

# Antibacterial Potential of Palm Wine (*Raphia hookeri* G. Mann & H. Wendl) against Common Diarrhoeagenic Bacteria

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## ABSTRACT

**Background and Objective:** Diarrhoeagenic bacteria are major causes of bacterial diarrhoea, particularly affecting children under five and the elderly in developing countries. Rising antibiotic resistance among these pathogens necessitates alternative therapies. This study investigated the antibacterial potential of palm wine, a locally fermented drink, against common diarrhoeagenic bacteria: *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella typhi*. **Materials and Methods:** An *in vitro* agar well diffusion assay was used to evaluate the growth-inhibitory effects of freshly tapped and fermented palm wine samples on the test bacteria. The antibiogram profiles of the pathogens, physicochemical properties, and microbial composition of the palm wine were determined using standard methods. Statistical analysis was conducted to assess significance at  $p < 0.05$ . **Results:** Both fresh and fermented palm wine exhibited significant antibacterial activity against all tested diarrhoeagenic bacteria, with inhibition zones ranging from 7.2 to 23.6 mm. The activity was greater than that of several antibiotics in the antibiogram assay, except for ciprofloxacin. *Escherichia coli* and *S. dysenteriae* were more susceptible than *S. typhi*. The pH of palm wine ranged from 3.08 to 5.58 over a 7-day fermentation period. Microbial analysis revealed seven bacterial species, including *Micrococcus* sp., *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus lactis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, and six fungal species, including *Saccharomyces cerevisiae*, *Aspergillus niger*, *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Neurospora crassa*. **Conclusion:** Palm wine demonstrates promising antibacterial activity against common diarrhoeagenic bacteria and may serve as a natural alternative for managing bacterial diarrhoea. Further research is warranted to isolate active compounds and evaluate their therapeutic potential.

## KEYWORDS

Diarrhoeagenic bacteria, palm wine, antibacterial activity

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## INTRODUCTION

Diarrhoeal diseases are a significant public health concern, especially in developing countries where access to safe drinking water and proper sanitation is often limited<sup>1</sup>. These diseases are a leading cause of morbidity and mortality, particularly among young children in low- and middle-income countries<sup>2</sup>. Diarrhoeagenic bacteria, such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp., are among the leading causes of diarrhoeal diseases<sup>3</sup>. Diarrhoeal diseases can lead to severe dehydration,



malnutrition, and even death, particularly in young children and immunocompromised individuals<sup>4</sup>. Although, diarrhoeal diseases can be self limiting however when the aetiology is due to diarrhoeagenic bacteria such as *Escherichia coli*, *Salmonella* spp. and *Shigella* spp., antibiotic therapy is normally employed. However, the increasing resistance of bacteria to conventional antibiotics has become a significant global health challenge<sup>5</sup>. This resistance has been attributed to the overuse and misuse of antibiotics, as well as the ability of bacteria to acquire and transfer resistance genes<sup>6,7</sup>. The emergence and spread of antibiotic-resistant diarrhoeagenic bacteria therefore pose a serious threat to public health, as they can lead to treatment failures, prolonged illness and increased healthcare costs<sup>8</sup>. Therefore, there is a pressing need to explore alternative antimicrobial agents, including those derived from natural sources<sup>9</sup>. In many rural areas where there is no quick access to conventional therapy, different herbs or natural products are explored based on oral traditions.

Palm wine, also known as palm toddy or palm nectar, is a traditional alcoholic beverage obtained from the sap of various palm tree species, primarily the African oil palm (*Elaeis guineensis*), coconut palm (*Cocos nucifera*), and date palm (*Phoenix dactylifera*)<sup>10,11</sup>. Other palm species from which palm wine is produced include the Palmyra palm (*Borassus flabellifer*), Nipa palm (*Nypa fruticans*), Raphia palm (*Raphia hookeri*) and Chilean wine palm (*Jubaea chilensis*), each imparting unique characteristics to the resulting beverage<sup>12</sup>. In traditional medicine systems across different cultures, palm wine has been used for treating various ailments such as malaria, jaundice, measles and hypertension<sup>13</sup>. Palm wine is believed to possess medicinal properties due to its rich nutrient composition, including vitamins, minerals, and bioactive compounds<sup>14</sup>. It is also reported to have cardioprotective effects due to its polyphenol content and ability to increase high-density lipoprotein (HDL) cholesterol levels<sup>15</sup>. Palm wine is also used in some communities to stimulate and boost milk production in lactating mothers and as a nourishing tonic for convalescent patients<sup>16</sup>. Although, palm wine is used to treat many ailments in folk medicine, there is no information on the antidiarrhoeal potential of palm wine. This study therefore evaluates palm wine for possible antibacterial activity against common diarrhoeagenic bacteria in Akure Metropolis, Ondo State, Nigeria in searching for an alternative and more effective therapy to conventional therapy for treating bacterial diarrhoea.

