight chamber for 5 days to grow (Wang *et al.*, 2023; Li *et al.*, 2025).

Extraction

Fungal inoculums obtained from pure culture of *Lasiodiplodia pseudotheobromae* were inoculated into 250 ml Erlenmeyer flask containing 250 ml potato dextrose broth and incubated for 21 days at 29±1°C with intermittent shaking. The fungal culture was filtered. Equal volume of ethyl acetate:methanol was added and homogenized. The secondary metabolites were extracted with ethyl acetate:methanol (1:1). The organic phase was evaporated and stored at 4°C for further use. The crude extract was then partitioned by adding n-hexane (NH), the mixture was thoroughly shaken and then separated using separating funnel. The two layers were collected in different beakers. Dichloromethane (DCM) was added to the remnant of the EA:MeOH extract, shaken and separated and lastly to the EA:MeOH extract, diethyl ether (DE) was added and also separated using separating funnel. The n-hexane (NH), dichloromethane (DCM) and diethyl ether (DE) fractions were further washed with water, and together with the marc dried over MgSO₄ and left to concentrate by air drying (Garuba *et al.*, 2018; Ameen *et al.*, 2021).

Thin Layer Chromatography

Thin Layer Chromatography (TLC) was carried out on a pre-coated TLC plate (silica gel 60 F₂₅₄ aluminum foil) on the LP₁-LP₄ fractions for the establishment of the purity of isolates. Spots on TLC were examined with a UV lamp operating at a wavelength of 366 nm for fluorescence and at 254 nm for fluorescence quenching spots (Ameen, 2015).

Antioxidant Analysis

The Antioxidant analysis of the LP₁-LP₄ fractions of *Lasiodiplodia pseudotheobromae* extract was carried out at the Central Research and Diagnostic Laboratory, Tanke, Tipper Garage, Ilorin, Kwara State Nigeria.

Total Antioxidant Capacity

The Total Antioxidant capacity (TAC) reagent was prepared by mixing equal volumes of 0.6 M Sulfuric Acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Samples were prepared with the concentration of 100-500 µg/mL. The reaction solution comprised of 100 µl of the sample and 1000 µl of the TAC reagent. The mixture was incubated in water bath for 1 hour at 95 °C, after which the absorbance was taken with the spectrophotometer at 695 nm upon cooling. The blank solution contained 1000 µl of the reagent and 0.1 ml of methanol while the control was the reagent only without sample and methanol. Standard curve is prepared with gallic acid in the concentration range of 1-100 µg/ml. The antioxidant activity of the samples is derived from the standard curve and expressed relative to that of the gallic acid in (mg/g Gallic acid) (Kumar *et al.*, 2014; Molole *et al.*, 2022).

Hydroxyl Radical Scavenging

The reaction mixture composed of 500 µL of 1,10 phenanthroline, 1000 µL of Sodium phosphate buffer, 500 µL of ferrous sulfate, 500 µL of hydrogen peroxide and 500 µL of different concentration of the sample 100-500 µg/mL. The content was incubated for 30 min at room temperature after which the absorbance was recorded at 536 nm against the reagent blank. Distilled water was used in place of sample as blank while ascorbic acid was used as positive control having the same concentration as the sample (Xiao *et al.*, 2022). The scavenging activity was calculated using equation 1.

$$\text{Scavenging Activity (\%)} = \frac{(\text{AbsCtrl} - \text{AbSample})}{(\text{AbsCtrl})} \times 100 \quad \text{Equation 1}$$

Where:

AbsCtrl = Absorbance of the Control and

AbSample = Absorbance of the sample or Standard

Spectroscopic Analysis

The IR spectroscopic analysis of the LP₁-LP₄ fractions of *L. pseudotheobromae* extract was carried out at the instrumentation room of the Department of Chemistry, while the UV spectroscopic analysis of the LP₁-LP₄ fractions of *L. pseudotheobromae* extract was carried out at the Central Research Laboratory, both at the University of Ilorin, Nigeria.

Results and Discussion

Thin Layer Chromatography of LP₁-LP₄ Fractions

Lasiodiplodia pseudotheobromae was successfully cultured under laboratory conditions, and the resulting extract obtained using a mixture of ethyl acetate and ethanol yielded a pinkish-colored solution. The fractions designated LP₁-LP₄ were subsequently analyzed by thin-layer chromatography (TLC) (Figure 1).

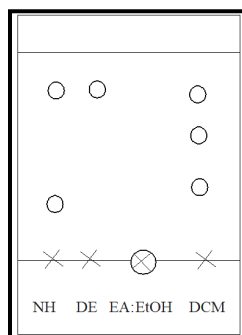


Figure 1: TLC Chromatogram of the LP₁-LP₄ fractions of *Lasiodiplodia pseudotheobromae*

TLC is an efficient, rapid, and cost-effective method that provides researchers with a prompt determination of the number of components present in a mixture. Additionally, TLC aids in verifying the identity of a compound within a mixture by comparing the R_f values of the unknown compound with those of known standards. A clear benefit of Thin Layer Chromatography (TLC) is its cost-effectiveness, coupled with its ability to deliver rapid and definitive results (Sasidharan *et al.*, 2011; Kowalska and Sajewicz, 2022). Figure 1 presents the TLC chromatogram for the LP₁-LP₄ fractions of *L. pseudotheobromae*, the TLC plate developed with n-hexane and diethyl ether in ratio 2:1 indicates the presence of two components in the NH fraction, one in DE fraction and three components in the DCM fraction. The R_f value obtained for the n hexane fraction (0.72, 0.80) were consistent with those reported in recent studies on endophytic fungi extracts, which also show a similar range of R_f values for non-polar compounds in the n hexane solvent (0.7-0.8) (Sharma *et al.* 2016). Similarly, the diethyl ether fraction exhibited R_f values 0.80 aligning with the findings of Owolabi, *et al.* (2018) on *Feretia apondanthera* root bark extracts, which also indicated the presence of polar compounds like flavonoids and tannins in the same range.

Spectroscopic Analysis of the Fractions

The UV and FTIR spectroscopic analyses of the LP₁-LP₄ fractions provided crucial insights into the functional groups and electronic transitions within the compounds present in the fractions. The UV-Vis spectra of the n-hexane (LP₁), diethyl ether (LP₂), ethyl acetate:ethanol (LP₃) and dichloromethane (LP₄) fractions are presented in Figures 2 – 9.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

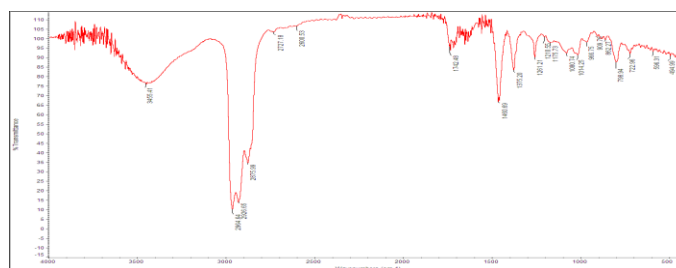


Figure 2: Infrared Spectroscopy Spectrum of N-Hexane fraction of the *L. pseudotheobromae* extract

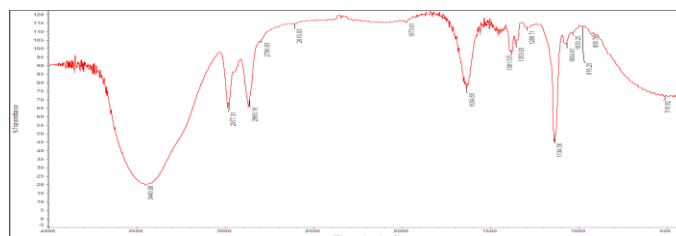


Figure 3: Infrared Spectroscopy Spectrum of Diethyl Ether fraction of the *L. pseudotheobromae* extract

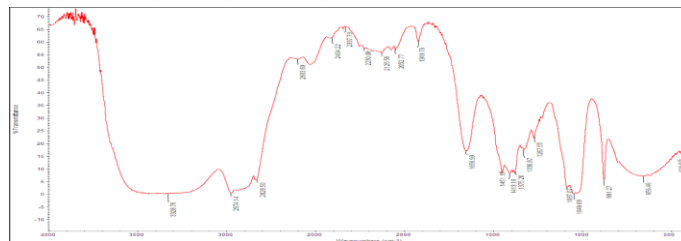


Figure 4: Infrared Spectroscopy Spectrum of ethyl acetate:ethanol fraction of the *L. pseudotheobromae* extract

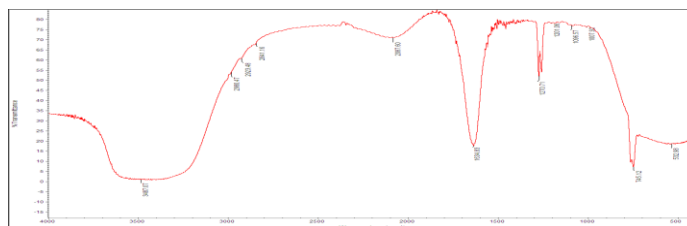


Figure 5: Infrared Spectroscopy Spectrum of dichloromethane fraction of the *L. pseudotheobromae* extract

Ultraviolet-Visible Spectroscopy Analysis

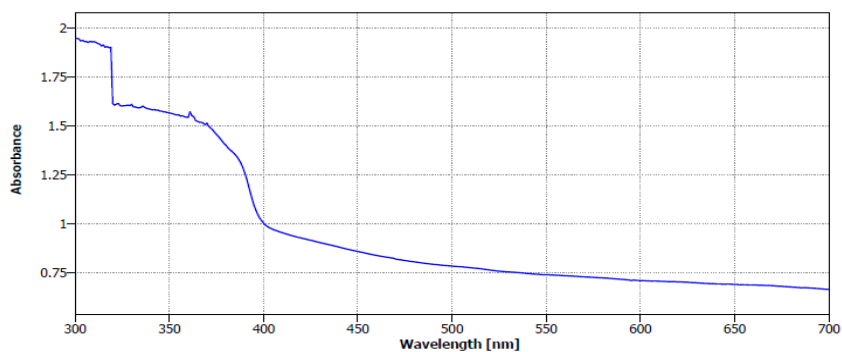


Figure 6: Ultraviolet Spectroscopy Spectrum of N-Hexane fraction of the *L. pseudotheobromae* extract

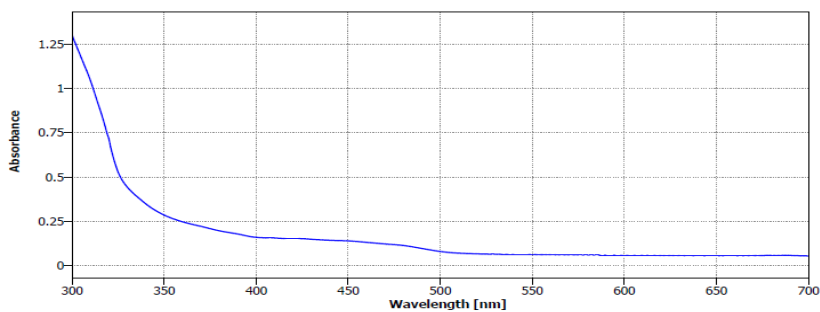


Figure 7: Ultraviolet Spectroscopy Spectrum of Diethyl Ether fraction of the *L. pseudotheobromae* extract

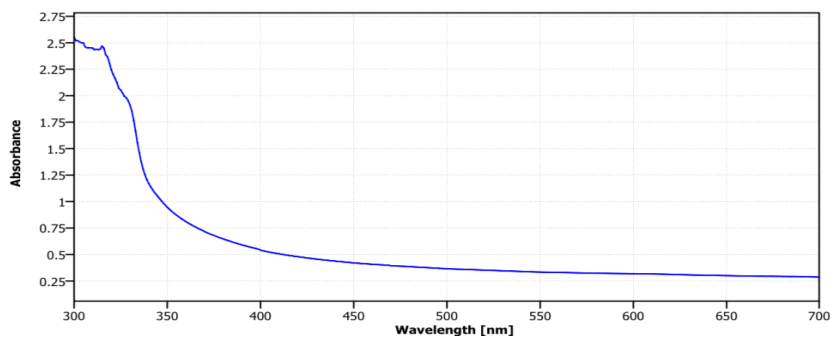


Figure 8: Ultraviolet Spectroscopy Spectrum of ethyl acetate: ethanol fraction of the *L. pseudotheobromae* extract

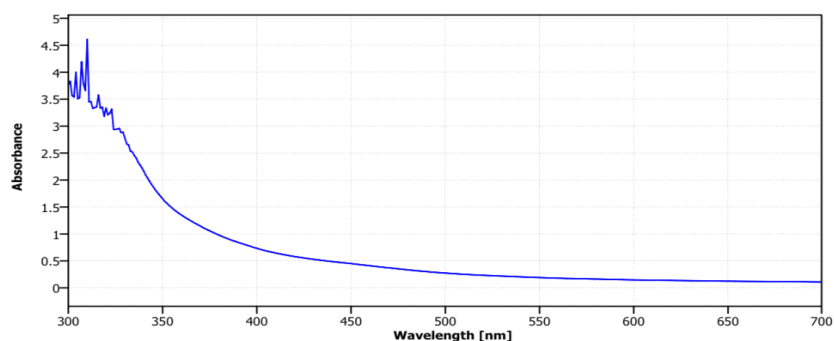


Figure 9: Ultraviolet Spectroscopy Spectrum of dichloromethane fraction of the *L. pseudotheobromae* extract

The UV-Vis spectra of the n-hexane, diethyl ether, dichloromethane and ethyl acetate:ethanol fractions exhibit prominent absorption peaks at 300 nm, 290 nm, 320 nm and 300 nm respectively. These peaks suggest the presence of conjugated systems such as polyphenols or terpenoids, which are known to absorb in this region due to π - π^* transitions. This finding aligns with previous studies that have identified these classes of compounds in endophytic fungal extracts, especially in non-polar fractions like n-hexane (Coates, 2000).

FTIR analysis further confirmed the presence of key functional groups in the fractions. The n-hexane fraction exhibited strong absorption bands around 3455 cm^{-1} (*O-H* stretch), 2964 cm^{-1} , and 2926 cm^{-1} (*C-H* stretches), indicating the presence of hydroxyl groups, aliphatic hydrocarbons, and fatty acids. The peaks at 1742 cm^{-1} suggest the presence of carbonyl groups (*C=O*), typical of esters or aldehydes, which are common in lipid-based compounds. The diethyl ether fraction displayed similar functional groups, indicating a mix of phenolic compounds, alcohols, and fatty acids, but also showed peaks that could correspond to unsaturated hydrocarbons or sulfur-containing compounds (thiols) (Shankar *et al.*, 2022; Segaran and Sathiavelu, 2023).

The EA: MeOH and dichloromethane fractions revealed strong *O-H* and *C=O* stretches, consistent with the presence of phenolic compounds, steroids, or polyphenols. These fractions also exhibited *C-H* stretches, further suggesting the presence of compounds with aromatic or aliphatic characteristics. The spectroscopic data strongly suggest that *Lasiodiplodia pseudotheobromae* produces a range of bioactive compounds, including polyphenols and terpenoids, which are well for their antioxidant properties (Segaran and Sathiavelu, 2023).

Antioxidant Analysis

The antioxidant activity of the LP1-LP4 fractions was evaluated using both the Total Antioxidant Capacity (TAC) and Hydroxyl Radical Scavenging Assays Table 1 and 2.

Total Antioxidant Capacity (TAC) Analysis**Table 1:** Total Antioxidant Capacity of LP₁-LP₄ fractions

SAMPLES	TAC (mg/100g)
N-Hexane	0.0722
Diethyl Ether	0.0241
DCM	0.0805
EA:EtOH	0.0246
STD Gallic Acid	3.0814

Table 2: Hydroxyl Radical Scavenging of LP₁-LP₄ fractions

SAMPLES	Concentrations (µg/ml)				
	100	200	300	400	500
N-Hexane	42.52768	48.61624	58.67159	62.91513	72.23247
Diethyl Ether	86.99262	91.69742	91.78967	95.66421	96.58672
DCM	78.04428	83.94834	87.91513	90.49816	96.03321
EA:EtOH	95.73247	97.60418	98.33948	98.89299	99.07749
STD Ascorbic Acid	78.57145	84.74025	88.81210	89.93505	91.83765

The TAC results demonstrated that the n-hexane and dichloromethane (DCM) fractions exhibited higher antioxidant capacities compared to the diethyl ether and EA:MeOH fractions. The efficacy of different fractions can be attributed to the presence and concentration of specific antioxidant compounds extracted by the solvents. For instance, the high antioxidant activity in n-hexane could be linked to the extraction of compounds like γ -sitosterol, as found in the n-hexane extract of *S. alba* leaves. Similarly, n-hexane and dichloromethane which are considered non-polar to moderately polar solvents might have extracted compounds with higher antioxidant potential in this particular study. It is also possible that the compounds extracted by n-hexane and DCM work synergistically to produce a higher antioxidant effect, even if the total quantity of antioxidant compounds in these fractions might not be the highest (Nawaz *et al.*, 2020; Ginting *et al.*, 2022; Kumar *et al.*, 2023). The relatively higher TAC value for the DCM fraction (0.0805 mg/100g) obtained in this study is in tandem with the works of Effiong *et al.*, (2024) and that of Asomadu *et al.*, (2024) who reported that DCM fraction of plant extracts contains more potent antioxidant compounds compared to the other fractions.

The hydroxyl radical scavenging assay provided a more detailed picture of the antioxidant potential of each fraction. The EA:MeOH fraction exhibited the highest scavenging activity (up to 99.08% at 500 µg/mL), followed closely by the DCM fraction, which also demonstrated significant scavenging capacity (96.03% at the same concentration). These results indicate that the polar fractions, particularly EA:MeOH, are rich in antioxidants, likely due to the high concentration of polyphenols and other polar bioactive compounds. The diethyl ether fraction also displayed notable antioxidant

activity (96.59%), despite its lower TAC value, suggesting the presence of compounds with selective radical scavenging abilities (Vu *et al.*, 2022; Verma *et al.*, 2022).

In comparison, the n-hexane fraction showed the least scavenging activity, reinforcing the suggestion that non-polar solvents are less effective at extracting antioxidant-rich compounds. Overall, the antioxidant assays highlight the strong potential of the polar fractions (EA:MeOH and DCM) as sources of natural antioxidants, which could be harnessed for therapeutic or health-related applications (Effiong *et al.*, 2024; Asomadu *et al.*, 2024).

Conclusion

This study demonstrated that *Lasiodiplodia pseudotheobromae* produces diverse bioactive metabolites with strong antioxidant potential, particularly concentrated in the polar fractions (EA:MeOH and DCM). Thin-layer chromatography and spectroscopic analyses confirmed the presence of phenolic compounds, polyphenols, and terpenoids, which are known contributors to antioxidant activity. The high hydroxyl radical scavenging activity observed in these fractions underscores their potential as valuable sources of natural antioxidants. Taken together, these findings highlight *L. pseudotheobromae* as a climate-resilient endophytic fungus with promising applications in sustainable drug discovery. Further isolation and structural elucidation of its bioactive compounds will be essential to unlocking its full therapeutic potential.

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**Effect of Ethanol Extract of Clove on the Nutritive and Storage
Attributes of West African Soft Cheese**

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Abstract

The microbial spoilage of cheese results in multi-billion naira loss and global food insecurity. The need to preserve West African soft cheese using clove ethanolic extract and determine its preservative potency is the theme of this study. Five treatments studied were varied levels of inclusion of Clove Ethanolic Extract (CEE 0, 0.5, 1, 1.5 and 2ml) variously into 200 ml milk in a Completely Randomized Design. Sodom apple was the coagulant used, but was fortified with different inclusion levels of CEE into 200ml of coagulating milk. Parameters evaluated include bacterial count, nutritional composition and sensory analysis. It was observed that increasing the inclusion level of clove extract improved ($p < 0.5$) the protein, fat and sensory properties, while reducing bacterial growth. The study recommended that clove ethanolic extract is a potent additive and its addition at 1 % of coagulating milk will enhance the nutritional, sensory and storage attributes of West African soft cheese.

Keywords: *Nutritive value, Sensory values, Bacterial attributes*

Introduction

Cheese is universally desired food item, a highly nutritious food product made by the coagulation of milk protein known as casein (Badmos *et al*, 2018). Cheese is one of the major products obtained from processing the milk of cows, goats, sheep, buffalos, camels and yaks (Akinloye and Adewumi, 2014). It is rich in proteins, lipids, sugars, vitamins and minerals and it serves as an excellent medium of milk preservation (Hidreth, 1977; Belewu, 2019). Cheese is presently a highly commercialized food item (Aworh, 2008; Badmos *et al.*, 2017). The shelf life of Cheese however varies with its moisture

content and soft cheese, with moisture content above 60% has a shelf life of 3 days when in whey (Adegoke *et al.*, 1992; Belewu *et al.*, 2005). This is due to the high moisture content and almost neutral pH making cheese, particularly soft cheese an ideal growth medium for bacteria and spoilage micro-organisms (Adesokan *et al.*, 2008). There is a continuous need to inhibit bacterial growth and extend the shelf life of soft cheese, beyond the use of synthetic preservatives which are implicated in health problems (Alvarez-Suarez *et al.*, 2010). Natural preservatives are a more preferred option. Such natural spices and herbal products include Alligator pepper (*Aframomum melegueta*), Locust bean husk (*Parkia biglobosa*) eucalyptus oil, Ginger (*Allium sativum*), lemon grass oil and Sorghum stalk extract [Joseph and Akinyosoye (1997); Belewu *et al.* (2005); Belewu *et al.* (2012); Ashaye *et al.* (2006) and Badmos *et al.*, 2017)]. Clove is another additive with strong antimicrobial properties (Cock and Cheeseman, 2018). It is one of the most valuable spices that have been used traditionally as food preservatives and for many other purposes. This plant represents one of the richest sources of phenolic constituents as eugenol, and eugenol acetate possessing great potential for pharmaceutical, cosmetics, food and agricultural applications (Suárez-Vega *et al.*, 2024). Clove contains appreciable amounts of volatile oil used for flavouring foods and pharmaceuticals. The antioxidant and antimicrobial activities of clove are higher than for many fruits and spices (Burst and Reinders, 2003) and it has remarkable antibacterial, antiviral, anti-inflammatory, anti-mutagenic and antiparasitic attributes (Cai and Wu, 1996; Mittal *et al.*, 2023). The thrust of this study is thus to evaluate the potential of clove extract as a preservative and determine its efficacy as an additive to improve the nutritive and sensory properties of West African soft cheese in storage.

Materials and Methods

Preparation of clove ethanolic ethanol extracts (CEE): Fresh dried clove obtained from Ilorin Market was destoned, cleaned and blended using Black & Decker smartgrind and cuisinart spice nut grinder to a powdery form in the Animal production laboratory of the University of Ilorin. The clove powder was immersed in absolute unadulterated ethanol at ratio of 1g of the clove powder to 4ml of the ethanol which was stirred thoroughly, placed in an electric shaker and allowed for 48hrs to become a homogenous mixture. The clove- ethanol mixture was then transferred, filtered (bottle top filter) and evaporated in a water bath which was set at 50°C until a thick extract was obtained. (Zakaria *et al.*, 2011) This process took approximately 7 days and reconstitution followed with a Dimethyl Sulfoxide (DMSO) at concentration of 1024mg/ml (Zakariyah *et al.*, 2017).

Milk: Fresh cow milk was obtained from the cattle herd in Kara, Okeose, adjacent to the Teaching hospital of University of Ilorin Teaching Hospital, under strict hygienic guidelines. The milk was transported in an ice jacket within 25 minutes to the department of Animal Production (latitude 08030'N and longitude 04035'E) laboratory.

Sodom Apple juice extraction: Some quantities of Sodom apple (*Calotropis procera*) leaves were collected, rinsed and weighed. Portions were weighed to 10g,

crushed with a sterilized enamel mortar and pestle with addition of 20 ml water. The resulting mash was squeezed out with a muslin cloth (Badmos and Joseph, 2012).

Table 1: The Treatment Plan

Treatments	Eth Ext (ml)	Percent Inclusion	Sodom Apple (ml)
CEE 0	0	0	3.5
CEE1	0.5	0.25	3.5
CEE 2	1.0	0.5	3.5
CEE 3	1.5	0.75	3.5
CEE 4	2.0	1	3.5

Note: CEE means Clove Ethanol extract and Eth Ext means Ethanol Extract

Treatment Plan: Milk coagulation for all cheese samples was effected by the addition of Sodom apple leave as the coagulant after which different inclusion levels of the ethanol extract of clove (0.5ml, 1.0ml, 1.5ml, 2.0ml) were also measured into 200ml of milk. The control sample had no (sodom apple coagulant only) inclusion of ethanol extract of clove.

Cheese Preparation: Five stainless cups were rinsed and 200ml of milk was measured and poured into each cup i.e. each cup contained 200ml of milk. The cups were placed into a water bath. The milk was heated to approximately 45 °C and 3.5ml of Sodom apple extract was strained into each cup. Coagulation started after some few minutes and fortification was done at this stage with the clove extract being added to the coagulating curd at different levels (0.5, 1.0, 1.5 and 2.0ml as described in the treatment plan). The control sample cheese had no extract added (0 ml).The curd was heated further for 10minutes to inactivate the plant enzyme and facilitate whey expulsion (Belewu 2005; Badmos and Joseph 2012). The cheese curds were removed from the cups and ladled individually into raffia baskets to enable drainage of whey and to give it desired shape. The cheese samples were labelled and prepared for proximate, microbial and sensory analyses. This procedure was repeated 3 times for precision.

Proximate Analysis: Proximate content of cheese were conducted, including the crude protein, moisture, fat and ash contents according to AOAC (2000) methods.

Total Bacterial Count (TBC): The methods of Fawole and Osho (2007) and Badmos *et al*, (2018) was followed, where the cheese samples of Days 1, 3 and 5 were taken aseptically and subjected to serial dilution of cheese samples, such that 1g of cheese was diluted in 9ml distilled water. The samples were smeared on Agar plates and incubated for 48 hours at 37 °C. Counting of viable bacterial colonies for the respective cheese samples followed and the values were expressed as colony forming units per gram. Characterization and identification of the isolates in cheese samples used standards microbiological procedures (Barrow, 2004 and Garrity *et al*, 2005).

Sensory Analysis: A Thirty-member trained panellist, consisting of University of Ilorin students were employed for the test. Cheese samples in white plastic cups were labelled and placed on benches in a way that there was no interference between the panellists (Lammond, 1970; Badmos and Abdulsalam, 2012) and water was provided for the panelists to rinse their mouth after each taste to avoid interference or overlap between samples. Evaluation was based on colour, taste, flavour, texture and overall acceptability. Scores were based on hedonic scale of 1-9 (1=dislike extremely and 9=like extremely).

Statistical Analysis: The results obtained were subjected to analysis of variance (ANOVA) using the completely randomized design and the means were compared by Duncan Multiple Range Test (Steel *et al.*, 1997)

Result and Discussion

Phytochemical screening of ethanol clove extract: The screening of the ethanol extract of clove showed that saponin, steroid, triterpenoid and glycoside are present in the extract (Table 2). Other phytochemicals were not observed. Lone and Jain (2022) earlier reported that clove; a native of Indonesia contains alkaloids, flavonoids, tannins, sterols and triterpenes in its dry bud. Dahiru and Paliwal (2021) reported saponins, alkaloids, phytosterols, flavonoid pigments, tannins, terpenoids in clove. Bharadwaj (2020) reported phytochemicals in clove to include tannins, saponins, terpenoids and cardiac glycosides. These observations confirm the phytochemical screening in the present study.

Table 2: Phytochemicals present in ethanol extracts of cloves

Phytochemicals	Present
Saponin	+
Steroid	+
Triterpenoid	+
Glycoside	+

NOTE: *Phytochemical precense is +*

Bacterial count: The bacterial study shows that the control (CEE 0) had the highest microbial count (113.50×10^{-6} cfu/g) of colony forming units of bacteria throughout the five storage days. It can be observed from Table 3 that the Total bacterial count (TBC) increased as the days of storage increased for all the cheese samples. The effect of increasing the inclusion level of clove is general decrease in bacterial count for the cheese samples and for the days of storage. The bacterial population increased daily as expected with increasing spoilage but the spoilage rate was inhibited with clove extract addition. This observation on bacterial growth is consistent with the previous studies reporting that clove oil reduced total bacteria content in cake (Ibrahim *et al.*, 2013) and buffalo meat (Naveena *et al.*, 2017).Clove contains eugenol and other phytochemicals with intense antioxidant activity that is as potent as BHA, pyrogallol and other synthetic

anti-oxidants (Dorman *et al.* 2000) as linolenic acid peroxidation was inhibited above 95 % at concentration of 15 µg/ml. The phytochemicals in clove in addition to denaturing proteins of microbes, changes cell membrane permeability by reacting with phospholipids. Burst and Reinders (2003) reported that clove oil inhibited the growth of some *E. coli* strains. Gupta *et al.* (2014) similarly observed that clove oil inhibited the growth of gram-positive bacteria in food including *Listeria monocytogenes*, *Streptococcus aureus* and *Escheria faecalis*. These reports can explain the lower bacterial content of the cheese samples with clove extract in the present study.

Table 3: Total Bacterial Counts (TBC) of cheese samples (10⁻⁶cfu/g)

Treatment	Day 1	Day 3	Day 5
CEE0	58.00 ^c	80.00 ^d	113.50 ^e
CEE 0.5	35.00 ^b	69.50 ^c	87.00 ^d
CEE 1	33.00 ^b	57.00 ^{bc}	67.00 ^c
CEE 1.5	31.00 ^{ab}	54.50 ^b	56.50 ^{ab}
CEE 2	28.00 ^a	48.00 ^a	52.00 ^a
SEM	1.14	1.22	1.09

^{a-e}Means sharing the same lowercase letters in the same column differ significantly ($p < 0.05$). CEE means Clove Ethanol Extract

Table 4: Proximate Analysis of West African soft cheese samples

Treatment	Moisture (%)	Fat (%)	CP (%)	Ash (%)
CEE0	65.46 ^a	12.57 ^d	10.97 ^a	2.22 ^c
CEE0.5	65.17 ^b	13.17 ^b	11.33 ^a	2.42 ^b
CEE 1	65.12 ^b	12.84 ^c	11.37 ^a	2.39 ^b
CEE 1.5	65.09 ^{bc}	13.43 ^a	11.05 ^a	2.40 ^b
CEE 2	64.82 ^c	13.09 ^b	11.28 ^b	3.41 ^a
SEM	2.19	4.72	3.04	0.04

^{a-e}Means sharing the same lowercase letters in the same row differ significantly ($p < 0.05$). CEE means Clove Ethanol Extract
CP means Crude Protein

Cheese Nutritive Content: Table 4 shows the proximate composition of clove extract treated cheese samples. The control cheese (0% clove) was highest in moisture and the clove inclusion generally reduced the moisture content of cheese. The opposite was the case for the protein and fat content of cheese samples, two nutrients which

increased generally but inconsistently with increasing inclusion of clove extract. The ash content of the CEE 2 cheese was highest. The high protein and fat content observed with increasing clove extract content is apparently due to inhibition of proteolysis and lipolysis that is associated with bacterial growth. Belewu *et al.* (2013) earlier reported higher crude protein, ether extract, ash and dry matter in cheese with inclusion of ether extract of garlic. A similar nutrient content improvement was observed by Badmos *et al.* (2020) with treatment of soft cheese with honey and *Aframomum melegueta* (alligator pepper). Earlier studies (Kikuzaki *et al.*, 1994; Belewu and Morakinyo, 2008) had similar conclusion. The extraction of fat during the ether extract preparation might also be responsible for higher fat content (Dari, 2009) of clove extract treated cheese.

Sensory Properties: Table 5 shows the Appearance, Flavour, Taste, Texture and General Acceptability of soft cheese with inclusion of clove ethanolic extract. CEE 0.5 was rated best in Taste, while CEE 2 was rated best in Appearance, Flavour, Texture and General acceptability. The stringent taste of clove, particularly in the absence of sugar apparently reduced the taste preference with increasing clove extract inclusion in cheese samples. Badmos *et al.* (2018) reported similar observation on the use of *Parkia biglobosa* husk extract additive for soft cheese. The high fat content of CEE 2 might have been responsible for the high cheese flavour rating. The effect of cheese fat on flavour and consumer preference was earlier reported (Childs and Drake, 2009; Johnson *et al.*, 2001).

Table 5: Sensory values of West African soft cheese samples

Treatment	Appearance	Flavour	Taste	Texture	G. Acceptability
CEE0	7.4 ^a	6.5 ^b	6.45 ^b	4.75 ^c	5.25 ^d
CEE0.5	6.95 ^b	6.6 ^b	6.85 ^a	6.45 ^b	6.1 ^b
CEE 1	7.35 ^a	6.9 ^a	6.4 ^b	6.55 ^b	6.85 ^{ab}
CEE 1.5	6.35 ^b	6.9 ^a	6.2 ^b	6.6 ^b	5.85 ^{bc}
CEE 2	7.45 ^a	7.02 ^a	6.2 ^b	6.95 ^a	7.2 ^a
SEM	2.32	3.02	3.04	2.81	2.55

^{a-h}Means sharing the same lowercase letters in the same row differ significantly ($p < 0.05$). CEE means Clove Ethanol Extract

Conclusion

The conclusion of this study is that ethanolic extract of clove improved the dry matter, fat and protein of cheese, reduced bacterial growth and enhanced sensory properties of soft cheese. Clove extract is thus a potent additive and preservative of cheese and is hereby recommended to be included into coagulating cheese curd at the rate of 2ml extract per 200ml milk.

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Synergistic Biodegradation of Petroleum Hydrocarbons by a Bacterial–Yeast Coculture Isolated from Engine Oil-Contaminated Soil

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Abstract

Petroleum hydrocarbon contamination persists as a major environmental concern, particularly in developing regions such as Nigeria where industrial waste disposal is poorly. While individual microbial strains exhibit crude oil biodegradation capacity, limitations such as incomplete breakdown and low biomass hinder application. This study evaluated the synergistic efficacy of a bacterial–fungal coculture of *Pseudomonas aeruginosa* and *Yarrowialipolytica* under laboratory conditions. Both organisms were isolated from spent engine oil–contaminated soil and subjected to 240-hour biodegradation assays in Bushnell-Haas medium with crude oil as the sole carbon source. Parameters assessed included total petroleum hydrocarbon (TPH) degradation, viable cell counts, biosurfactant production, enzymatic activity, and hydrocarbon transformations via GC-MS and FTIR. The coculture achieved complete TPH degradation (100%) by 240 hours, surpassing monocultures (70–74%), and exhibiting enhanced biosurfactant production (81.3%) as well as broader aliphatic/aromatic breakdown. Principal Component Analysis confirmed strong correlations between degradation, enzymatic activity, and emulsification. Findings highlight the promise of microbial consortia as scalable, sustainable platforms for crude oil remediation.

Key words: Crude Oil Biodegradation; Microbial Coculture; *Pseudomonas aeruginosa*; *Yarrowialipolytica*; Biosurfactant Production; Petroleum Hydrocarbon Remediation

Introduction

Crude oil pollution presents a persistent environmental and public health challenge, particularly in oil-producing regions such as the Niger Delta in Nigeria. Frequent spills have led to severe soil degradation, aquatic ecosystem disruption, and biodiversity loss (Amadi *et al.*, 2019; Nwachukwu *et al.*, 2021). Among existing remediation strategies, microbial bioremediation has emerged as a promising, eco-friendly, and cost-effective alternative to physicochemical approaches. However, its practical application remains hindered by inconsistent degradation rates, particularly

when relying on single microbial isolates (Okoh & Trejo-Hernández, 2020). Monocultures often exhibit limited enzymatic diversity, narrow substrate specificity, and poor adaptability under fluctuating environmental conditions, leading to incomplete mineralization of complex hydrocarbon mixtures (Eze & Chikere, 2023).

Pseudomonas aeruginosa is widely recognized for its robust biosurfactant production and hydrocarbon-degrading enzymes, enabling it to solubilize and oxidize diverse petroleum fractions. In contrast, *Yarrowialipolytica* possesses strong emulsifying capacity and oxidizes long-chain alkanes through cytochrome P450 and peroxisomal β -oxidation pathways. While each organism exhibits notable hydrocarbon-degrading potential, their individual limitations such as incomplete mineralization and reduced efficiency under stress conditions restrict overall bioremediation performance. Recent studies suggest that combining bacterial and fungal strains into coculture systems can expand metabolic diversity, improve substrate accessibility, and accelerate degradation kinetics (Adekunle *et al.*, 2021; Obuekwe *et al.*, 2020). However, despite growing global interest in bacterial–fungal cocultures for petroleum remediation (Bezza & Chirwa, 2020), there remains limited empirical data on the interaction between *P. aeruginosa* and *Y. lipolytica* under Nigerian environmental conditions (Nwinyi *et al.*, 2022). Addressing this gap, the present study investigates the synergistic performance of a bacterial–yeast coculture isolated from engine oil–contaminated Nigerian soil, with the aim of providing insights into its potential as a sustainable remediation strategy.

In Nigeria, remediation strategies continue to focus primarily on physicochemical methods or the application of indigenous monocultures, which are often inadequate for fully mineralizing recalcitrant hydrocarbons (Onwubuya & Okpokwasili, 2022). Moreover, the lack of localized studies assessing microbial consortia, biosurfactant activity, enzyme expression, and transformation dynamics using advanced analytical techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR) impedes the development of targeted, scalable bioremediation frameworks (Umeh, Adewumi, & Anya, 2022).

This study addresses this critical gap by investigating the coculture synergy between *P. aeruginosa* and *Y. lipolytica*, both isolated from Nigerian petroleum-contaminated soil. It explores their complementary metabolic activities biosurfactant-mediated hydrocarbon solubilization and oxidative degradation and evaluates their collective performance in degrading crude oil under controlled conditions. By integrating microbial kinetics with spectroscopic and chromatographic evidence, the study aims to provide a comprehensive understanding of functional synergy and biodegradation efficiency.

Ultimately, this research seeks to inform sustainable bioremediation strategies that are both locally adaptable and scientifically validated, contributing to environmental restoration efforts in oil-impacted regions across sub-Saharan Africa.

Materials and Methods

Microbial Strains and Coculture Preparation

Pseudomonas aeruginosa and *Yarrowialipolytica* were isolated from soil contaminated with spent engine oil from a mechanic workshop using selective enrichment. Ten grams of soil were added to 100 mL of sterile Bushnell-Haas medium (BHM) with 1% (v/v) crude oil and incubated at 30 °C, 150 rpm for 7 days. Enriched cultures were serially diluted and plated on nutrient agar (NA) and potato dextrose agar (PDA) for bacterial and yeast isolation. Plates were incubated at 30 °C for 24–72 hours. Distinct colonies were purified by repeated streaking and preserved on NA and PDA slants at 4 °C. Subcultures were freshly prepared for experiments, and isolates were identified morphologically, biochemically, and molecularly.

A single colony of *P. aeruginosa* was cultured in 100 mL Luria–Bertani (LB) broth at 37 °C for 12 hours. *Y. lipolytica* was cultured in yeast peptone dextrose (YPD) broth at 30 °C. Cultures were standardized to OD₆₀₀ = 0.5 ($\approx 1 \times 10^8$ CFU/mL for *P. aeruginosa*, $\approx 1 \times 10^7$ CFU/mL for *Y. lipolytica*) based on 0.5 McFarland standard.

For cocultures, 1% *Y. lipolytica* and 3% *P. aeruginosa* inocula were added to 250 mL Erlenmeyer flasks containing 100 mL BHM (per liter: 0.2 g MgSO₄, 0.02 g CaCl₂, 1.0 g KH₂PO₄, 1.0 g K₂HPO₄, 1.0 g NH₄NO₃, 0.05 g FeCl₃; pH 7.0 $\pm$ 0.2). The inoculum ratio (1:3, yeast to bacteria) was selected based on previous reports that higher bacterial density facilitates faster biosurfactant production and hydrocarbon solubilization, while lower yeast proportion prevents excessive biomass accumulation that may limit oxygen transfer. Treatments included bacterial monoculture, yeast monoculture, coculture, and abiotic control. Cultures were incubated at 30 °C, 160 rpm for 240 hours, with samples taken at 0, 48, 96, 144, 192, and 240 hours.

Viable Microbial Counts

Cell counts were determined via serial dilution and plating on NA and PDA. Plates were incubated at 30 °C for 24–48 hours, and results expressed in CFU/mL.

Crude Oil Biodegradation and Residual TPH Estimation

Degradation was assessed gravimetrically. Ten milliliters of culture were extracted with n-hexane, evaporated at 60 °C in pre-weighed dishes, and residual oil weighed. Percentage degradation was calculated per APHA (2017).

Emulsification Index (E24%)

Two milliliters each of culture supernatant and kerosene were vortexed for 2 minutes and left for 24 hours. Emulsified layer height was measured and E24% calculated.

Enzymatic and Biochemical Assays

Enzyme assays were conducted at 144 and 240 hours. Lipase activity was determined using *p*-nitrophenylpalmitate (pNPP, 1 mM final concentration) as substrate, incubated for 15 min at 37 °C and monitored at 410 nm. Catechol 2,3-dioxygenase

activity was assayed using catechol (0.5 mM) as substrate, with formation of 2-hydroxymuconic semialdehyde measured at 375 nm after 10 min incubation. Monooxygenase activity was assessed via the indole assay (2 mM indole, 30 min incubation, absorbance at 540 nm). Alkane hydroxylase activity was evaluated by monitoring NADH oxidation (0.2 mM NADH) at 340 nm, following 5 min incubation at 30 °C.). Total protein was measured by the Bradford assay. pH was recorded using a digital meter (HANNA HI2211).