## MATERIALS AND METHODS

**Study area:** This study was conducted in Akure Metropolis, Ondo State, Nigeria. Akure is located in the Southwestern part of Nigeria at approximately 7.25°N Latitude and 5.19°E Longitude, within the tropical rainforest zone. The metropolis experiences a tropical climate with a mean annual rainfall of about 1,500 mm and a mean annual temperature of approximately 26°C. The study was carried out between May and October 2024 at the Department of Microbiology, Federal University of Technology, Akure (FUTA). Palm wine samples were sourced from Raphia palm trees (*Raphia hookeri*) within Akure metropolis, while all laboratory analyses were conducted in the Microbiology research laboratories at FUTA.

**Source of palm wine samples:** Fresh palm wine samples (n = 4) were obtained from Raphia palm trees (*Raphia hookeri*) in Akure Metropolis, Ondo State, Nigeria. The samples were collected using sterilized 1L capacity sample bottles with screw caps to minimize fermentation before laboratory analysis<sup>14</sup>. This collection method has been shown to significantly reduce fermentation rates, preserving the initial characteristics of the palm wine<sup>14</sup>. The palm wine was tapped using traditional methods by local palm wine tappers. Typically, the sap is collected from the cut flower of the palm tree, with a container fastened to the flower stump to collect the sap<sup>10</sup>. The freshly tapped palm wine was immediately transported to the laboratory in an ice cooler to maintain its original properties.

**Source of test organisms:** The diarrhoeagenic bacteria used in this study were obtained from the Bacterial Stock Culture Unit of the Department of Microbiology, Federal University of Technology, Akure. These include *Salmonella typhi*, *Shigella dysenteriae*, and *Escherichia coli*.

**Antibiotics:** The following antibiotics were used for the antibiotic susceptibility profile studies: Chloramphenicol (30 µg), Ciprofloxacin (30 µg), Septrin (30 µg), Amoxicillin (30 µg), Perfloxacin (30 µg), Sparfloxacin (30 µg), Streptomycin (30 µg), Augmentin (30 µg), Gentamycin (30 µg), Tarvid (30 µg). These antibiotics were chosen based on their broad-spectrum activity and common use in treating diarrheal diseases<sup>1</sup>. Stock solutions of antibiotics were prepared according to the Clinical and Laboratory Standards Institute (CLSI) guidelines<sup>17</sup>.

**Determination of the antibiogram profile of the test diarrhoeagenic bacteria:** This was carried out using standard antibiotic discs on the test bacterial species inoculated on Mueller-Hinton agar plates. Standardized bacterial suspensions of  $1 \times 10^8$  CFU/mL determined according to 0.5 McFarland standard were used for the assay. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the zones of inhibition around each antibiotic disc was measured in millimeters.

**Assessment of growth inhibitory activity of palm wine on test diarrhoeagenic bacteria:** The agar well diffusion method was used to assess the growth-inhibitory activity of palm wine on the test organisms. One millilitre (1 mL) of standardized bacterial suspension ( $1 \times 10^8$  CFU/mL) was inoculated into three sterile Petri dishes. Molten Nutrient Agar (20 mL, 45-50°C) was poured into each plate and swirled gently to ensure even distribution of the organisms. After solidification, 6 wells (8 mm in diameter) were bored in each agar plate using a sterile corkborer. Into separate wells, 0.1 mL of each of the four palmwine samples was introduced, one sample per well. Sterile distilled water was put in the fifth well (negative control) while Ciprofloxacin (CPX 30 µg) was used as the positive control. The plates were incubated at 37°C for 18-24 hrs. After incubation, the diameter of the zones of inhibition around each well was measured in millimeters. This assay was repeated every 18-24 hrs for 7 days to determine the effect of fermentation duration on the antibacterial activity of palm wine on the test organisms.