Gas Chromatography–Mass Spectrometry (GC-MS)

Hexane-extracted samples were dried over anhydrous sodium sulfate and concentrated. GC–MS analysis was performed on an Agilent 7890B GC system coupled with a 5977A MSD detector, equipped with a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The oven program was set from 60 °C to 300 °C at a rate of 10 °C/min. Injector and detector temperatures were 250 °C and 280 °C, respectively. Samples were injected in splitless mode (1 µL injection volume), with helium as the carrier gas at a constant flow rate of 1.0 mL/min. Data were processed using the NIST Library.

Fourier Transform Infrared (FTIR) Spectroscopy

Dried extracts were pelletized with KBr and analyzed on a PerkinElmer Spectrum Two spectrometer (4000–400 cm⁻¹, 4 cm⁻¹ resolution) to detect hydroxyl, alkene, nitrile, and carbonyl groups.

Molecular Identification and Phylogenetic Analysis

Genomic DNA was extracted using the Zymo Fungal/Bacterial MiniPrep Kit following the manufacturer's protocol. The bacterial 16S rRNA gene was amplified using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), while the yeast 18S rRNA gene was amplified using primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS8 (5'-TCCGCAGGTTTCACC TACGGA-3'). PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. Amplicons were sequenced, and sequences were analyzed using BLAST (NCBI). Phylogenetic trees were constructed in MEGA X using the Neighbor-Joining method with 1000 bootstrap replicates.

Statistical and Multivariate Analysis

Triplicate results were analyzed via one-way ANOVA with Tukey's test ($p < 0.05$) using GraphPad Prism 9.5. Boxplots, radar charts, and PCA were used to visualize relationships among TPH degradation, enzymatic activity, and biosurfactant production. R Studio (v4.3.1) with FactoMineR and ggplot2 was used for PCA after data normalization. FTIR and GC-MS overlays were employed for comparative interpretation.

Results

Biodegradation Performance Over Time

Figure 1 shows the temporal trends of TPH degradation, OD600, emulsification index, and residual oil concentration in *P. aeruginosa*, *Y. lipolytica*, and coculture systems over 240 hours. The coculture achieved complete TPH degradation by 240 hours and outperformed monocultures in biomass accumulation and biosurfactant production. Residual oil levels declined more rapidly in the coculture, indicating enhanced hydrocarbon breakdown. Among the monocultures, *Y. lipolytica* showed moderate performance, while *P. aeruginosa* was least efficient. These findings highlight the synergistic advantage of the coculture in enhancing biodegradation kinetics.

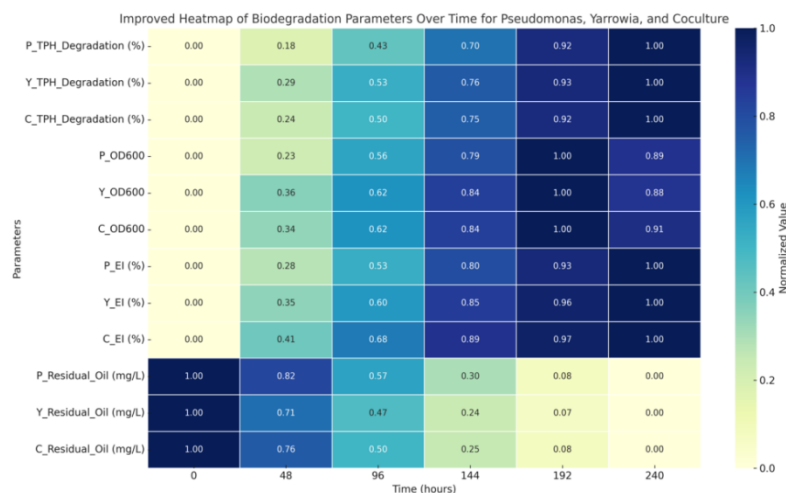


Figure 1: Biodegradation Parameters Over Time for *Pseudomonas*, *Yarrowia*, and Coculture Systems

Viable Cell Count Trends

Table 1 presents microbial population dynamics. Initial CFUs were 1.2×10^5 (*P. aeruginosa*), 9.5×10^4 (*Y. lipolytica*), and 2.15×10^5 (coculture). Growth accelerated after 48 hours, peaking at 2.7×10^6 CFU/mL in the coculture by 192 hours. Although a slight decline was observed at 240 hours, coculture viability remained highest. Statistical analysis (one-way ANOVA with Tukey's post-hoc test) confirmed that coculture CFU counts were significantly higher ($p < 0.05$) compared with monocultures at 144–240 hours. Moreover, CFU trends closely paralleled the extent of TPH degradation, suggesting that the enhanced biomass in the coculture contributed directly to improved hydrocarbon breakdown and sustained metabolic activity.

Table 1: Viable Microbial Counts in Monoculture and Coculture During Biodegradation of Hydrocarbons by *P. aeruginosa* and *Y. lipolytica*

Time (hrs)	<i>P. aeruginosa</i> (CFU/mL)	<i>Y. lipolytica</i> (CFU/mL)	Total (Co-culture)
0	1.2×10^5	9.5×10^4	2.15×10^5
48	4.6×10^5	3.1×10^5	7.7×10^5
96	9.0×10^5	6.4×10^5	1.54×10^6
144	1.5×10^6	1.2×10^6	2.7×10^6
192	1.6×10^6	1.1×10^6	2.7×10^6
240	1.4×10^6	9.0×10^5	2.3×10^6

Principal Component Analysis (PCA)

Figure 2 illustrates the PCA biplot of biodegradation parameters. Coculture treatments (96–240 hours) clustered in regions associated with high enzymatic activity, OD600, biosurfactant production, and TPH degradation. In contrast, monocultures correlated with higher residual TPH and lower functional parameter values. Biosurfactant and enzyme vectors aligned closely, confirming their co-involvement in degradation. PCA highlights the metabolic synergy and superior efficiency of the coculture.

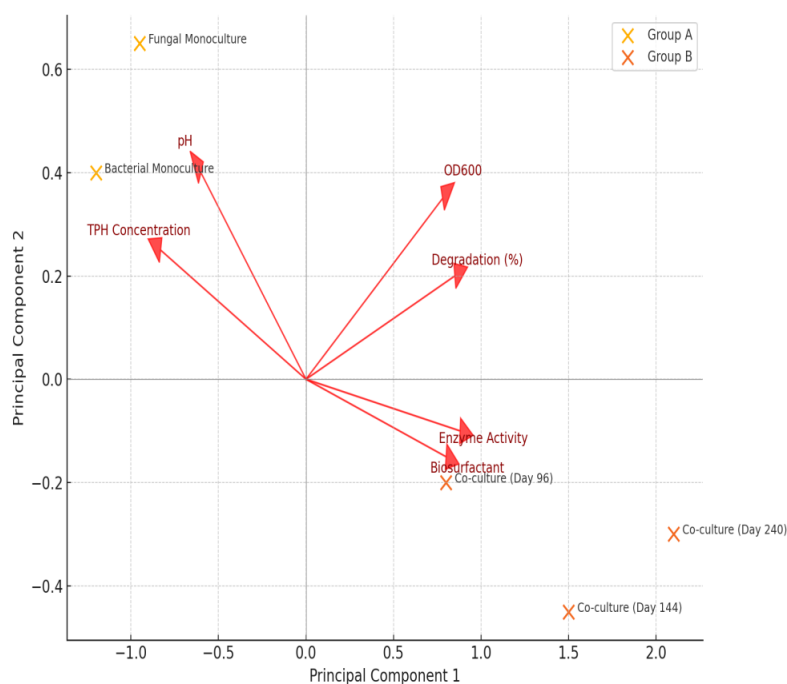


Fig. 2: PCA Biplot Showing the Relationship Between Biodegradation Treatments and Key Parameters.

Biochemical Shifts via Radar Chart

Figure 3 compares biochemical markers at 144 and 240 hours. E24% rose from 72.4% to 81.3%, pH dropped slightly (6.2 to 5.9), and total protein decreased (0.72 to 0.60 mg/mL). Minor declines in enzyme activities suggest metabolic peak occurred before 240 hours, likely due to substrate depletion and subsequent stabilization. Although only two time points were assayed, the trends align with observed CFU dynamics and TPH degradation, supporting this interpretation.

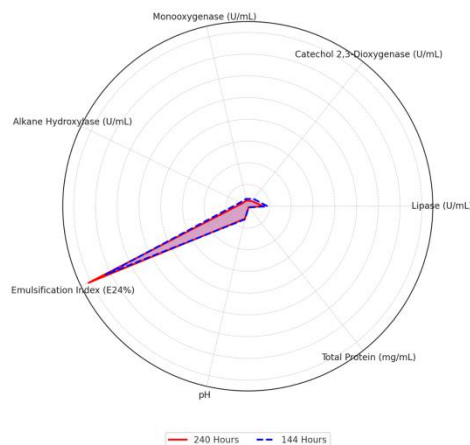


Fig. 3: Radar Chart of Biochemical Parameters at 144 and 240 Hours with Distinct Colors.

Foot note: Increased emulsification, slight pH drop, minor enzyme decline, and reduced protein suggest peak biodegradation occurred before 240 hours.

TPH Degradation Comparison

Figure 4 shows TPH degradation across treatments. The coculture achieved the highest and most consistent performance (87–91%), followed by *P. aeruginosa* (70–74%) and *Y. lipolytica* (63–66%). The abiotic control showed minimal degradation (2–5%), confirming that hydrocarbon removal was microbially driven. One-way ANOVA indicated significant differences among treatments at 240 hours ($p < 0.01$), with post-hoc tests confirming that coculture degradation was significantly higher than monocultures (*P. aeruginosa*, $p = 0.004$; *Y. lipolytica*, $p = 0.002$). Coculture also displayed the least variability, indicating stable performance. Moreover, degradation progression across time points showed a steady increase in TPH removal, with coculture reaching ~45% by 96 hours and ~76% by 144 hours, surpassing monocultures at each interval.

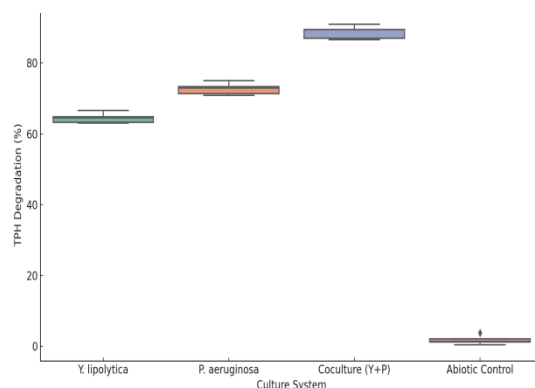


Fig. 4: Boxplot of TPH Degradation (%) Across Culture Systems.

GC-MS Analysis of Mono Culture

Figure 5 and Table 4.5 display TIC data for *P. aeruginosa*. Early retention peaks (7–9 min) indicated degradation of light alkanes. Most activity occurred between 13–15.8 min, targeting mid-chain hydrocarbons. Late-phase peaks (16.7–19.9 min) revealed persistent long-chain alkanes like nonadecane, indicating incomplete degradation.

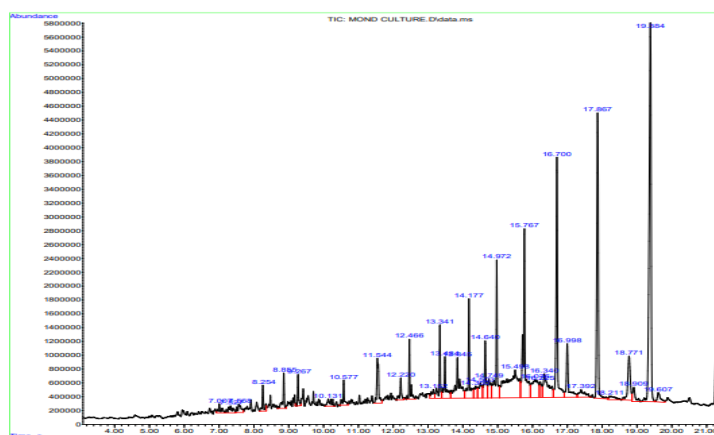


Fig. 5: GC-MS Total Ion Chromatogram (TIC) of Crude Oil Biodegradation by Mono Culture (*P.aeruginosa*)

GC-MS Profile of Coculture Biodegradation

Figure 6 shows the TIC for the coculture system. Hydrocarbons were broadly degraded across 4–21.2 min. Mid-phase peaks (11–14 min) and sharp peaks (14–16.7 min) reflect effective breakdown of both mid- and long-chain hydrocarbons. Late peaks were reduced compared to monocultures, indicating improved heavy hydrocarbon degradation.

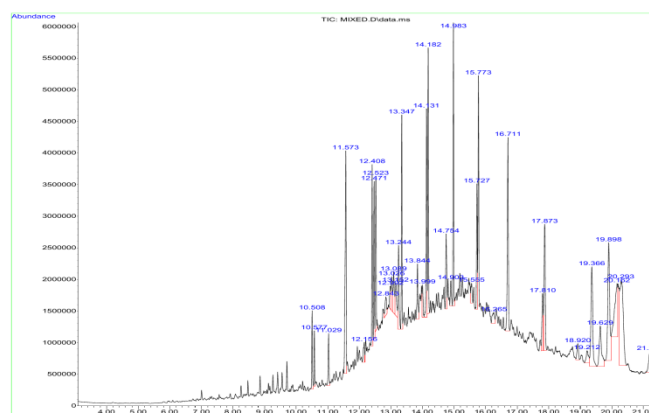


Fig. 6: The TIC for the coculture system.

Identified Compounds in Coculture

Table 6 lists the key compounds detected, including hexadecane, nonadecane, 1-nonadecene, and branched alkanes. The presence of both saturated and unsaturated hydrocarbons, together with oxygenated derivatives, indicates complex and cooperative degradation pathways involving oxidation and biosurfactant mediation.

Table 2: Hydrocarbon Compounds Identified from GC-MS Analysis of Coculture Biodegradation Profile

Compound Name	Retention Time (min)	CAS Number	Class/Type
Hexadecane	10.6–13.3	544-76-3	Saturated alkane
Nonadecane	12.5	629-92-5	Long-chain alkane
1-Nonadecene	12.4	18435-45-5	Unsaturated alkene
Heptadecane, 2,6,10,15-tetramethyl-	12.5	54833-48-6	Branched alkane
Pentadecane, 2,6,10-trimethyl-	11.0	3892-00-0	Branched alkane
Tetradecane	12.5–13.3	629-59-4	Medium-chain alkane

Monoculture vs Coculture Comparison

The coculture exhibited broader metabolite diversity, degraded both light and heavy hydrocarbons (10–19.9 min), and produced more biosurfactant-related compounds (e.g., phthalates). In contrast, monoculture activity was limited to 7–15 min, targeting fewer compounds with weaker biosurfactant signals. Table: Comparative Biodegradation Analysis confirms the coculture’s higher efficiency, metabolite range, and emulsification.

Table 3:Comparative Biodegradation Analysis: Monoculture vs Coculture

Criteria	Monoculture	Coculture (Mixed Culture)	Interpretation
Number of major peaks	Moderate (mainly early retention times)	High (broad peaks between 10–20 min)	Coculture shows richer degradation profile
Retention Time Range	~7.0 – 15.0 minutes	~10.0 – 19.9 minutes	Coculture degrades both light and heavy fractions
Hydrocarbon classes identified	Mainly linear and branched alkanes (decane, citronellol)	Branched alkanes, alkenes, alcohols, esters, phthalates	Coculture handles a broader metabolic range
Metabolite diversity	Low to moderate	High: alcohols, esters, unsaturated intermediates	Coculture produces more transformation products
Biotransformation indicators	Some alcohol and ester formation	Strong signals of oxidation (fatty acids, biosurfactants)	Coculture shows stronger metabolic conversion
Evidence of biosurfactant action	Minimal (few polar metabolites)	Present (e.g., phthalic acid esters, fatty alcohols)	Suggests higher emulsification capability in coculture
Degradation of heavy fractions	Limited	Confirmed (presence of hexadecane, nonadecane, etc.)	Coculture more effective in degrading heavier hydrocarbons

FTIR Spectral Differentiation

FTIR spectra (Figs. 7 & 8) show functional group changes. Monoculture spectra exhibited basic C–H, C=C, and O–H bands. Coculture spectra displayed intensified bands at 2109, 2202, and 3336 cm^{-1} , reflecting active hydroxyl, nitrile, and oxidation-related transformations, suggesting deeper biodegradation.

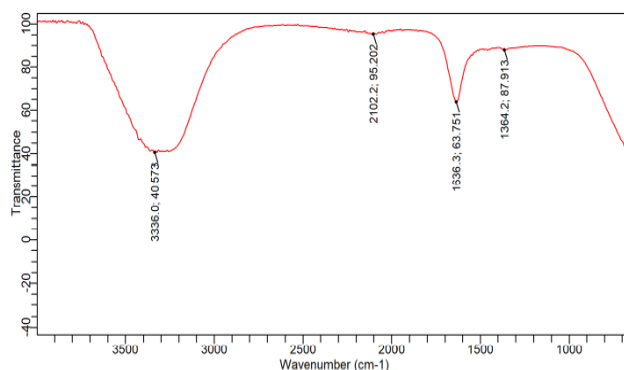


Figure X:FTIR Spectrum of Monoculture (*Pseudomonas aeruginosa*) During Crude Oil Biodegradation

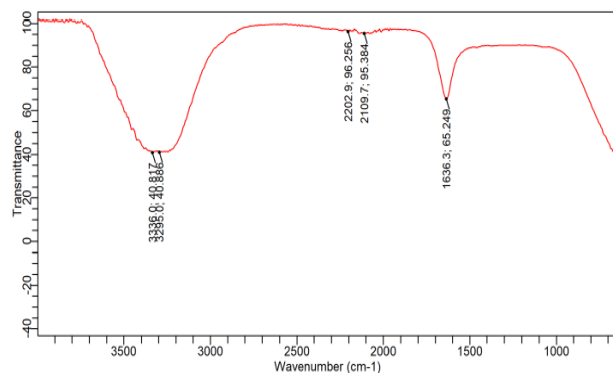


Figure X: Fig.FTIR Spectrum of Coculture (*Pseudomonas aeruginosa* and *Yarrowialipolytica*) During Crude Oil Biodegradation

Table 4: FTIR Spectral Characteristics of Monoculture and Coculture in Hydrocarbon Biodegradation

Monoculture FTIR Peaks

Peak	Wavenumber (cm ⁻¹)	Intensity	Interpretation
1	1364.21	87.91	C–H bending (methyl/methylene groups) — alkanes
2	1636.30	63.75	C=C or N–H bending — aromatics/proteins

3	2102.22	95.20	C≡C or C≡N stretches — alkynes or nitriles
4	3335.97	40.57	O–H or N–H stretch — alcohols or amines

Coculture

1	1636.30	65.25	Same C=C/N–H as monoculture
2	2109.67	95.38	Slight shift; alkynes/nitriles — enhanced activity
3	2202.86	96.26	Strong nitrile or isocyanate bond — increased transformation
4	3294.97	40.89	N–H/O–H stretch — protein or biosurfactant presence
5	3335.97	40.82	Same as monoculture — persistent hydroxyl functional groups

Integration of GC-MS and FTIR Findings

The combined analysis (Table: Synergy Between GC-MS and FTIR Results) confirms robust oxidation, broad degradation range, and enhanced biosurfactant production in the coculture. GC-MS detected esters and alcohols, while FTIR revealed strong O-H and nitrile peaks together affirming high metabolic versatility. While GC-MS provides high-resolution separation and identification of individual metabolites, it is limited to volatile and thermally stable compounds. FTIR, by contrast, cannot resolve individual metabolites but offers rapid functional group detection across complex mixtures. By integrating both techniques, we overcome these limitations: GC-MS identifies discrete compounds, whereas FTIR validates functional transformations and highlights chemical groups that may not be GC-MS detectable. This complementary approach strengthens the evidence for cooperative hydrocarbon degradation pathways in the coculture.

Table 5: Synergy Between GC-MS and FTIR Results

Observation	GC-MS Evidence	FTIR Evidence	Interpretation
Metabolite diversity	Esters, alcohols, phthalates	Hydroxyl and carbonyl peaks	Active oxidation & surfactant metabolism
Broader hydrocarbon degradation	Long-chain alkanes, unsaturated compounds	Stronger alkyne/nitrile peaks	Enhanced enzymatic breakdown in coculture
Surfactant activity	Presence of phthalate esters	Broad O–H/N–H absorption (3300 cm ⁻¹)	Supports increased solubility of hydrocarbons

Proposed Biodegradation Pathway

The pathway diagram (Fig. X) outlines sequential hydrocarbon degradation: *P. aeruginosa* initiates oxidation through enzymes such as alkane hydroxylase and catechol 2,3-dioxygenase, while *Y. lipolytica* enhances emulsification and contributes additional activities via lipase and monooxygenase. Annotated intermediates detected in this study include fatty alcohols, esters, and oxygenated alkanes, confirming stepwise hydrocarbon breakdown. Their synergy enables deeper and faster degradation, especially for complex fractions. Notably, potential bottlenecks were observed in the transition from long-chain alkanes to shorter-chain alcohols and acids, suggesting that enzyme turnover rates (e.g., alkane hydroxylase activity) may represent rate-limiting steps. Targeting these steps through strain engineering or enzyme supplementation could further enhance coculture efficiency in future applications.

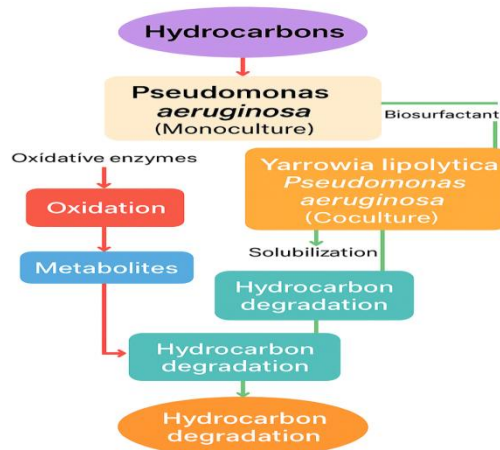


Figure X: Biodegradation Pathway of Hydrocarbons in Monoculture and Coculture Systems.

Discussion

This study evaluated the biodegradation potential of *Pseudomonas aeruginosa*, *Yarrowialipolytica*, and their coculture system in the degradation of crude oil, using multiple analytical techniques including microbial counts, GC-MS, FTIR, enzymatic assays, and PCA. The results confirmed the superiority of the bacterial-fungal coculture in promoting effective crude oil degradation compared to monocultures. This chapter discusses these findings in relation to previous studies, theoretical expectations, and biochemical mechanisms underlying microbial hydrocarbon degradation.

The consistent increase in Total Petroleum Hydrocarbon (TPH) degradation observed across time highlights the progressive metabolic adaptation of the microbial systems. Notably, the coculture system achieved complete TPH degradation (100%) by 240 hours, while monocultures achieved only 70–74% (*P. aeruginosa*) and 63–66% (*Y.*

lipolytica) degradation. These results support prior findings that microbial consortia exhibit enhanced degradation efficiencies through complementary metabolic functions (Bezza&Chirwa, 2020).

The improved biodegradation performance of the coculture can be attributed to the dual action of oxidative enzymatic degradation by *Y. lipolytica* and biosurfactant-mediated emulsification by *P. aeruginosa*. Biosurfactants play a critical role in increasing the bioavailability of hydrophobic hydrocarbon molecules, thereby enhancing microbial access and degradation rates (Okoh& Trejo-Hernández, 2020). This synergy was evident in the elevated Emulsification Index (E24%), which rose from 72.4% at 144 hours to 81.3% at 240 hours.

Microbial growth patterns from Table 4.1 show a clear increase in viable cell counts for all systems, with the coculture system peaking at 2.7×10^6 CFU/mL at 192 hours, significantly higher than the monoculture values. This indicates that the coculture not only promoted enhanced biodegradation but also sustained microbial proliferation over time. The synergistic growth pattern supports the idea that co-metabolism provides more stable energy yields and shared metabolic intermediates, which encourage mutualistic growth (Umehet *al.*, 2022).

The PCA biplot provides a statistical visualization of the relationship between biodegradation parameters and microbial treatments. The positioning of the coculture treatments along the positive PC1 axis, associated with enzyme activity, OD₆₀₀, biosurfactant production, and degradation percentage, further emphasizes their superior performance. In contrast, the monocultures were associated with higher residual TPH and low functional parameter values, reflecting limited degradation capacity.

This finding supports earlier observations by Bezza and Chirwa (2020), who reported that microbial consortia showed strong positive associations between biosurfactant activity, pH variation, and degradation metrics, which are essential in breaking down recalcitrant hydrocarbons. In our study, coculture performance was also linked with slight pH shifts, suggesting environmental parameters play a role in system stability. Since pH and temperature are known to influence enzyme activity, microbial growth, and biosurfactant efficiency, their fluctuations could directly impact degradation efficacy. Maintaining near-neutral pH and moderate temperatures (around 30 °C) appears favorable for coculture stability, but further testing across wider ranges would clarify tolerance thresholds and potential environmental constraints for field application. Biochemical parameters presented in the radar chart show a clear metabolic shift from 144 to 240 hours. Although enzyme activities such as Lipase and Monooxygenase slightly declined after peaking, this reduction was not indicative of loss of function but rather a consequence of substrate depletion. This pattern is in line with microbial enzyme regulation mechanisms, where synthesis declines after the exhaustion of target substrates (Das & Chandran, 2019). Moreover, the decline in total protein concentration may indicate enzymatic turnover or microbial protein catabolism in response to environmental shifts.

The GC-MS results for the coculture system revealed the degradation of a wider range of hydrocarbon classes, including hexadecane, nonadecane, 1-nonadecene, and phthalate esters, with retention times spanning ~10.0 to 19.9 minutes. These results suggest efficient transformation of both saturated and unsaturated hydrocarbons. In contrast, the monoculture GC-MS profile exhibited more limited transformation with most peaks clustered between 7.0–15.8 minutes, indicating partial degradation of lighter fractions.

FTIR spectra further confirmed this difference. The coculture displayed stronger and broader peaks at 2202.86 cm^{-1} (nitrile/isocyanate) and $3294.97\text{--}3335.97\text{ cm}^{-1}$ (hydroxyl/amino) than the monoculture (Table: FTIR Spectral Characteristics). These peaks reflect enhanced oxidative and biosurfactant-related biochemical activity, confirming that coculture biodegradation is more complete and chemically diverse.

The synergy between GC-MS and FTIR findings underscores the multidimensional efficiency of the coculture system. While GC-MS quantified the presence and transformation of complex hydrocarbons, FTIR characterized the functional group evolution, providing a holistic view of microbial degradation pathways. The presence of esters, alcohols, and phthalates in GC-MS and the hydroxyl and nitrile peaks in FTIR jointly indicate oxidative transformation and emulsification processes at work, as supported by earlier studies (Alabiet *et al.*, 2023; Zhang *et al.*, 2021).

The proposed hydrocarbon degradation pathway demonstrates how the dual mechanisms of *P. aeruginosa* (biosurfactant production) and *Y. lipolytica* (oxidative metabolism) intersect to promote substrate solubilization, oxidation, and eventual mineralization. *P. aeruginosa* biosurfactants reduce hydrocarbon surface tension, dispersing hydrophobic molecules and making them more bioavailable. This increased solubilization accelerates uptake by *Y. lipolytica*, which further oxidizes long-chain alkanes into fatty alcohols and acids, thereby complementing bacterial enzymatic breakdown. The synergy emerges because biosurfactant-mediated dispersion enhances substrate accessibility, while yeast-driven oxidation expands the metabolic range, ensuring more complete degradation of both aliphatic and aromatic fractions. Nonetheless, a limitation of this study is that assays were conducted under controlled laboratory conditions with constant pH, temperature, and nutrient supply factors that differ significantly from fluctuating field environments. Such variations may affect biosurfactant stability, enzyme expression, and overall coculture performance. Future work should therefore validate these interactions under dynamic field-relevant conditions to confirm scalability and robustness.

Conclusion and Recommendation

This study demonstrated the enhanced biodegradation potential of a bacterial–fungal coculture involving *Pseudomonas aeruginosa* and *Yarrowia lipolytica* isolated from engine oil–contaminated soil. The coculture system exhibited superior crude oil degradation efficiency, achieving complete TPH removal within 240 hours, alongside enhanced microbial biomass, biosurfactant production, and enzymatic activity. GC-MS

and FTIR analyses confirmed broader hydrocarbon degradation and more intensive biochemical transformations compared to monocultures. Principal Component Analysis further revealed strong correlations between biodegradation performance and functional biomarkers, underscoring the synergistic interaction between the two organisms. These findings highlight the coculture's potential as a sustainable and effective bioremediation strategy for petroleum-contaminated environments. Importantly, the observed 240-hour degradation window provides a practical timeframe for considering cocultures in remediation programs, balancing both efficacy and operational feasibility. From an ecological perspective, such bioremediation minimizes secondary pollution risks associated with chemical or physical methods, while economically, microbial consortia offer a low-cost and scalable alternative for managing oil spills. Future research should explore field-scale applications, optimization of environmental parameters (e.g., pH, temperature, and nutrient availability), and integration with existing remediation technologies to maximize efficiency and system stability.

Acknowledgement

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Morphoanatomical characterisation and fibre properties of *Luffa cylindrica* and *Luffa acutangula* in Kwara State, Nigeria

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Abstract

Luffa, a genus of tropical and subtropical climbing plants within the cucumber family (Cucurbitaceae), exhibits considerable morphological variation influenced by environmental conditions. Despite its economic potential, scientific information on the thermal and mechanical properties of *Luffa* fibres remains scarce. This study evaluated the leaf morpho-anatomical features and fibre quality of *Luffa cylindrica* and *Luffa acutangula*, based on mature fruits collected from eight Local Government Areas of Kwara State, Nigeria. The seeds were cultivated under a randomized complete block design. Morphological assessment revealed greater variability in *L. cylindrica*, particularly in stem girth, leaf dimensions, and other qualitative traits. Anatomical examination showed that both species are amphistomatic; however, *L. cylindrica* possessed trichomes on both leaf surfaces, unlike *L. acutangula*. Mechanical analysis indicated that *L. cylindrica* exhibited higher tensile strength, modulus, flexural strength, and hardness, whereas *L. acutangula* demonstrated greater impact energy and elongation. These results highlight *L. cylindrica* as a superior species for industrial fibre applications and underscore the need to encourage its cultivation and commercial exploitation.

Keywords: *Luffa cylindrica*, *Luffa acutangula*, fibre quality, morphology, anatomy, mechanical properties.

Introduction

The genus *Luffa*, belonging to the family *Cucurbitaceae*, comprises economically important climbing vines widely cultivated in tropical and subtropical regions for food, fibre, and diverse industrial applications (Cheng et al., 2020). The fruits, upon maturation and drying, yield a fibrous, sponge-like network that serves as a natural source of luffa fibres (Barirega & Van Damme, 2014). These fibres have been reported to serve multiple

purposes, including as materials for engine filters, steel helmet linings, insulation, packaging, and industrial filtration (Daniel & Yadav, 2016).

In Nigeria, *Luffa cylindrica* (smooth loofah) and *Luffa acutangula* (ridged loofah) are the most commonly cultivated species. Both exhibit remarkable adaptability and versatility, particularly in their use as household sponges and reinforcement materials in bio-composite production (Wambua *et al.*, 2003). However, taxonomic delimitation between *L. cylindrica* and *L. acutangula* remains problematic when based solely on vegetative morphology due to their close resemblance and the potential for interspecific hybridization (Ahmed, 2018). Morphological variation in *Luffa* is influenced by a complex interplay of genetic and environmental factors (Widen & Sheinmann, 2006), making a comprehensive understanding of such variation essential for germplasm characterization and the identification of superior genotypes suited to specific applications. Anatomical features of leaves and stems can further provide valuable taxonomic and diagnostic information.

With the increasing global shift toward sustainable, renewable, and biodegradable resources, Nigeria is exploring bio-based materials as alternatives to synthetic counterparts. *Luffa* fibres, which are natural, abundant, and cost-effective, have attracted attention as potential substitutes for synthetic fibres in various industrial applications (Mohanty *et al.*, 2000). Nonetheless, a detailed understanding of the morpho-anatomical and mechanical properties of *Luffa* species is required to optimize their use in bio-composites, textiles, and agro-industrial systems.

Morphological traits, epidermal features, and anatomical markers play a central role in plant taxonomy (Zaman *et al.*, 2025) and have been applied successfully in resolving taxonomic ambiguities (Mbagwu & Edeoga, 2006). The fibrous vascular endocarp of *Luffa* has drawn significant attention due to its biodegradability, high cellulose content, and suitability for use in industrial composites (Tanobe *et al.*, 2005). However, environmental variation and genetic differences can substantially influence the mechanical properties of the fibres (Siqueira *et al.*, 2010). Comparative studies on the morpho-anatomical and mechanical characteristics of *Luffa* species, particularly those cultivated across different ecological regions such as Nigeria, remain limited.

This study, therefore, aims to bridge this knowledge gap by conducting a comprehensive investigation into the morpho-anatomical characteristics and fibre quality of *Luffa cylindrica* and *Luffa acutangula* accessions collected and cultivated across various Local Government Areas of Kwara State, Nigeria.

Materials and Methods

Study Area Description

The study was carried out in Kwara State, North-Central Nigeria, located between latitudes 8°30'N and 8°59'N and longitudes 4°20'E and 4°59'E. The region lies within the transitional zone between the southern forest and the northern Guinea savanna vegetation belts. The soils are predominantly loamy and lateritic, with annual rainfall ranging from 990.3 mm to 1,318 mm, occurring mainly between April and November (Adeleke, 2024).

Plant Material Collection and Identification

Mature, dried fruits of twenty accessions of *Luffa acutangula* and *Luffa cylindrica* were collected from eight Local Government Areas (LGAs) across Kwara State. The LGAs were randomly selected using a table of random numbers to ensure representative sampling across the three Senatorial Districts (Table 1). The geographic coordinates of each collection site were recorded using the integrated GPS feature of a Samsung Galaxy A52 device (Snapdragon 720G chipset supporting GPS, GLONASS, Galileo, and BDS). Collected specimens were identified and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, where voucher specimens were also deposited for reference.

Table 1: *Luffa* species accession code numbers, place of collection, and coordinates

Accession code No	Location	Coordinates
01 OGD	Ogidi, IL-West	8.21' N, 4.26' E
02 UIL	Unilorin Garden, IL-South	9.54' N, 4.45' E
03 AGB	Agbooba Area, IL-West	8.43' N, 4.50' E
04 ADW	Adewole Estate, IL-West	8.51' N, 4.42' E
05 SHK	Sheik Abdulkadri College, IL-West	8.29' N, 4.46' E
06 APL	Apalara Area, IL-West	8.25' N, 4.39' E
07 OKF	Okefoma Area, IL-West	8.40' N, 4.42' E
08 ARP	Airport Road, IL-West	8.49' N, 4.59' E
09 ALG	Alagbado Hospital, IL-South	9.40' N, 5'00' E
10 OKL	Okelele Area, IL-East	10.28' N, 5.07' E
11 AGS	Agric Sango, IL-South	9.43' N, 4.20' E
12 KSU	Kwasu Malete, Moro	9.64' N, 4.99' E
13 OLJ	Oloje Estate, IL-West	8.39' N, 4.19' E
14 OKY	Okeoyi, IL-East	10.26' N, 5.04' E
15 OFG	Offa Garage, IL-South	9.86' N, 4.95' E
16 OKS	Oke Ose, IL-East	10.85' N, 4.69' E
17 OMP	Omupo, Ifelodun	9.04' N, 4.98' E
18 OFF	Offa COHT, Offa	9.92' N, 4.95' E
19 EIY	Eiyenkorin, Asa	9.45' N, 4.47' E
20 AJS	Ajasepo ipo, Irepodun	10.04' N, 4.80' E

Note: - 14 OKY and 15 OFG are *Luffa acutangula* while other accessions are *Luffa cylindrica*.

Experimental Design and Agronomic Practices

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replicates per accession. It was conducted at the Botanical Garden of the Department of Plant Biology, University of Ilorin, Nigeria. Mature fruits of each *Luffa* species were freshly harvested and air-dried (Plate 1), after which the seeds were extracted and treated by soaking in a 1% fungicide solution for 30 minutes to prevent soil-borne infections.

The treated seeds were sown on ridges at an inter-row spacing of 3 m and intra-row spacing of 1 m, following the method of Kumar et al. (2013), with four seeds dibbled per hole. Standard agronomic practices were maintained throughout the growing period. Manual irrigation was carried out twice daily during the dry season (November to February) to maintain adequate soil moisture using borehole water. Two weeks after germination, a basal application of NPK 20:10:10 fertilizer was made at a rate of 3 g per plant stand (equivalent to 8.2 kg/ha). Weed control was achieved through manual weeding at four-week intervals, following the procedures described by Bal et al. (2004).



A



B



C



D

Plate 1: Photographs of the fruit with the seeds of *Luffa cylindrical* (A and C) and *Luffa acutangula* (B and D)

Morphological Data Collection

Morphological traits, including seedling leaf dimensions (measured at two weeks after planting, WAP) and mature leaf dimensions and stem girth (at twelve WAP), were recorded. To ensure data uniformity, measurements followed a standardized protocol: stem girth was measured 5 cm above the soil surface using a digital caliper, while leaf length and width were taken from the fourth fully expanded leaf using a metric ruler. All measurements were carried out between 08:00 and 10:00 hours to minimize diurnal variation.

To avoid measurement bias, a blinded and randomized approach was adopted. Individual plants were identified using randomized codes, and their measurement sequence was varied daily (Radford et al., 1974). Qualitative morphological traits—such as leaf colour, flower colour, fruit shape, and seed characteristics—were visually assessed and recorded using descriptive terms based on established taxonomic descriptors (Zalapa et al., 2006).

Foliar Anatomical Studies

Fresh, mature leaves from each accession were collected at 90 days after planting and fixed in nitric acid for 24 hours to facilitate maceration. Epidermal peels were obtained using fine forceps, stained with safranin following the procedure of Hilu and Randall (1984), and mounted on clean glass slides. Microscopic observations were made under an Olympus light microscope at 40× magnification, and photomicrographs were captured using an Amscope digital camera (Model MU1000B, Amscope, USA).

Quantitative anatomical parameters—including stomatal length, stomatal width, stomatal density, stomatal size, and epidermal cell dimensions—were measured using calibrated eyepiece graticules, following established methods (Baker & Silverton, 1982; Gill & Nyawuame, 1990).

Micrometry Calibration and Measurement Standardization

Calibration was performed using an eyepiece micrometer in conjunction with a stage micrometer to establish a field of view of 0.152 mm² at 40× magnification. This reference area served as the basis for calculating stomatal and epidermal cell densities. Stomatal density (SD) was determined as the number of stomata per square millimetre, based on counts within the 0.152 mm² field of view (Stace, 1966). Epidermal cell shape was described according to the classification system of Dilcher (1974). The stomatal index (SI) was calculated as:

$$SI = \frac{S}{S + E} \times 100$$

where *S* represents the number of stomata and *E* the number of ordinary epidermal cells per unit area (Wilkinson, 1979).

The mean stomatal size (SS) was determined by measuring the length and width of guard cells. The methods follow those of Franco (1939).

$$SS = L \times B \times K$$

where L = length, B = breadth, and K = Franco's constant (0.79)

Mechanical Properties of *Luffa* Fibre Composites

Fibre-reinforced composites were produced by blending *Luffa* fibres with polypropylene resin in a 1:1 ratio. The mixture was processed using a Two-Roll Mill Machine and subsequently compression-moulded at 170 °C. Mechanical performance tests—including tensile, flexural, impact, and hardness assessments—were carried out in accordance with relevant ASTM standards (ASTM D790, D256, and D2240).

Tensile strength and percentage elongation were determined using a Monsanto Tensometer (Model T-10, Monsanto, U.K.), while flexural strength and modulus were evaluated with a Testometric Universal Testing Machine. Impact strength was measured using a Resil Impact Tester on notched specimens (10 × 4 cm) employing a 1 J hammer. Hardness was assessed using a Muer Durometer following the ASTM D2240 protocol.

Data Analysis

Mean values and standard errors were computed for all morphological, anatomical, and mechanical parameters. Statistical analyses were performed using SPSS version 25. One-way Analysis of Variance (ANOVA) was employed to determine significant differences among *Luffa* accessions at the 5% probability level ($p < 0.05$), following the procedures described by Rohlf (2009).

Results

Morphological Features of *Luffa* Accessions

Marked morphological variation was observed among the twenty accessions of *Luffa cylindrica* and *L. acutangula* collected from eight Local Government Areas across Kwara State. Quantitative traits assessed included stem girth, mature leaf length and width, as well as seedling leaf length and width (Table 2).

Seedling traits exhibited comparatively narrow variation. The highest seedling leaf length (4.10 cm) was recorded in accession 13OLJ, while the lowest (3.63 cm) occurred in 14OKY. Similarly, seedling leaf width ranged from 2.05 cm in accession 09ALG to 1.88 cm in 20AJS.

Stem girth showed considerable variability, ranging from 3.98 cm in accession 09ALG (Ilorin South) to 7.52 cm in 12KSU (Moro). Mature leaf traits demonstrated even greater diversity. The longest leaves (16.50 cm) were observed in accessions 01OGD (Ogidi) and 04ADW (Adewole), whereas accession 15OFG (Ilorin South) produced the shortest leaves (9.95 cm). In terms of leaf width, accession 01OGD exhibited the broadest leaves (13.03 cm), while 09ALG had the narrowest (6.40 cm).