**Isolation, characterization, and total viable counts of microorganisms present in fermenting palm wine bacteria:** Serial dilutions of palm wine samples were prepared ( $10^{-1}$  to  $10^{-6}$ ) using sterile physiological saline. An aliquot of 0.1 mL of appropriate dilutions was pour-plated on Nutrient Agar (NA) and De Man, Rogosa and Sharpe (MRS) agar. NA plates were incubated aerobically at 37°C for 24 hrs, while MRS agar plates were incubated anaerobically using an anaerobic jar with a gas-generating kit at 37°C for 48-72 hrs. After incubation, the total viable bacterial colonies were counted, and distinct colonies were selected and sub-cultured to obtain pure isolates<sup>18</sup>. The pure colonies were characterized using a combination of morphological, physiological, and biochemical tests as described by Cheesbrough<sup>19</sup>.

**Fungi:** Serial dilutions of palm wine samples were prepared as for the isolation of bacterial species. An aliquot of 0.1 mL of appropriate dilutions was pour-plated on Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/L) to inhibit bacterial growth. Plates were incubated at room temperature (25-28°C) for 3-5 days. After incubation, total viable fungal colonies were counted, and distinct fungal colonies were selected and sub-cultured on fresh PDA plates to obtain pure isolates. Pure cultures were maintained on PDA slants at 4°C for characterization<sup>12</sup>. The fungal isolates were characterized using a combination of morphological and physiological methods as described by Kurtzman *et al.*<sup>20</sup> and Samson<sup>21</sup>.

**Evaluation of microbial succession in fermenting palm wine:** A microbial succession study was conducted to understand the changes in microbial populations during palm wine fermentation. The methods of Santiago-Urbina *et al.*<sup>22</sup> and Oluwole *et al.*<sup>23</sup> were adapted for the evaluation.

**pH determination:** The pH of palm wine samples was measured using a digital pH meter (Hanna Instruments, USA) following the method described by AOAC<sup>24</sup>.

**Total titratable acidity (TTA) determination:** The total titratable acidity was determined using the method described by Nielsen<sup>25</sup>. The TTA measurements were taken at 24 hrs intervals for 7 days to track changes during fermentation.

**Statistical analysis:** All experiments were performed in triplicate, and the data were expressed as Mean  $\pm$  Standard Deviation (SD) to represent the variability of the measurements. The mean value provides an estimate of the central tendency, while the standard deviation quantifies the spread or dispersion of the data around the mean. One-way Analysis of Variance (ANOVA) was used to determine if there were significant differences among the treatments or experimental groups. ANOVA is a statistical method used to compare the means of three or more independent groups by analyzing the variance within and between the groups.

Statistical significance was set at  $p < 0.05$ , and means with the same superscript letter in a column were not considered significantly different.

## RESULTS

**Antibiogram profiles of the test diarrhoeagenic bacteria:** Out of all the antibiotics tested on the test diarrhoeagenic bacteria, Ciprofloxacin exerted the highest growth inhibitory activity on all of them, with values ranging from 17.0 mm for *E. coli* to 28.0 mm for *Sh. dysenteriae* (Table 1). Antibiotics like Chloramphenicol, Amoxicillin, Streptomycin, Augmentin, and Gentamycin did not inhibit the growth of *E. coli*; Septrin did not inhibit the growth of *S. typhi*, while Chloramphenicol, Septrin, Amoxicillin, Streptomycin and Augmentin did not inhibit the growth of *Sh. dysenteriae*.

The antibiotic susceptibility profiles of the test organisms are presented in Table 1. The results showed varied responses of the three bacterial pathogens to the ten antibiotics tested. Ciprofloxacin demonstrated the highest inhibition zones against all three organisms, with 17.0 mm against *E. coli*, 19.0 mm against *Salmonella typhi*, and 28.0 mm against *Shigella dysenteriae*. Tarivid also showed broad-spectrum activity with inhibition zones of 13.0 mm (*E. coli*), 15.0 mm (*S. typhi*), and 10.0 mm (*S. dysenteriae*). Perfloxacin exhibited moderate activity against all tested organisms (8.0-14.0 mm). Sparfloxacin showed activity against *E. coli* (10.0 mm), *S. typhi* (8.0 mm), and *S. dysenteriae* (10.0 mm). However, several antibiotics demonstrated organism-specific activity or complete resistance. Septrin showed activity only against *E. coli* (10.0 mm), while Chloramphenicol, Amoxicillin, Streptomycin, and Augmentin displayed activity against *S. typhi* alone, with zones of inhibition ranging from 4.0-10.0 mm. Gentamycin showed limited activity against *S. typhi* (8.0 mm) and *S. dysenteriae* (5.0 mm). The varying susceptibility patterns indicate differential antibiotic resistance among the test organisms.