Table 2: Quantitative morphological features of the 20 accessions of *Luffa* species at 2WAP and 12WAP

ACC NO	STGTH (CM)	LFLGTH (CM)	LFWDT (CM)	SLFLGTH (CM)	SLFWDT (CM)
01OGD	5.99 ± 0.32 ^{abcd}	16.50 ± 0.67 ^a	13.03 ± 0.39 ^a	3.97 ± 0.12 ^a	2.00 ± 0.06 ^a
02UIL	5.74 ± 1.22 ^{abcd}	11.10 ± 0.60 ^{fgh}	7.37 ± 0.56 ^{ef}	3.87 ± 0.18 ^a	1.92 ± 0.06 ^a
03AGB	4.72 ± 0.97 ^{cd}	14.97 ± 0.41 ^{abcd}	10.40 ± 0.49 ^{bc}	3.97 ± 0.27 ^a	1.92 ± 0.04 ^a
04ADW	4.38 ± 0.68 ^d	16.50 ± 0.51 ^a	10.97 ± 0.41 ^{ab}	4.00 ± 0.44 ^a	1.97 ± 0.07 ^a
05SHK	4.86 ± 0.48 ^{bcd}	14.87 ± 1.29 ^{abcd}	10.20 ± 0.98 ^{bc}	4.20 ± 0.21 ^a	1.93 ± 0.13 ^a
06APL	4.30 ± 0.65 ^d	15.73 ± 0.46 ^{abc}	10.00 ± 0.26 ^{abc}	3.83 ± 0.12 ^a	1.98 ± 0.07 ^a
07OKF	4.22 ± 0.30 ^d	14.70 ± 1.08 ^{abcd}	8.63 ± 1.79 ^{bcdet}	3.93 ± 0.22 ^a	1.83 ± 0.03 ^a
08ARP	4.26 ± 0.29 ^d	16.40 ± 0.79 ^{ab}	10.93 ± 0.89 ^{ab}	3.77 ± 0.12 ^a	2.02 ± 0.09 ^a
09ALG	3.98 ± 0.50 ^d	13.33 ± 1.22 ^{cdef}	6.40 ± 0.49 ^f	3.80 ± 0.36 ^a	2.05 ± 0.23 ^a
10OKL	5.52 ± 0.82 ^{abcd}	14.03 ± 0.98 ^{abcd}	9.30 ± 1.10 ^{bcd}	3.67 ± 0.28 ^a	1.97 ± 0.07 ^a
11SNG	5.39 ± 0.53 ^{abcd}	13.47 ± 0.62 ^{cdef}	8.20 ± 0.89 ^{cdef}	3.73 ± 0.19 ^a	1.97 ± 0.09 ^a
12KSU	7.52 ± 0.97 ^a	13.93 ± 1.41 ^{abcd}	9.30 ± 0.75 ^{bcd}	3.80 ± 0.06 ^a	1.93 ± 0.09 ^a
13OLJ	7.21 ± 1.37 ^{abc}	12.63 ± 1.33 ^{defg}	9.07 ± 0.45 ^{bcd}	4.10 ± 0.10 ^a	1.90 ± 0.06 ^a
14OKY	6.49 ± 0.47 ^{abcd}	10.60 ± 0.32 ^{gh}	9.33 ± 0.43 ^{bcd}	3.63 ± 0.09 ^a	2.00 ± 0.06 ^a
15OFG	4.20 ± 0.33 ^d	9.95 ± 0.09 ^h	7.33 ± 0.64 ^{ef}	3.80 ± 0.06 ^a	1.80 ± 0.06 ^a
16OKS	5.20 ± 1.45 ^{abcd}	11.20 ± 0.68 ^{efgh}	7.63 ± 0.48 ^{def}	3.67 ± 0.17 ^a	1.98 ± 0.12 ^a
17OMP	6.41 ± 0.34 ^{abcd}	13.13 ± 0.56 ^{cdef}	8.03 ± 0.74 ^{cdef}	3.87 ± 0.09 ^a	1.93 ± 0.09 ^a
18OFF	5.77 ± 0.98 ^{abcd}	14.07 ± 0.73 ^{abcd}	10.20 ± 0.61 ^{bc}	3.67 ± 0.20 ^a	1.92 ± 0.09 ^a
19EIY	6.34 ± 0.54 ^{abcd}	13.87 ± 0.52 ^{abcde}	8.10 ± 0.26 ^{cdef}	3.70 ± 0.29 ^a	2.03 ± 0.03 ^a
20AJS	7.34 ± 0.23 ^{ab}	13.67 ± 0.72 ^{bcdet}	9.67 ± 0.49 ^{bcd}	4.03 ± 0.13 ^a	1.88 ± 0.06 ^a

Morphological Variation among *Luffa* Accessions

Table 2 presents the mean values, standard errors, and Duncan's multiple range test rankings for the morphological traits of the twenty *Luffa* accessions. Different letters along each column indicate significant differences among accessions at $P < 0.05$. Abbreviations used include: Acc. Cod. No. = Accession Code Number; STGTH = Stem Girth; LFLGTH = Mature Leaf Length; LFWDTH = Mature Leaf Width; SLFLGTH = Seedling Leaf Length; and SLFWDTH = Seedling Leaf Width.

Qualitative morphological traits revealed distinct taxonomic differences between *L. cylindrica* and *L. acutangula* (Table 3). All accessions exhibited dark green leaves and bright yellow flowers, except accessions 14OKY and 15OFG (both *L. acutangula*), which were characterized by light green leaves and pale-yellow flowers.

Fruit morphology also differentiated the species: *L. cylindrica* produced elongated, cylindrical fruits, whereas *L. acutangula* bore elongated but spindle-shaped fruits. Seed colour varied from black to brown in both species, with greyish-black seeds occurring exclusively in *L. acutangula*. Seed shape further distinguished the taxa—*L. cylindrica* accessions had predominantly oblong seeds, while *L. acutangula* accessions possessed ovate seeds.

Table 3: Qualitative morphological features of the 20 accessions of *Luffa* species

Acc. No.	Leaf colour	Flower colour	Fruit shape	Seed colour	Seed shape
01OGD	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
02UIL	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
03AGB	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
04ADW	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
05SHK	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
06APL	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
07OKF	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
08ARP	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
09ALG	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
10OKL	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
11SNG	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
12KSU	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
13OLJ	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
14OKY	Light green	Light yellow	Elongated and spindle	Greyish Black	Ovate
15OFG	Light green	Light yellow	Elongated and spindle	Greyish Black	Ovate
16OKS	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
17OMP	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
180FF	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
19EIY	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong
20AJS	Dark green	Bright yellow	Elongated and cylindrical	Black and Brown	Oblong

Leaf Anatomical Features

The results of the microscopic examination of foliar epidermal structures are presented in Plates 2 and 3. All *Luffa* accessions examined were amphistomatic, bearing stomata on both the adaxial and abaxial epidermal surfaces, though in unequal distribution. The predominant stomatal type observed on both surfaces was **staurocytic**. Epidermal cell walls exhibited both polygonal and irregular shapes, polygonal forms being more common on the adaxial surface, while irregular outlines predominated on the abaxial side. Unicellular, non-glandular trichomes were present on both epidermal surfaces in *L. cylindrica*, whereas in *L. acutangula* they were restricted to the adaxial surface.

Quantitative anatomical parameters including stomatal length, width, density, index, size, and epidermal cell dimensions are summarized in Tables 4 and 5. On the adaxial surface, stomatal length ranged from 8.20 μm^2 in accession 14OKY to 11.20 μm^2 in 12KSU and 05SHK. Stomatal width was highest (9.40 μm^2) in accessions 13OLJ, 19EIY, and 20AJS, and lowest (5.00 μm^2) in 03AGB. The highest stomatal density (136.84 mm^{-2}) and epidermal cell density (547.37 mm^{-2}) were recorded in accession

05SHK. The stomatal index peaked in 11SNG (21.63%), while the largest stomatal size ($81.67 \mu\text{m}^2$) occurred in 13OLJ.

On the abaxial surface, epidermal cell length varied from $4.40 \mu\text{m}^2$ (10OKL) to $15.80 \mu\text{m}^2$ (05SHK), with the greatest epidermal cell width ($14.60 \mu\text{m}^2$) also recorded in 10OKL. The highest stomatal density on the abaxial surface occurred in accession 15OFG, while the lowest (22.37 mm^{-2}) was observed in 09ALG. The stomatal index ranged from 3.78% (09ALG) to 11.70% (05SHK). Notably, *L. acutangula* accessions (14OKY and 15OFG) consistently exhibited lower stomatal and epidermal dimensions compared with *L. cylindrica*, highlighting species-level anatomical differentiation.

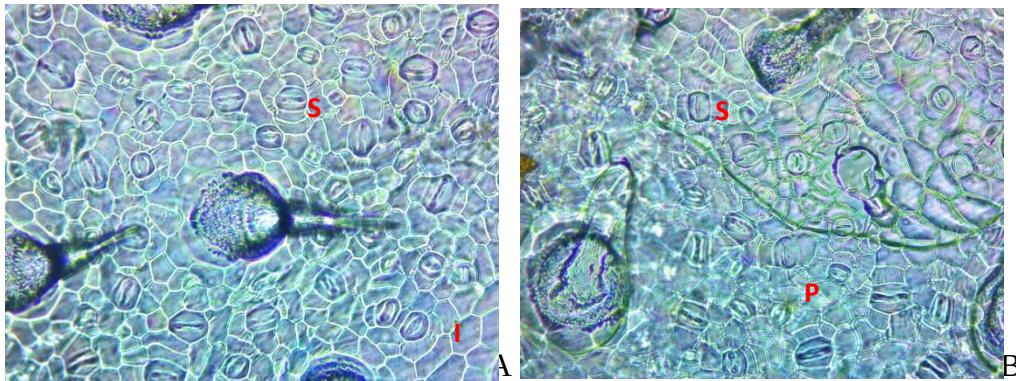


Plate 2: Photomicrographs of the *Luffa cylindrica* (A: Abaxial surface; B: Adaxial surface. S: Staurocytic stomata T: Trichome; P: Polygonal epidermal cell; I: Irregular epidermal cell)

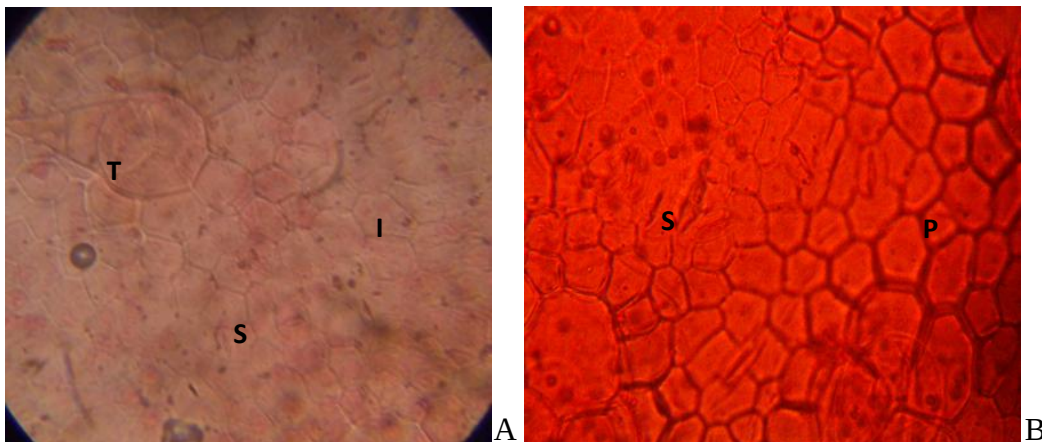


Plate 3: Photomicrographs of the *Luffa acutangula* (A: Abaxial surface; B: Adaxial surface. S: Staurocytic stomata; T: Trichome; P: Polygonal epidermal cell; I: Irregular epidermal cell)

Table 4: Leaf anatomical features of the 20 accessions of two *Luffa* species

Acc. Cod. No	Leaf Surface	STLT(mm ²)	STWTV(mm ²)	STD	ECLT(mm ²)	ECWT(mm ²)
01OGD	Upper	10.20±0.58 ^{abcdef}	9.00±0.58 ^a	98.68±6.58 ^{bcd}	17.60±0.75 ^{abcde}	11.60±1.21 ^{cd}
	Lower	9.40±0.40 ^{cdefgh}	5.00±0.40 ⁱ	44.74±5.26 ^{ghijkl}	10.80±0.49 ^{lmn}	11.80±0.58 ^{cd}
02UIL	Upper	10.40±0.51 ^{abcde}	6.20±0.51 ^{fghi}	86.84±3.22 ^{bcd}	18.20±1.16 ^{abc}	10.20±0.66 ^{cdeh}
	Lower	8.40±0.75 ^{gh}	5.60±0.75 ^{ghi}	22.37±2.63 ⁱ	12.20±1.20 ^{klmn}	8.20±0.49 ^{ghi}
03AGB	Upper	8.60±0.24 ^{gh}	5.00±0.24 ⁱ	69.74±2.63 ^{defghij}	19.60±0.51 ^{ab}	11.80±1.07 ^{cd}
	Lower	11.20±0.58 ^a	5.60±0.58 ^{ghi}	47.37±6.3 ^{fghij}	14.80±1.24 ^{defghijk}	11.20±0.58 ^{ghi}
04ADW	Upper	10.20±0.37 ^{abcdef}	7.00±0.37 ^{efgh}	102.63±7.67 ^{bcd}	14.40±0.75 ^{efghijk}	12.80±1.50 ^{bcd}
	Lower	8.20±0.20 ^{hi}	5.20±0.20 ⁱ	31.58±2.46 ⁱ	13.40±0.87 ^{ijklmn}	8.20±0.37 ^{bc}
05SHK	Upper	11.20±0.58 ^a	5.40±0.58 ^{hi}	136.84±5.26 ^a	20.40±0.51 ^a	11.00±0.45 ^{cdefg}
	Lower	8.40±0.24 ^{gh}	12.20±0.24 ^a	80.26±6.71 ^{def}	15.80±0.37 ^{cdefghij}	10.20±0.20 ^{cdefgh}
06APL	Upper	9.60±0.24 ^{bcddefgh}	6.60±0.24 ^{efghi}	86.84±3.22 ^{bcd}	18.20±1.16 ^{abc}	10.20±0.66 ^{cdefgh}
	Lower	8.20±0.49 ^{hi}	5.60±0.49 ^{ghi}	76.32±5.49 ^{defgh}	12.20±1.20 ^{klmn}	8.20±0.49 ^{ghi}
07OKF	Upper	10.80±0.37 ^{abc}	7.20±0.37 ^{cdefgh}	86.84±6.71 ^{bcd}	17.40±0.68 ^{abcdef}	12.80±1.66 ^{bc}
	Lower	8.60±0.40 ^{gh}	5.00±0.40 ⁱ	50.00±4.46 ^{efghijkl}	11.80±0.66 ^{klmn}	9.20±0.49 ^{defghi}
08ARP	Upper	10.60±0.40 ^{abcd}	7.80±0.40 ^{bcddef}	77.63±13.22 ^{defg}	18.00±1.67 ^{abcd}	11.20±1.24 ^{def}
	Lower	8.80±0.37 ^{fghi}	6.40±0.37 ^{efghi}	25.00±3.83 ⁱ	10.20±1.02 ⁿ	8.40±0.51 ^{fghi}
09ALG	Upper	10.20±0.20 ^{abcdef}	9.00±0.20 ^b	82.90±5.43 ^{cde}	18.00±1.05 ^{abcd}	11.80±0.86 ^{cd}
	Lower	9.20±0.20 ^{defgh}	7.00±0.20 ^{defgh}	22.37±1.61 ⁱ	10.60±1.12 ^{mn}	7.40±0.24 ⁱ
10OKL	Upper	10.20±0.20 ^{abcdef}	5.80±0.20 ^{ghi}	92.10±8.58 ^{abcd}	14.00±1.30 ^{ghijkl}	17.80±1.24 ^a
	Lower	8.60±0.40 ^{gh}	5.40±0.40 ^{hi}	28.95±1.61 ⁱ	4.40±0.24 ^o	14.60±1.60 ^b
11SNG	Upper	10.60±0.24 ^{abcd}	7.20±0.24 ^{cdefg}	119.74±6.38 ^{ab}	13.60±0.98 ^{hijklm}	8.80±0.49 ^{efghi}
	Lower	8.40±0.24 ^{gh}	6.20±0.24 ^{fghi}	30.26±3.35 ⁱ	14.20±1.74 ^{fghijk}	10.40±0.51 ^{cdefgh}
12KSU	Upper	11.20±0.37 ^a	8.00±0.37 ^{bcdde}	100.00±9.16 ^{bcd}	17.80±0.73 ^{abcd}	10.40±1.60 ^{cdefgh}
	Lower	8.60±0.40 ^{gh}	5.40±0.40 ^{hi}	25.00±3.83 ⁱ	10.60±0.40 ^{mn}	8.80±0.58 ^{efghi}
13OLJ	Upper	11.00±0.45 ^{ab}	9.40±0.45 ^b	92.11±9.30 ^{bcd}	16.80±1.11 ^{bcddefgh}	11.20±0.37 ^{cdef}
	Lower	8.80±0.20 ^{fghi}	5.40±0.20 ^{hi}	42.10±5.34 ^{hijkl}	14.20±1.07 ^{fghijk}	8.40±0.51 ^{fghi}
14OKY	Upper	8.20±0.37 ^{hi}	5.40±0.37 ^{hi}	72.37±2.94 ^{defghi}	15.60±0.87 ^{cdefghij}	9.20±0.49 ^{defghi}
	Lower	7.00±0.45 ⁱ	6.40±0.45 ^{efghi}	34.21±4.83 ^{kl}	13.20±0.73 ^{ijklmn}	10.80±0.37 ^{cdefgh}
15OFG	Upper	8.40±0.40 ^{gh}	7.20±0.40 ^{cdefg}	68.42±3.36 ^{defghijk}	14.00±0.71 ^{cdefghij}	9.00±0.45 ^{defghi}
	Lower	9.20±0.37 ^{defgh}	8.60±0.37 ^{bcd}	38.16±4.83 ^{ijkl}	13.80±0.80 ^{ijklmn}	8.40±0.51 ^{fghi}
16OKS	Upper	9.60±0.24 ^{bcddefgh}	8.40±0.24 ^{bcd}	72.37±2.94 ^{defghi}	15.60±0.87 ^{bcddefghi}	9.20±0.49 ^{defghij}
	Lower	8.20±0.20 ^{hi}	5.00±0.20 ⁱ	34.21±4.83 ^{kl}	13.20±0.73 ^{ijklmn}	10.80±0.37 ^{cdefgh}
17OMP	Upper	10.40±0.24 ^{abcde}	8.80±0.24 ^{bc}	98.68±11.39 ^{bcd}	16.60±1.63 ^{bcddefghi}	11.80±0.66 ^{cd}
	Lower	8.80±0.37 ^{fghi}	5.20±0.37 ⁱ	36.84±4.46 ^{kl}	13.20±0.97 ^{ijklmn}	8.60±0.60 ^{fghi}
18OFF	Upper	10.40±0.40 ^{abcdef}	9.20±0.40 ^b	117.10±6.38 ^{abc}	17.20±1.20 ^{abcdef}	11.40±0.75 ^{cde}
	Lower	9.00±0.32 ^{efgh}	5.40±0.32 ^{hi}	31.58±2.46 ⁱ	12.60±0.75 ^{ijklmn}	8.40±0.40 ^{fghi}
19EIY	Upper	9.60±0.24 ^{bcddefgh}	9.40±0.24 ^b	115.79±7.95 ^{abc}	17.40±0.51 ^{abcd}	11.40±0.51 ^{cde}
	Lower	8.80±0.37 ^{fghi}	6.20±0.37 ^{fghi}	31.58±2.46 ⁱ	13.20±0.97 ^{ijklmn}	8.00±0.32 ^{hi}
20AJS	Upper	9.80±0.58 ^{abcdefg}	9.40±0.58 ^b	98.69±9.76 ^{bcd}	18.00±0.55 ^{abcd}	11.80±1.11 ^{cd}
	Lower	8.40±1.03 ^{gh}	7.20±1.03 ^{cdefg}	34.21±3.83 ^{kl}	12.80±0.80 ^{ijklmn}	8.40±0.68 ^{fghi}

Mean values, standard error and different letters along columns depicting significant difference at 0.05 level using ANOVA test. Acc. Cod. No. = Accession Code Number, STLT = Stomata length, STWT = Stomatal width, STD= Stomatal Density, ECWT = Epidermal cell width, ECLT = Epidermal cell length, U = Upper leaf surface, L = Lower leaf surface.

Table 5: Leaf anatomical features of the 20 accessions of two *Luffa* species

Acc. Cod. No	Surface	ECD	STSZ (μm^2)	STID (%)	ECS	Trichomes
01OGD	Upper	426.31±10.69 ^{cde}	72.09±4.69 ^{ab}	18.83±1.31 ^{abcde}	Polygonal	Present
	Lower	572.37±5.89 ^a	36.91±2.94 ^{ijklm}	7.24±0.84 ^{kl}	Polygonal	Present
02UIL	Upper	436.84±11.32 ^{bcde}	51.35±6.89 ^{defghi}	16.63±0.8 ^{bcdefg}	Polygonal	Present
	Lower	552.63±25.05 ^a	36.75±3.28 ^{ijklm}	3.90±0.47 ^j	Irregular	Present
03AGB	Upper	438.16±29.17 ^{bcde}	33.92±2.82 ^{lm}	13.87±0.79 ^{fghi}	Polygonal	Present
	Lower	576.32±10.32 ^a	49.31±3.60 ^{efghijk}	7.56±0.95 ^{kl}	Irregular	Present
04ADW	Upper	482.89±17.85 ^{bcd}	56.54±7.23 ^{cdefgh}	17.45±0.81 ^{abcdefg}	Polygonal	Present
	Lower	584.21±5.66 ^a	33.61±2.96 ^{lm}	5.12±0.37 ^{kl}	Irregular	Present
05SHK	Upper	547.37±13.71 ^a	47.74±4.14 ^{fghijkl}	20.02±0.79 ^{abc}	Polygonal	Present
	Lower	601.32±13.26 ^a	80.41±6.64 ^a	11.70±0.65 ^{hij}	Irregular	Present
06APL	Upper	436.84±11.32 ^{bcde}	50.26±6.26 ^{efghij}	16.63±0.80 ^{bcdefg}	Polygonal	Present
	Lower	568.42±21.48 ^a	35.81±1.52 ^{ijklm}	9.99±6.38 ^{ijk}	Irregular	Present
07OKF	Upper	485.53±7.02 ^{bc}	61.72±7.80 ^{bcddef}	15.15±1.06 ^{defgh}	Polygonal	Present
	Lower	567.11±13.39 ^a	33.61±3.50 ^{lm}	8.11±0.70 ^{kl}	Polygonal	Present
08ARP	Upper	460.53±8.58 ^{bcd}	64.70±5.33 ^{bcd}	14.30±2.25 ^{efghi}	Polygonal	Present
	Lower	561.84±19.25 ^a	44.45±5.61 ^{hijklm}	4.25±0.60 ^l	Polygonal	Present
09ALG	Upper	440.79±12.82 ^{bcde}	72.24±4.58 ^{ab}	15.82±0.87 ^{cdefgh}	Polygonal	Present
	Lower	569.74±11.51 ^a	50.57±4.02 ^{defghij}	3.78±0.27 ^l	Irregular	Present
10OKL	Upper	432.89±20.00 ^{bcde}	46.64±3.88 ^{ghijklm}	17.61±1.69 ^{abcdefg}	Polygonal	Present
	Lower	564.48±12.02 ^a	36.28±1.37 ^{klm}	4.89±0.32 ^l	Irregular	Present
11SNG	Upper	436.84±31.52 ^{bcde}	60.15±4.23 ^{abcdefg}	21.63±0.93 ^a	Polygonal	Present
	Lower	543.42±28.80 ^a	40.83±1.31 ^{ijklm}	5.33±0.67 ^{kl}	Polygonal	Present
12KSU	Upper	463.16±25.45 ^{bcd}	70.51±4.42 ^{abc}	17.90±1.78 ^{abcdef}	Polygonal	Present
	Lower	565.79±10.81 ^a	36.59±2.89 ^{ijklm}	4.24±0.65 ^l	Irregular	Present
13OLJ	Upper	384.21±20.13 ^e	81.67±6.38 ^a	19.48±2.29 ^{abcd}	Polygonal	Present
	Lower	553.95±11.09 ^a	37.54±5.61 ^{ijklm}	6.99±0.72 ^{kl}	Polygonal	Present

14OKY	Upper	436.84±14.80 ^{bcd}	34.39±2.66 ^{lm}	14.21±0.30 ^{efghi}	Polygonal	Absent
	Lower	560.53±7.62 ^a	34.24±3.52 ^{klm}	5.76±0.84 ^{kl}	Polygonal	Present
15OFG	Upper	465.79±27.55 ^{bcd}	47.43±3.17 ^{ghijkl}	12.95±0.96 ^{ghi}	Polygonal	Absent
	Lower	486.84±30.65 ^b	62.19±3.31 ^{bcd}	7.26±0.73 ^{kl}	Irregular	Present
16OKS	Upper	436.84±14.80 ^{bcd}	63.45±3.16 ^{bcd}	14.21±0.30 ^{efghi}	Polygonal	Present
	Lower	560.53±7.62 ^a	32.04±1.54 ^m	5.76±0.84 ^{kl}	Irregular	Present
17OMP	Upper	436.84±28.27 ^{de}	71.93±3.72 ^{ab}	18.55±2.15 ^{abcde}	Polygonal	Present
	Lower	565.79±12.48 ^a	35.96±3.22 ^{klm}	6.10±0.67 ^{kl}	Irregular	Present
18OFF	Upper	425.00±10.73 ^{bcd}	74.60±3.35 ^{ab}	21.62±1.16 ^a	Polygonal	Present
	Lower	567.11±9.63 ^a	38.01±1.33 ^{ijklm}	5.27±0.38 ^{kl}	Polygonal	Present
19E1Y	Upper	438.16±20.87 ^{bcd}	70.83±3.43 ^{ab}	20.91±1.61 ^{ab}	Polygonal	Present
	Lower	563.16±10.73 ^a	43.35±5.72 ^{hijklm}	5.32±0.43 ^{kl}	Irregular	Present
20AJS	Upper	423.68±10.11 ^{de}	71.61±2.82 ^{ab}	18.79±1.51 ^{abcde}	Polygonal	Present
	Lower	569.74±12.76 ^a	46.96±5.74 ^{ghijklm}	5.71±0.72 ^{kl}	Polygonal	Present

Mean values, standard error and different letters along columns depicting significant difference at 0.05 level using ANOVA test. Acc. Cod. No. = Accession Code Number, ECD = Epidermal cell density, STSZ = Stomatal size, STID = Stomatal index, ECS = Epidermal cell shape.

Mechanical Properties of Fibres from 20 Accessions of *Luffa* Species

Analysis of variance (Table 6) revealed statistically significant differences ($p < 0.05$) among the 20 *Luffa* accessions across all measured mechanical parameters, including tensile stress, tensile strain, percentage elongation, tensile modulus, flexural strength, impact energy, and hardness.

Among the accessions, 02UIL exhibited the highest tensile stress (17.82 N/mm²), closely followed by 01OGD (Ogidi, Giwa LGA) with 17.72 N/mm², while the lowest tensile stress was recorded in 14OKY (*L. acutangula*, 13.66 N/mm²). Accession 03AGB showed the highest tensile strain (0.09) and percentage elongation (9.06%), reflecting superior extension capacity before failure. Conversely, 06APL recorded the lowest tensile strain, while 05SHK exhibited the lowest percentage elongation (4.49%).

For tensile modulus, 18OFF displayed the highest value (338.03 N/mm²), whereas 03AGB had the lowest (187.08 N/mm²). Flexural strength peaked in 01OGD (58.81 N/mm²) and was lowest in 15OFG (45.65 N/mm²). Impact energy was highest in 14OKY (1.29 J) but lowest in 11SNG (0.76 J). Regarding hardness, 18OFF exhibited the greatest value (96.71 Hv), followed by 01OGD (96.44 Hv), while 07OKF showed the lowest (91.01 Hv).

Overall, *Luffa cylindrica* accessions demonstrated superior mechanical performance, particularly in tensile strength, modulus, flexural strength, and hardness—

attributes that make them more suitable for applications requiring rigidity and load-bearing capacity. In contrast, *Luffa acutangula* accessions, characterized by higher impact energy and elongation, appear better suited for uses demanding flexibility and shock absorption.

Table 6: Mechanical properties of fibres of the 20 accessions of *Luffa* species

Acc. No.	TStr (N/mm ²)	T Strain	% Elong	T Mod (N/mm ²)	FL Strt (N/mm ²)	Impact (J)	Hardness (Hv)
01OGD	17.72±0.05 ^a	0.06 ±0.00 ^{cde}	6.64 ±0.17 ^{ef}	279.56±0.69 ^e	58.81 ±0.42 ^a	0.92±0.04 ^{hi}	96.44 ±0.51 ^{ab}
02UIL	17.82±0.09 ^a	0.08 ±0.01 ^{ab}	7.63 ±0.10 ^c	238.36±1.32 ^{ij}	57.48 ±0.48 ^b	0.78±0.01 ^k	93.70 ±0.12 ^{efgh}
03AGB	17.51±0.24 ^a	0.09 ±0.00 ^a	9.06 ±0.07 ^a	187.08±1.08 ^p	56.03 ±0.03 ^{cd}	0.87±0.01 ^{ij}	94.19 ±0.10 ^{defg}
04ADW	17.70±0.09 ^a	0.07 ±0.00 ^{abcd}	6.97 ±0.17 ^{def}	261.52±0.62 ^g	49.37 ±0.27 ^h	0.95±0.02 ^{gh}	94.67 ±2.34 ^{bcdef}
05SHK	13.66±0.31 ^f	0.06 ±0.00 ^e	4.49 ±0.27 ^j	307.26±3.16 ^b	49.47 ±0.25 ^h	1.06±0.02 ^{de}	96.06 ±0.02 ^{abc}
06APL	14.36±0.29 ^e	0.05 ±0.00 ^e	4.89 ±0.02 ^{ij}	287.05±0.04 ^c	54.75 ±0.22 ^{fg}	1.08±0.01 ^{cde}	92.05 ±0.02 ^{hi}
07OKF	16.85±0.11 ^c	0.06 ±0.00 ^{cde}	5.99 ±0.03 ^{gh}	282.40±0.73 ^d	55.88±0.14 ^{cde}	0.88±0.02 ^{ij}	91.01 ±0.02 ⁱ
08ARP	17.53±0.17 ^a	0.09 ±0.00 ^a	8.46 ±0.13 ^b	206.44±0.26 ⁿ	58.42 ±0.24 ^a	0.79±0.00 ^k	94.91 ±0.12 ^{abcdef}
09ALG	14.73±0.12 ^e	0.07 ±0.00 ^{abcd}	7.23 ±0.21 ^{cd}	210.16±0.17 ^m	57.47 ±0.04 ^b	0.84±0.01 ^j	96.01 ±0.01 ^{abc}
10OKL	14.66±0.16 ^e	0.07 ±0.00 ^{abcd}	6.28 ±0.14 ^{fg}	240.91±0.29 ⁱ	56.54 ±0.26 ^c	0.91±0.00 ^{hi}	96.01 ±0.01 ^{abc}
11SNG	16.61±0.19 ^c	0.07 ±0.00 ^{abcd}	6.60 ±0.28 ^{ef}	237.26±0.11 ^j	56.20 ±0.19 ^c	0.76±0.00 ^k	96.05 ±0.05 ^{abc}
12KSU	14.67±0.15 ^e	0.07 ±0.01 ^{bcd}	5.68 ±0.14 ^h	273.81±0.37 ⁱ	54.80 ±0.13 ^{fg}	0.98±0.00 ^{fg}	95.70 ±0.13 ^{abcd}
13OLJ	15.31±0.16 ^d	0.07 ±0.01 ^{bcd}	5.93 ±0.04 ^{gh}	251.03±0.56 ^h	55.37 ±0.37 ^{def}	0.89±0.00 ^{ij}	94.43 ±0.06 ^{cdef}
14OKY	13.66±0.17 ^f	0.07 ±0.00 ^{abcd}	6.68 ±0.13 ^{ef}	207.25±0.16 ⁿ	49.48 ±0.25 ^h	1.29±0.01 ^a	91.45 ±0.12 ⁱ
15OFG	14.68±0.20 ^e	0.08 ±0.00 ^{ab}	7.61 ±0.21 ^c	195.14±0.08 ^o	45.65 ±0.21 ⁱ	1.13±0.04 ^{bc}	93.17 ±0.06 ^{fgh}
16OKS	16.63±0.06 ^c	0.07 ±0.00 ^{abcd}	6.80 ±0.08 ^{def}	239.22±0.20 ^{ij}	55.30±0.18 ^{defg}	0.96±0.02 ^{gh}	95.14 ±0.06 ^{abcde}
17OMP	17.42±0.24 ^{ab}	0.08 ±0.00 ^{abc}	8.31 ±0.18 ^b	212.45±0.28 ^{lm}	55.13 ±0.06 ^{efg}	1.07±0.01 ^{de}	95.15 ±0.05 ^{abcde}
180FF	16.92±0.05 ^{bc}	0.06 ±0.01 ^{de}	5.10 ±0.34 ⁱ	338.03±0.74 ^a	56.56 ±0.08 ^c	1.17±0.01 ^b	96.71 ±0.10 ^a
19Eiy	16.50±0.18 ^c	0.08 ±0.00 ^{abc}	7.68±0.09 ^c	223.73±0.28 ^k	56.55 ±0.29 ^c	1.10±0.01 ^{cd}	94.57 ±0.08 ^{cdef}
20AJS	12.69±0.34 ^g	0.06 ±0.00 ^{cde}	5.68 ±0.03 ^h	213.55±0.29 ^l	54.58 ±0.12 ^g	1.03±0.01 ^{ef}	92.56 ±0.05 ^{ghi}

Mean values, standard error and different letters columns depicting significant difference at $P < 0.05$ level using ANOVA test. Acc. No. = Accession Code Number, TStr = Tensile stress, Tstrain = Tensile strain, %Elong = Percentage elongation, Tmod = Tensile Modulus, Fl str = Flexural Strength, Impact = Impact energy (strength), Hardness = Hardness, $\pm$ Standard error.

Discussion

The morphological variations observed among the 20 *Luffa* accessions examined in this study corroborate previous findings on the genus, particularly in relation to fruit shape, seed colour, and general growth habit (Adie *et al.*, 2013). The wide range of stem girth values (3.98–7.52 cm) observed reflects substantial morphological diversity within the genus, consistent with the report of Partap *et al.* (2012), who attributed the morphological variability in *Luffa* germplasm from India to adaptation across diverse agro-ecological zones. Similar intraspecific variations in stem diameter within *L. cylindrica* and *L. acutangula* have been documented by Kumar and Pandit (2022) and Tyagi *et al.* (2016), who linked these differences to genetic diversity and environmental influence. Likewise, Varalakshmi *et al.* (2015) highlighted considerable variation in stem thickness and vine length among *L. acutangula* germplasm, identifying these as key indicators of adaptive evolution and promising traits for selection in domestication and breeding programs.

The relatively smaller leaf size in accession 15OFG (*L. acutangula*) compared to other accessions may reflect either intrinsic species-level genetic variation or specific environmental responses. This agrees with the findings of Ikechukwu and Benjamin (2004), who emphasised the diagnostic importance of vegetative characters in Cucurbitaceae taxonomy.

The distinct qualitative morphological features observed further strengthen classical taxonomic delineations within the *Luffa* genus. The contrast between the dark green leaves and bright yellow flowers of *L. cylindrica* and the light green leaves and pale-yellow flowers of *L. acutangula* validates Heiser's (1979) descriptions. Additionally, the cylindrical fruits of *L. cylindrica* and the spindle-shaped, ribbed fruits of *L. acutangula* are defining species-level traits, as reported by Marr *et al.* (2005). The differences in seed form and colour—predominantly oblong and black/brown in *L. cylindrica* versus ovate and sometimes greyish-black in *L. acutangula*, provide further diagnostic cues, echoing Bisognin's (2002) observation on the value of seed morphology in taxonomic identification and germplasm characterization.

Foliar anatomical features revealed substantial inter-accessional diversity, particularly in epidermal cell wall configurations and stomatal characteristics. The predominance of polygonal cells on the adaxial surface and irregular forms on the abaxial surface aligns with Kadiri *et al.* (2013), though the occasional occurrence of polygonal cells on both surfaces in certain accessions suggests possible micro-environmental or developmental influences.

The consistent occurrence of staurocytic stomata across all accessions aligns with earlier observations by AbdulRahaman *et al.* (2011), who identified both staurocytic and actinocytic types within *Luffa*. The predominance of the staurocytic form in this study may reflect the taxonomic stability of this stomatal type within the genus, while minor deviations could indicate genetic heterogeneity or ecological adaptation.

The generally higher stomatal index on the abaxial surface compared to the adaxial surface represents a typical xeromorphic adaptation for minimizing water loss (Abubakar *et al.*, 2011). However, accessions from wetter environments, such as Sango (11SNG), exhibited a relatively higher adaxial stomatal index, possibly reflecting reduced selective pressure for water conservation and indicating microclimatic adaptation.

Variation in trichome type, density, and distribution among the accessions further emphasizes their distinctiveness. The presence of trichomes on both leaf surfaces in *L. cylindrica* accessions contradicts earlier reports by AbdulRahaman *et al.* (2011) and may represent either ontogenetic variation or ecotypic differentiation. As noted by Shield (1950), dead trichomes provide protection against excessive transpiration, suggesting that future studies should investigate trichome physiology and functional morphology in *Luffa* species.

The mechanical properties of *Luffa* fibres recorded in this study fall within the range reported in related literature, albeit with significant inter-accessional variation. The tensile stress values (13.66–17.82 N/mm²) are comparable to those reported by Saw *et al.* (2013) and Panneerdhass *et al.* (2014), though somewhat lower than those of alkali-treated fibres (Vignesh *et al.*, 2015; Tanobe *et al.*, 2015). The use of untreated fibres in the present study likely accounts for these lower values, as chemical treatments enhance fibre strength by removing amorphous materials and improving cellulose alignment (Demir *et al.*, 2006; Seki *et al.*, 2011).

Accessions such as 03AGB, which showed higher tensile strain and elongation, demonstrated greater flexibility, an attribute desirable in cushioning and impact-absorbing composites. The wide range in tensile modulus (187.08–338.03 N/mm²) indicates variability in fibre stiffness, likely influenced by genetic factors, environmental conditions, and fibre maturity (Mohanty *et al.*, 2000). Flexural and impact strengths also varied considerably, corroborating reports by Ichetaonye *et al.* (2015) and Panneerdhass *et al.* (2014). Notably, accession 14OKY (*L. acutangula*) exhibited the highest impact energy (1.29 J) but lower tensile strength and hardness, suggesting a tough yet less rigid fibre. Hence, *L. acutangula* fibres may be more suitable for dynamic or flexible applications, while *L. cylindrica* fibres, with higher tensile strength and hardness, are better suited for structural or load-bearing uses.

Overall, the findings reaffirm that fibre mechanical performance is governed by a complex interplay of genetic constitution, growth environment, and post-harvest processing (Saheb & Jog, 1999). The results underscore the potential for targeted selection of *Luffa* accessions for industrial applications based on specific mechanical attributes.

Conclusion

This study comprehensively evaluated the morphological, anatomical, and mechanical characteristics of *Luffa* species collected across various locations in Kwara State, Nigeria. Significant intra- and interspecific variations were observed, reflecting a rich reservoir of genetic diversity within the studied accessions. Morphological and anatomical traits provided reliable taxonomic markers distinguishing *L. cylindrica* from *L. acutangula*, while differences in stomatal structure, trichome distribution, and mechanical strength highlighted adaptive and functional specializations.

Mechanical assessments revealed that *Luffa* fibres differ significantly in tensile strength, modulus, and impact resistance, suggesting their suitability for different industrial applications. *L. cylindrica* accessions such as 01OGD and 02UIL should be prioritised for high-strength composites and abrasive materials, whereas *L. acutangula* accessions like 03AGB and 14OKY, with greater elongation and impact energy, are better suited for flexible and shock-absorbing materials. High-performing accessions (e.g., 01OGD for tensile strength and 18OFF for hardness) should be conserved and considered as parent lines in breeding programmes aimed at developing improved *Luffa* cultivars with optimised fibre qualities for bio-composite industries.

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Prevalence of Refractive Errors and Attitude Towards Usage of Spectacles Among Commercial Vehicle Drivers in University of Ilorin, Kwara State, Nigeria

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Abstract

This cross-sectional descriptive study determined the prevalence of refractive errors among 114 male commercial car drivers aged 18-72 years (mean 45 ± 9.0 years) at the University of Ilorin motor park and assessed their attitudes toward corrective lenses. Comprehensive eye examinations including visual acuity tests, ocular health examinations, and refractions were conducted. Structured questionnaires were administered and data analyzed using IBM SPSS software version 22.0. Results showed 25.44% prevalence of refractive errors, with hyperopia being most common (13.16%). Regarding attitudes, 74.56% of drivers believed glasses improved vision while 4.39% viewed them as fashion accessories. Most drivers (89.47%) indicated willingness to wear prescribed glasses, though some lacked interest. The findings emphasize the need for periodic eye screening and ocular health awareness programs for drivers to enhance road safety.

Keywords: Refractive errors, Attitude, Spectacles, Commercial car drivers.

Introduction

According to the World Health Organization (2004), it is estimated that roughly 314 million individuals globally experience vision impairment caused by eye conditions or refractive errors that have not been properly corrected. These refractive errors constitute the foremost cause of visual impairment globally, with their impact extending across all age groups and ethnic populations (Dairo *et al.*, 2019).

In most countries worldwide, driving serves as the predominant mode of transportation, facilitating daily tasks and has significant influence on life quality (Bowers *et al.*, 2005). The World Health Organization data from 2004 revealed that approximately 1.2 million casualties resulted from vehicular accidents globally each year (Dairo *et al.*, 2019). This makes traffic safety a critical global health challenge, with

inadequate visual acuity among road users representing one of the major risk factors contributing to the occurrence of road accidents (Peden, 2005).

Commercial vehicles are designed specifically for transporting goods or fare-paying passengers from one destination to another. For average Nigerians, road safety remains a paramount concern, with commercial vehicle drivers playing an important role in maintaining safe transportation conditions (Adekoya *et al.*, 2009). However, it is not farfetched to understand that effective driving fundamentally depends on adequate vision, as operating or driving a vehicle demands intensive visual processing alongside multiple skill sets encompassing sensory perception, cognitive processing, physical coordination, and adaptive capabilities (Azumamah *et al.*, 2014).

Visual impairment, including reduced acuity or constricted visual fields, can significantly impair a driver's capacity for safe vehicle operation, particularly on modern busy highways with high traffic speeds (Emerole and Nneli, 2013). The clarity of vision or visual acuity can be broadly defined as one's ability to distinguish fine details which represents the most critical element of visual function. This function is commonly evaluated through standardized testing where participants identify increasingly smaller symbols or letters displayed on examination charts positioned at 3-6 meters away (Norman, 2002).

Nigeria's Federal Road Safety Commission (FRSC) established driver's licensing standards in 1990, specifying that commercial vehicle operators must maintain minimum visual acuity of 6/9 in their better seeing eye and 6/12 in their worst eye. Consequently, having visual acuity at 6/9 or better levels of vision is essential for all drivers to obtain driving license, as this standard will enhance their ability for timely recognition and interpretation of road signs, thereby facilitating appropriate decision-making and safe vehicular maneuvering (Pepple and Adio, 2013).

Visual perception is the primary sensory component influencing a driver's decision-making capabilities during vehicle operation. In environments where regular health screenings, particularly eye examinations, are not regularly conducted, numerous drivers may remain unaware of their visual deficiencies or that their sight could be enhanced through corrective optical devices (Rollison *et al.*, 2018). Even when drivers discover they have visual impairments and receive prescriptions for corrective eyewear, various obstacles may still prevent them from consistently using the recommended spectacles (Uwadia, 2015).

Therefore, examining the prevalence of refractive disorders among commercial vehicle operators at the University of Ilorin becomes critically important, given that students' safety depends on drivers having adequate vision recommended for driving. Additionally, understanding these drivers' attitudes toward eye care services and their compliance with prescribed corrective lenses for driving purposes represent essential areas for research investigation.

Materials and Methods

This study was a cross sectional descriptive study conducted at the University of Ilorin involving 114 commercial drivers (buses, cars/taxis, and tricycles) with a minimum of one-year driving experience. An eye screening exercise was done at the University general motor park between 20th–25th March, 2023 by licensed Optometrists. Each participant received a comprehensive ocular assessment, which included measurement of visual acuity at both distance and near using Snellen's charts, external and internal eye health evaluations performed with a penlight and ophthalmoscope respectively, and objective refraction conducted with a streak retinoscope followed by subjective refraction.

Normal vision for driving was defined as visual acuity of 6/9 or better in the eye with a better sight, which aligns with the criteria set by Nigeria's FRSC for driving license eligibility (Pepple and Adio, 2013). Visual impairment was categorised as visual acuity less than 6/18 but equal to or greater than 6/60 in the better seeing Eye, following the International Classification of Diseases (ICD-10) guidelines. Commercial drivers who required additional assessments were directed to the Optometry Clinic within the Department of Optometry and Vision Science at the University of Ilorin.

Qualitative data were collected using researcher-administered structured questionnaires and the details of the questionnaires were interpreted in the local languages of the drivers who did not understand English language. This approach was implemented to gather participants' personal information, driving experience records, traffic collision history, engagement with vision care services, and their perceived obstacles to using prescribed eyewear while operating vehicles. Before conducting the vision assessment activities, approval was requested and secured from the ethics review committee of the Department of Optometry and Vision Science and the Dean of Student Affairs was informed formally of the need to use the University of Ilorin Park for this research and permission was granted. The Chairmen of both the National Union of Road Transport Workers (NURTW) and Road Transport Employers' Association of Nigeria (RTEAN), University of Ilorin chapters were also informed via written letters and physical meetings, permissions were granted by both parties and a date was fixed for the eye screening exercise.

Eligible subjects were all well informed, the procedures were explained to them and their consents obtained before conducting the tests.

Data obtained from this study were analyzed with International Business Machine Corporations Statistical Packages for Social Sciences (IBM SPSS) software version 22.0, and variables were summarized using mean and standard deviation.

Results

A total number of 150 questionnaires and examination forms were distributed to the commercial drivers for this study, however, only 114 answered the questionnaires and turned up for the examinations representing a response rate of 76%. All (100%) of the participants were males.

Table 1: Age distribution of the Participants

Age (years)	Frequency	Percentage
18-28	4	3.51%
29-39	18	15.79%
40-50	51	44.74%
51- 61	34	29.82%
62-72	7	6.14%
Grand Total	114	100.00%

Table 1 shows the age distribution of the drivers. The participants were aged between 18–72 with a mean age of 45 ± 9.0 . Majority 51 (44.74%) of the participants were between the ages of 40 – 50.

Table 2: Driving Experience of the Participants

Experience (years)	Frequency	Percent
1-10	44	38.60%
11-20	36	31.58%
21-30	25	21.93%
31-40	9	7.89%
Grand Total	114	100.00%

Table 2 shows the driving experience (in years) of the drivers. All drivers had driving experience ranging from 1–40 years with a mean of 16.18 ± 9.9 years.

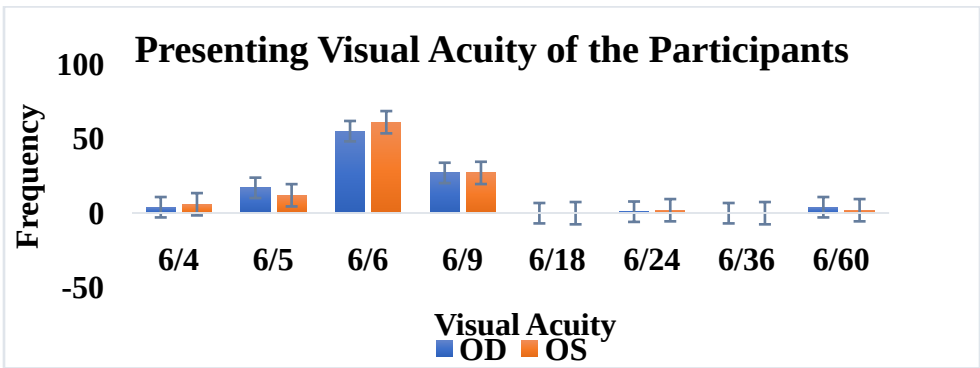


Figure 1: Multiple bar chart showing the distribution of participants' visual acuity for both eyes

Figure 1 shows the distribution of visual acuity of the participants for each eye. Using the minimum standard of visual requirement for driving as required by the Federal Road Safety Commission (FRSC) for commercial drivers in Nigeria, 110 (96.41%) individuals passed the minimum requirement of 6/9 in the better eye and 6/12 (with or without the use of corrective lenses). Only 4 (3.51%) failed this requirement.

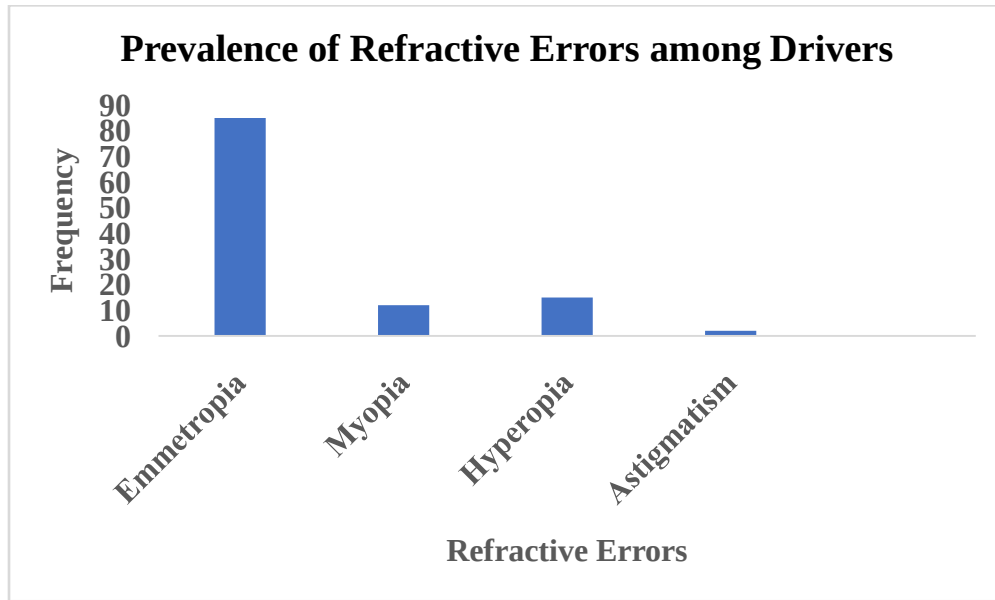


Figure 2 shows the prevalence of the refractive error among the participants, out of 114 drivers, only 29 suffered from refractive errors, indicating a prevalence of 25.44%. The most common type of refractive error encountered was hyperopia which accounted for 13.16%, followed by myopia which accounted for 10.53% and the least common was astigmatism which accounted for 1.75%.

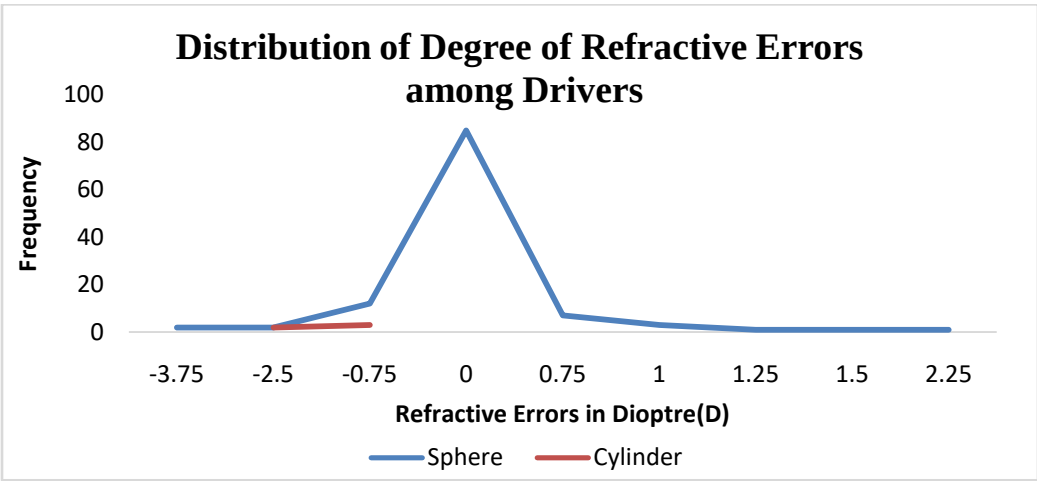


Figure 3: Distribution of degree of refractive errors among drivers

Figure 3 gives the detailed distribution of the range of refractive errors found among the drivers. The highest degree of myopia recorded was 3.75DS while the highest degree of hyperopia encountered was 2.25DS.

Table 3: Attitude towards the Use of Corrective Lenses

Use of corrective lenses	Reason	Frequency	Percent
No		12	10.53%
	Not interested	7	6.14%
	Public discrimination	5	4.39%
Yes		102	89.47%
	If recommended	39	34.21%
	Indifferent	5	4.39%
	Cosmetically	2	2.63%
	Vision improvement	42	36.84%
	Eye protection	7	6.14%
Grand Total		114	100.00%

Table 3 shows the participants' attitude toward the use of corrective lenses, 102 (89.47%) participants would wear glasses for various reasons if they were given while 12 (10.53%) would not wear spectacles even if they were prescribed to them, because they had no interest in wearing glasses as well as public discrimination.

Discussion

For decades now, numerous studies on the prevalence of refractive errors among drivers of various studied populations have been carried out internationally and within Nigeria. A similar study was conducted by Bekibele *et al.* (2007), among motor vehicle drivers of public institutions, using the College of Medicine, University of Ibadan, Nigeria as a case study.

Visual performance, particularly visual acuity, is a critical factor in ensuring safe driving, especially for commercial drivers who spend extended periods on the road. Numerous studies have established a strong connection between diminished visual acuities and an increased risk of road accidents (Norman, 2002).

In this study, the visual acuity (VA) presentation of the participants in Figure 1 shows that majority 76 (66.67%) of the drivers had normal visual acuity of 6/6 or better and about 96.41% of the participants had visual acuity recommended for driving in their better eyes. Only 4 drivers (3.51%) did not have the minimum visual acuity requirement for driving among the examined commercial drivers. This finding has similarities with the studies conducted by Uwadia (2015) and Adekoya *et al.*, (2009) where low prevalence rates of 5.1% (of 227 drivers tested) and 11.5% (of the total 399 drivers tested) were recorded respectively as having inadequate visual acuities (VA) required for driving.

Our study revealed that out of the 114 drivers tested, only 29 had refractive errors, indicating a prevalence of 25.44%. This prevalence is similar to a study carried out among truck drivers in India by Sabherwal *et al.*, (2020) who reported a prevalence of 26.4%. Erdoğan *et al.*, (2011) also reported a relatively similar prevalence of refractive error (21.5%) in their study conducted in Turkey among heavy vehicle drivers. However, Bekibele *et al.*, (2007) and Adekoya *et al.*, (2009) reported lower prevalence of refractive error (16.7% and 6.0%) in their studies which were carried out in University of Ibadan, Nigeria and Ilorin metropolis in Nigeria among drivers respectively.

The most common type of refractive error encountered was hyperopia which accounted for 13.16%, followed by myopia (10.53%) and the least common was astigmatism which was 1.75%. This is in tandem with studies carried out by Kumar *et al.* (2022) and Isawumi *et al.*, (2011) that reported hyperopia as the most common refractive error with prevalence of 8.2%, and 19.7% respectively.

Uwadia (2015) reported a prevalence of refractive error of 13.5% among fuel truck drivers in Oyo, Nigeria despite the fact that the number of participants was higher (227) than our study, he also reported hyperopia to be the most prevalent refractive error. It is worthy of note that the lower prevalence of refractive error recorded by Uwadia (2015) could be as a result of the studied population used in his study which comprised Fuel truck drivers who were more likely to have undergone comprehensive eye screening by the companies that employed them before they were entrusted with their vehicles, this may have resulted to the lower value of prevalence that was reported.

The attitude of drivers towards wearing spectacles has been a subject of interest for researchers, in this study, the majority of participants expressed agreement with wearing spectacles or corrective lenses when driving commercial vehicles. They acknowledged the significance of maintaining optimal visual performance for safe driving, particularly when transporting passengers within an academic setting. Dougherty *et al.*, (2019) also reported that drivers who recognize the importance of maintaining optimal visual acuity are more likely to have positive attitudes toward wearing spectacles. However, some individuals held the viewpoint that they would refrain from wearing glasses due to their lack of interest in using corrective lenses while driving, and also because they wished to avoid potential public discrimination associated with wearing glasses.

Conclusion

At the end of this study, the prevalence of refractive errors among commercial vehicle drivers in the University of Ilorin motor park was found to be 25.44% of which hyperopia had the highest prevalence of all refractive errors. It was also revealed that the attitudes of the drivers and public opinions play essential roles in the acceptance of wearing of corrective lenses by commercial vehicle drivers. However, clear vision is fundamental to safe driving, as visual impairment significantly heightens road accident risks by limiting hazard detection, distance judgment, and navigation abilities. Without routine eye screenings and corrective lens usage, many drivers will remain unaware of their vision deficiencies or available treatments, creating widespread safety concerns on roadways. Therefore, regular vision assessments and optical corrections are critical public safety measures.

Acknowledgment

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Conflict of Interest

There is no conflict of interest associated with this study.

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Assessment Of Generation, Composition, and Physicochemical Properties of Municipal Solid Waste in Ilorin, Kwara State, Nigeria

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Abstract

Rapid urbanization and population growth in Ilorin, Kwara State, Nigeria, have led to a substantial increase in the generation of Municipal Solid Waste (MSW). This study evaluates the rate of MSW generation, its physical and percentage composition, and the physicochemical characteristics of decomposed municipal solid waste (DMSW) from two major dumpsites: the University of Ilorin (UI) and Gbagede (GB). The MSW generation rate for Ilorin metropolis was estimated at approximately 760,000 tons/year. Physical classification revealed a predominance of paper waste (19.6%) at UI, attributed to academic activities, and thermocol (25.2%) at GB, indicating industrial sources. Proximate and ultimate analyses of DMSW showed significant variations in moisture content, ash content, pH, and heavy metal concentrations. Notably, iron levels (3.67–6.93 mg/L) and other heavy metals exceeded national environmental standards, classifying the waste as hazardous. The alkaline pH levels (7.85–8.7) and high conductivity values indicate the influence of anaerobic decomposition and mineral leaching. The findings underscore the need for improved waste segregation, monitoring, and environmentally sound management practices in the region.

Keywords: Municipal Solid Waste, Waste Composition, Heavy Metals, Dumpsite, Physicochemical Properties, Words Count 170

Introduction

The quantity of solid waste has increased significantly, and its characteristics have evolved due to accelerated urbanization, industrialization, and population growth in most cities of developing countries (Adeniran, K.A., 2005). Over the years, Ilorin Metropolis has experienced rapid urbanization, sprawling settlements, and population

growth, all of which have contributed to an increase in solid waste generation. Solid waste is a byproduct of population growth, massive urban expansion, and changing lifestyles. As population and income levels rise, so does the production of goods and services, resulting in the discharge of effluents into the environment.

The United States Environmental Protection Agency (U.S.E.P.A.) defines waste as any garbage or refuse, sludge from wastewater treatment plants, water supply treatment facilities, or air pollution control systems, as well as other discarded materials. These materials include solid, liquid, semi-solid, or gaseous substances resulting from industrial, commercial, mining, agricultural, or community activities. Various sources of waste have been identified, including animal waste, hazardous waste, industrial and medical waste, food waste, and nonhazardous waste (New Jersey, 2012). Other sources include municipal (household), commercial, and institutional waste originating from communities. For example, household waste is generated by private residences, commercial waste arises from establishments like restaurants, stores, and markets, and institutional waste is produced by schools, hospitals, research institutions, and public buildings.

Solid waste can be classified under categories such as sludge from sewage treatment plants, household waste, and construction or demolition debris. Other types include vegetative waste from nurseries and gardens, animal and food processing waste, dry industrial waste, and septic tank or sewage sludge (New Jersey, 2012). More recently, electronic waste (e-waste) has been added to these categories.

Solid waste is classified as hazardous if it exhibits any of the following characteristics: ignitability, corrosiveness, reactivity, or toxicity. Universal wastes, a subset of hazardous waste, can be collected under streamlined conditions. Examples include batteries, pesticides, mercury-containing equipment, and lamp bulbs (U.S.E.P.A., 1976). Multi-hazardous waste, containing combinations of chemical, radioactive, or biological hazards, requires special management. Such waste may be regulated by agencies like In Texas, nonhazardous waste is classified into three categories: Class 1, Class 2, and Class 3. Class 1 waste, regulated by the Texas Commission on Environmental Quality (TCEQ), poses potential threats to human health and the environment if not properly managed. Examples include water contaminated with ethylene glycol, petroleum hydrocarbon-contaminated soil, and ignitable liquids. Class 2 waste, often accepted at local landfills, includes depleted aerosol cans, non-radioactive surgical waste, and food packaging waste from manufacturing. Class 3 waste, which is insoluble, non-reactive, and non-decomposing, poses minimal threats. Examples include rocks, bricks, glass, dirt, and certain plastics. Waste management guidelines are provided by the United States Environmental Protection Agency (U.S. EPA, 2006). Additionally, regulations from the Texas Department of State Health Services (TDSHS) provide further guidance on nonhazardous waste handling.

Municipal Solid Waste (M.S.W.) refers to everyday trash, including packaging, yard waste, glass, paper, food waste, appliances, and batteries. It excludes construction

debris, wastewater treatment sludge, and nonhazardous industrial waste (Beatrice et al., 2013). Waste is generally defined as any material with no direct value to the producer that must be discarded (D.E.F.R.A., 2004).

Developed countries generate more waste than developing countries but manage it more efficiently due to higher public awareness, better funding, and advanced technologies. In developing countries, however, municipal solid waste (MSW) collection and disposal remain problematic, with methods such as uncontrolled dumpsites and open burning still widely practiced, generating significant environmental and public health concerns (Rattanamonon, 2003). According to Yuan (2014), daily per capita MSW generation varies with the level of economic development:

- Highly developed cities: 1.2–1.8 kg/person/day
- Industrial cities: 0.7–1.2 kg/person/day
- Medium-income cities: 0.5–0.75 kg/person/day
- Low-income cities: 0.3–0.6 kg/person/day

Specific Objective

To:

1. Determine the physical and percentage composition of Municipal Solid Waste in the study area.
2. Evaluate the physicochemical properties of decomposed Municipal Solid Waste.
3. Assess the ecotoxicity of decomposed Municipal Solid Waste by analyzing heavy metal concentrations. The findings of this study are expected to guide the formulation of policies and decision-making for efficient waste management techniques.

Materials and Methods

Study Area and Design

This study was conducted in Ilorin Metropolis, the capital of Kwara State, located in Nigeria's transition zone between the forest and savannah regions. Ilorin is situated at Latitude 8°30'0"N and Longitude 4°35'0"E, occupying an area of approximately 100 km² (Oyegun, 1992). It spans three local government areas: Ilorin West, Ilorin East, and Ilorin South, encompassing about twenty political wards. Historically, Ilorin was founded between 1600 and 1700 AD, becoming the capital of Kwara State in 1967. Since then, it has experienced significant growth in population and economic activities, making it a microcosm of Nigeria (Jimba, 1981).

Proximate Analysis

Moisture Content

The moisture content of decomposed waste was determined by weighing 0.1 kg of the sample into a pre-weighed dish and drying it in an oven at 105°C until a constant weight was achieved (ASTM D3173). This procedure was repeated in triplicate, and the

moisture content was calculated as the percentage loss in weight before and after drying (Amin, 2012).

Volatile Matter Content

The volatile matter content was determined by igniting the sample at 500°C. The triplicate samples used for moisture content analysis were placed in a furnace for 40 minutes at 500°C. After combustion, the samples were weighed to determine ash and dry weight. The volatile solids were calculated as the difference between the dried solids and the ash (Amin, 2012).

$$\% \text{ VM} = \frac{\text{Dry Sample Weight} - \text{Ash Weight}}{\text{Dry Sample Weight}} \times 100$$

Fixed Carbon Content

Fixed carbon is determined by removing the volatile mass from the sample's original mass, as expressed in equation (3) (Amin, 2012).

Fixed Carbon (Wt% wet basis) = 100 – Wt% Moisture Content + Wt% Ash + Wt% Volatile matter

Percent Saturation

Sub-sample of the paste was dried in an oven at 1050 °C, and the weight loss was used to determine the saturation percentage (amount of water added to the waste) as follows:

$$S = \frac{w - d}{d} \quad 4$$

Where:

W = wet weight of sample

d = dry weight of sample

Ultimate Analysis

Conductivity, pH, and Total Dissolved Solid

Twenty grams of the sample were weighed into a 50 mL beaker, followed by the addition of 20 mL of distilled water. The mixture was stirred continuously for 30 minutes and then covered for one hour to allow the majority of the suspended clay to settle out. The pH and conductivity of the suspension were measured to the nearest tenth of a pH unit, while the Total Dissolved Solids (TDS) were calculated using a conversion factor of 0.67 (Volker, 1988).

Digestion of Decomposed solid sample

Decomposed Municipal Solid Waste samples were subjected to acid digestion in a digestion vessel, with the process performed in triplicate. Acid-digested solid waste

samples were prepared using modified standard procedures designed to determine total metal content (NEPM, 1999). Aqua regia (a 3:1 mixture of hydrochloric acid and nitric acid) was used as the digestion medium, and the samples were heated in an oven at 105°C for two hours. After digestion, the samples were filtered and diluted to 50 mL in a standard volumetric flask. The filtrate was then analyzed using an Atomic Absorption Spectrophotometer (AAS) at the University of Lagos, Nigeria (Kanmani, 2002).

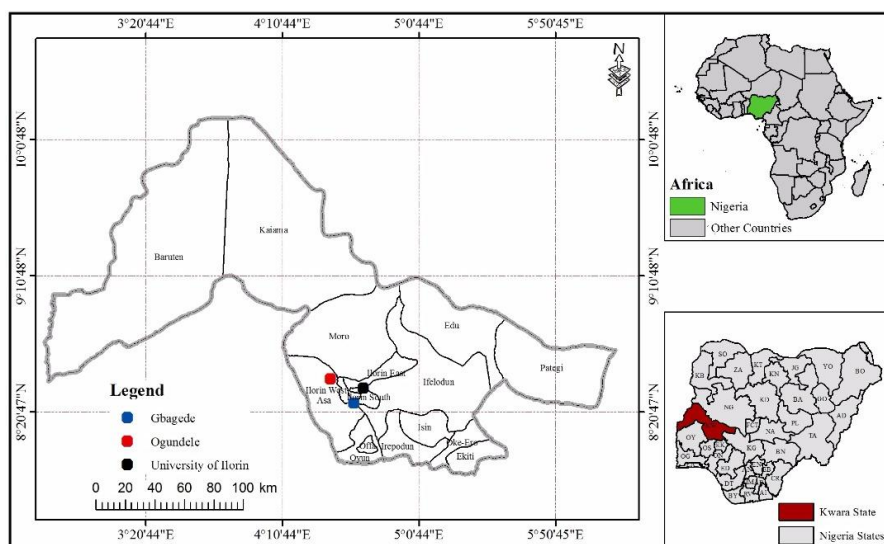


Figure 1: Map of Ilorin indicating the three dumpsites in Ilorin metropolis

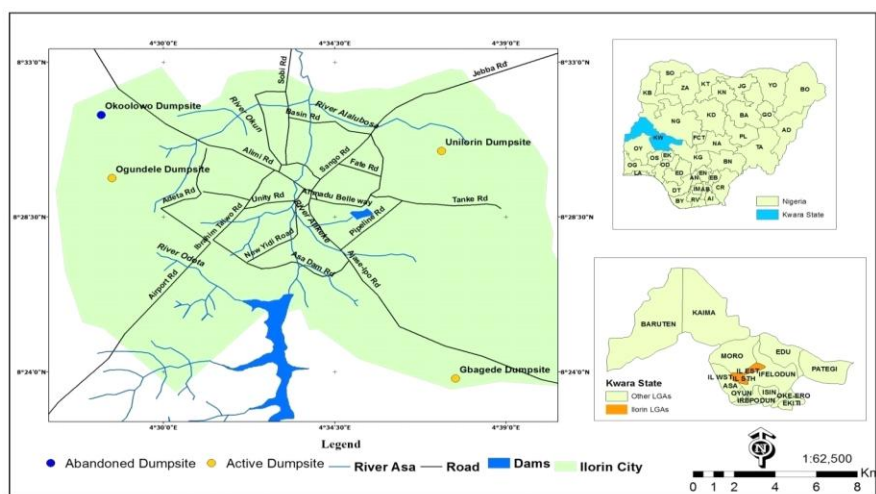


Figure: 2: Map of Ilorin metropolis indicating local governments Area and the dumpsites
Source: Kwara State Ministry of Land and Urban Development (2014)

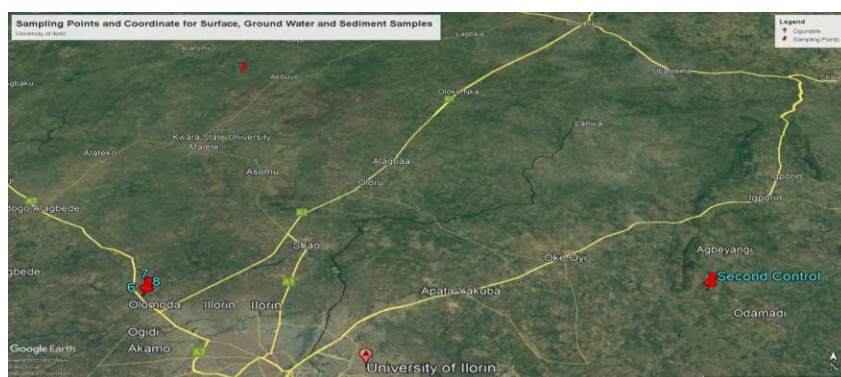


Figure 3: Sampling points for Water Soil and Sediments

Determination of rate of generation of MSW

Ilorin metropolis is classified as an industrial city, and the waste generated per person per day ranges from **0.7 to 1.2 kg/person/day**, according to data from the Kwara State Ministry of Environment.

To calculate the municipal solid waste (MSW) generation for Ilorin metropolis:

- **Population of Ilorin metropolis (M):** 2,185,494 (NPC, 2007)
- **MSW generation rate (GR):** 1.2 kg/person/day (upper limit for industrial cities)

The daily waste generated in Ilorin metropolis can be calculated as:

$$\text{Daily MSW (kg/day)} = \text{GR} \times \text{M} = 1.2 \times 2,185,494 \approx 2,622,592.8 \text{ kg/day}$$

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The annual MSW generation (GT) in tons per year is calculated as:

$$\text{GT} = \text{GR} \times \text{M} \times 10^{-3} \times 365$$

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Substituting the values:

$$\text{GT} = 1.2 \times 2,185,494 \times 10^{-3} \times 365 \approx 956,649 \text{ tons/year}$$

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Where:

- **GT:** Annual generation of MSW (tons/year)
- **GR:** MSW generation coefficient (kg/person/day)
- **M:** Population of the city

Thus, Ilorin metropolis generates approximately 9.57×10^5 tons of municipal solid waste per year.

Results and Discussion

Determination of rate of generation of MSW

The daily generation rate was calculated based on the quantity of waste arriving at the landfill facility, using the formula:

$$GT = 1.529845.8 \times 2.185,494 \times 10^{-3} \times 365 = (7.6 \times 10^5 \text{ tons/year}).$$

From this figure, the landfill is handling approximately $1.529845.8 \times 2.185,494 \times 10^{-3}$ tons of waste per day.

- Based on recalculations using waste generation per capita and the estimated population of Ilorin, the corrected MSW generation rate is approximately **0.76** million tons per year (7.6×10^5 tons/year)

The primary sources of Municipal Solid Waste (MSW) are residential, institutional, and commercial waste. The type, generation, and quantity of waste are significantly influenced by culture and societal consumption patterns (Amin *et al.*, 2012). The municipal solid waste (MSW) generation rate (GR) previously reported for Ilorin metropolis was 0.11 kg/person/day, according to Ciwat Engineering Ltd., the consultants who designed the Gbagede dumping site. This value was determined by dividing the total quantity of waste generated in the city per day by the population of the metropolis. However, this figure has since increased to 1.5 kg/person/day due to rapid urbanization, sprawling settlements, and significant population growth.

This growth has triggered a substantial increase in solid waste generation. The rapid expansion of urban areas, changing lifestyles, and rising population and income levels have led to higher production of goods and services, resulting in the discharge of large quantities of waste into the environment.



Figure 6: University of Ilorin Active Dumpsite (Active) (Source: University of Ilorin Health & Environmental Services)



Figure 7: Gbagede Active Dumpsite (Active) (Source: Kwara State Ministry of Environment)

Physical Composition of MSW at the University of Ilorin and Gbagede dumpsites

The composition of waste by weight percentage (%) ranges from 0.29% to 25.2%, with thermocole having the highest value (25.2%) and plastic bags the lowest (0.29%). Food waste follows closely at 20.9%, while textile gunny bags account for 19.6%. Other components include construction waste (7.1%), paper and cardboard (5.94%), glass (4.7%), wood (3.6%), plastic containers (3.3%), other plastics (2.97%), leather (2.75%), ferrous metals (1.63%), inert matter (1.41%), and non-ferrous metals (0.742%) (Table 1).

The waste stream at the Gbagede dumping site contains a high percentage of organic waste, which can be effectively utilized as organic manure through composting, either via aerobic or anaerobic processes. This highlights the municipality's role in aggregating waste from various communities.

Table 1: Physical Classification of Municipal Solid Wastes at Gbagede Dumping Site

S/No	Composition	Weight (kg)	Composition by Weight (%)	Volume (10 ³ M ³)	Composition by Volume (%)	Density (Kg/m ³)
1	Food waste (mixed)	140.00	20.80	89.20	26.68	1569.50
2	Inert matter	9.50	1.41	5.54	1.66	1714.80
3	Paper and card board	40.00	5.94	15.37	4.60	2602.50
4	Textile and gunny bags	132.00	19.60	27.68	8.29	4768.80
5	Wood	24.00	3.60	16.00	4.79	1500.00
6	Construction waste	48.00	7.10	120.00	35.92	400.00

7	Glass	31.50	4.70	18.30	5.48	1721.30
8	Leather	18.50	2.75	18.50	5.54	1000.00
9	Metal – Ferrous	11.00	1.63	2.75	0.82	4000.00
10	Metal – Non-Ferrous	5.00	0.74	0.59	0.18	8474.60
11	Other plastics	20.00	2.97	1.67	0.49	11976.00
12	Plastic bags	2.00	0.29	0.44	0.13	4545.50
13	Plastic container	22.20	3.30	1.37	0.41	16204.30
14	Thermocole	169.50	25.20	16.62	4.97	10198.60
TOTAL		673.2	100.02	334.03	99.96	70675.90

The percentage composition of paper and cardboard recorded at Unilorin (19.6%) is linked to the type of material predominantly used in an academic environment typical of Unilorin. In contrast, the 25.2% of thermocol recorded at the Gbagede dumping site is associated with the source of the MSW, which includes industrial waste such as nylon from packaged water companies and other industries within Ilorin metropolis.

Table 2: Physical Classification of Municipal Solid Wastes at University of Ilorin

S/No	Composition	Weight (kg)	Composition by Weight (%)	Volume (10 ³ M ³)	Composition by Volume (%)	Density (Kg/m ³)
1	Food waste (mixed)	25.800	18.400	16.430	25.670	1570.290
2	Inert matter	3.500	2.500	2.042	3.190	1714.010
3	Paper and card board	27.500	19.600	10.56	64.500	2604.170
4	Textile and gunny bags	10.900	7.800	2.290	3.580	4759.830
5	Wood	0.300	0.214	0.200	0.312	1500.000
6	Construction waste	3.250	2.320	8.125	12.690	400.000
7	Glass	15.500	11.060	8.990	14.040	1103.990
8	Leather	8.200	5.850	8.200	12.810	1000.000
9	Metal – Ferrous	19.000	13.600	4.750	7.420	4000.000
10	Metal – Non-Ferrous	4.000	2.850	0.470	0.734	8510.600
11	Other plastics	2.000	1.430	0.170	0.265	7547.170
12	Plastic bags	1.000	0.713	0.220	0.344	4545.450
13	Plastic container	8.700	6.200	0.540	0.844	16111.110
14	Thermocole	10.500	7.500	1.030	1.609	10194.170
TOTAL		72.150	100.040	64.017	148.008	65560.790

Tables 1 and 2 above show that the MSW at the University of Ilorin dumping site could be a valuable source of raw materials for a recycling plant and energy recovery. This conclusion is supported by the percentage composition of the organic and inorganic fractions at the site, which includes a higher percentage of biodegradable matter such as paper, cardboard, and mixed food waste, alongside smaller amounts of non-biodegradable matter like metal, ferrous glass, and thermocol (i.e., nylon) at the University of Ilorin dumping site in Ilorin South Local Government. The high percentage of paper, cardboard, mixed food waste, ferrous metals, glass, and thermocol can be attributed to the student population on campus, which increases the quantity of paper and cardboard generated at the University of Ilorin dumpsite. Thermocol (i.e., nylon), textiles, gunny bags, and food waste originate from various industries in Ilorin, the capital of Kwara State. The Gbagede dumping site, in contrast, is associated with increased population density, consumption patterns, lifestyle behaviors, and economic development. At this site, thermocol, mixed food waste, textiles, and gunny bags dominate the waste stream, which has the highest percentage of organic waste compared to the University of Ilorin dumping site. In countries with high per capita income, organic matter is the major component of waste, followed by inert matter, wood, leather, metal and non-metal ferrous materials, plastic bags, and plastic containers, which reflect the characteristics of the Gbagede dumping site in Ifelodun Local Government Area of Kwara State.

Composition of MSW at the University of Ilorin and Gbagede (GB) Dumpsites

The composition of municipal solid waste (MSW) at the University of Ilorin (UI) and the GB dumpsites was determined through physical sorting and percentage weight analysis. The detailed breakdown is as follows:

- **University of Ilorin (UI) Dumpsite:**
 - Paper and Cardboard: **19.6%**
 - Plastics: **17.2%**
 - Organic Waste: **23.4%**
 - Metals: **9.8%**
 - Glass: **5.5%**
 - Others (e.g., textiles, rubber, e-waste, etc.): **24.5%**
- **Gbagede (GB) Dumpsite:**
 - Thermocol: **25.2%**
 - Plastics: **21.1%**
 - Organic Waste: **19.8%**
 - Metals: **11.3%**
 - Glass: **7.4%**

- Others: **15.2%** These compositions highlight differences based on site activities, with UI having more paper waste due to its academic environment, while GB has more industrial-related waste materials.

Table 3: The percentage weight analysis

Waste Type	University of Ilorin (UI) Dumpsite (%)	Gbagede (GB) Dumpsite (%)
Paper and Cardboard	19.6	-
Thermocol	-	25.2
Plastics	17.2	21.1
Organic Waste	23.4	19.8
Metals	9.8	11.3
Glass	5.5	7.4
Others (e.g., textiles, rubber, e-waste, etc.)	24.5	15.2

Note:

- The **UI Dumpsite** reflects higher paper and organic waste due to academic-related activities.
- The **GB Dumpsite** shows a higher proportion of thermocol and industrial waste, indicative of more commercial/industrial activities in that area.

Proximate and Ultimate Analysis

The low volatile matter at the University of Ilorin ($4.0300 \times 10^{-4}\%$) compared to the higher volatile matter at Oko Olowo ($5.6406 \times 10^{-1}\%$) suggests a lower average ash content (84.36 %) at the University of Ilorin, in contrast to the higher average ash content (89.21 %) at Oko Olowo. The pH value (7.85–8.7) indicates alkalinity, which may be due to the decrease in the concentration of free volatile acids from the anaerobic decomposition of solid waste, as fatty acids can be partially ionized and contribute to higher pH values. Alkalinity in landfills has been linked to the decomposition of carbonate and bicarbonate, leading to the release of large amounts of mineral species into the surrounding soil, resulting in relatively high levels of total dissolved solids and conductivity at the dumping sites (Kanmani, 2012). The moisture content recorded at Oko Olowo and the University of Ilorin dumping sites was (0.3696 ppm and 0.0003 ppm), respectively. The higher moisture content at Oko Olowo is likely due to the disposal of large quantities of vegetable waste over time, compared to the University of Ilorin, where higher volumes of paper and cardboard are recorded (Table 2). The variations in parameters such as volatile matter and fixed carbon (Table 3) are likely due to the different ages of the dumping sites and the varying waste compositions from different regions and locations.

Table 3: Proximate and Ultimate analysis of Unilorin and Olo- Olowo Decomposed Municipal Solid Waste (DMSW)

S/N	Parameters	Result of Sample From UI	Result of Sample From Gbagede (GB)
1	Odour	Sewage Odour	Sewage Odour
2	Colour	Grey	Grey
3	Nature	Sandy loamy	Sandy loamy
4	pH	8.7000	7.8500
5	Conductivity	166 μ /cm	430 μ /cm
6	Total Dissolved Solid	111.2200 mg/l	289.3000 mg/l
7	Bulk Density	1.0007 kg/l	1.0010 kg/l
8	Percent Saturation	0.8588 %	0.1125 %
9	Moisture Content	0.0003 ppm	0.3696
10	Ash Content	89.2100 %	84.3600 %
11	Volatile Matter	0.0004	0.5641
12	Fixed Carbon	10.7800 %	12.3213 %

Conclusion

Based on the findings and conclusions of this study, it was determined that the MSW at both the University of Ilorin (UI) and Gbagede was categorized into biodegradable and non-biodegradable waste. The study revealed a higher concentration of biodegradable matter at UI, while non-biodegradable waste predominated at Gbagede. This implies that the MSW at UI could serve as a valuable source of energy when subjected to aerobic or anaerobic digestion, with the residue after digestion being suitable for use as organic manure. In contrast, the MSW from Gbagede is a good source of raw materials for the recycling industry, given the higher percentage of inorganic waste, particularly thermocol (nylon).

The heavy metals found at both sites classified the MSW as hazardous, as the recorded values exceeded the Federal Ministry of Environment Standards (FMES). Furthermore, the physical classification results align with the pH values of 7.85–8.7 for the decomposed municipal solid waste at both dumping sites, indicating alkalinity. This alkalinity may be attributed to the decrease in the concentration of free volatile acids due to the anaerobic decomposition of the solid waste, as fatty acids can be partially ionized

and contribute to higher pH levels. Alkaline pH is commonly observed at landfills and is associated with the decomposition of carbonate and bicarbonate, which typically leads to the release of a large number of mineral species into the surrounding soil, contributing to relatively high levels of total dissolved solids and conductivity at the dumping sites.

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