**Growth inhibitory effect of palm wine on common diarrhoeagenic bacteria:** The palm wine samples used in this study demonstrated growth inhibition of all the test diarrhoeagenic bacteria. The observed zones of inhibition ranged from 8.20 to 23.10 mm on *E. coli*, 7.30 to 21.80 mm on *S. typhi* and 7.20 to 23.60 mm on *Shigella dysenteriae* (Table 2-4, respectively). The highest growth inhibitory effect was observed by day 7 fermented palm wine on the test organisms. The growth inhibition mediated by the palm wine samples was superior to that mediated by antibiotics like Chloramphenicol, Amoxicillin,

Table 1: Antibiotic susceptibility profiles of *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae*

Antibiotics	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Shigella dysenteriae</i>
Septrin	10.0	0.0	0.0
Chloramphenicol	0.0	8.0	0.0
Sparfloxacin	10.0	8.0	10.0
Amoxicillin	0.0	10.0	0.0
Perfloxacin	8.0	12.0	14.0
Streptomycin	0.0	10.0	0.0
Ciprofloxacin	17.0	19.0	28.0
Augmentin	0.0	4.0	0.0
Gentamycin	0.0	8.0	5.0
Tarivid	13.0	15.0	10.0

Table 2: Growth inhibitory effects of fermenting palm wine on *E. coli*

Treatment	Diameter zones of inhibition (mm)/duration of fermentation (days)						
	1	2	3	4	5	6	7
PW1	8.50±0.20 <sup>b</sup>	10.30±0.20 <sup>b</sup>	12.70±0.20 <sup>b</sup>	14.93±0.25 <sup>b</sup>	16.43±0.25 <sup>b</sup>	18.53±0.25 <sup>c</sup>	20.23±0.25 <sup>c</sup>
PW2	8.20±0.20 <sup>b</sup>	10.00±0.20 <sup>b</sup>	12.30±0.20 <sup>b</sup>	14.50±0.20 <sup>b</sup>	16.00±0.20 <sup>b</sup>	17.40±0.20 <sup>c</sup>	19.80±0.20 <sup>c</sup>
PW3	8.20±0.20 <sup>b</sup>	9.70±0.20 <sup>b</sup>	12.00±0.20 <sup>b</sup>	14.10±0.20 <sup>b</sup>	17.60±0.20 <sup>c</sup>	18.70±0.20 <sup>c</sup>	20.40±0.20 <sup>c</sup>
PW4	8.70±0.20 <sup>b</sup>	10.20±0.20 <sup>b</sup>	13.60±0.30 <sup>b</sup>	15.80±0.30 <sup>b</sup>	17.30±0.30 <sup>c</sup>	19.40±0.30 <sup>c</sup>	23.10±0.30 <sup>c</sup>
CPX	16.30±0.32 <sup>c</sup>	16.32±0.40 <sup>c</sup>	15.60±0.98 <sup>c</sup>	16.40±0.45 <sup>c</sup>	17.00±0.66 <sup>c</sup>	16.40±0.26 <sup>b</sup>	16.80±0.35 <sup>b</sup>
WATER	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

PW1: Palm wine sample 1, PW2: Palm wine sample 2, PW3: Palm wine sample 3, PW4: Palm wine sample 4, CPX: Ciprofloxacin, Results were expressed in Mean±Standard deviation of three replicates, and means with same superscript in a column are not significantly different ( $p>0.05$ )

Table 3: Growth inhibitory effects of fermenting palm wine on *S. typhi*

Treatment	Diameter zones of inhibition (mm)/duration of fermentation (days)						
	1	2	3	4	5	6	7
PW1	7.80±0.20 <sup>b</sup>	9.50±0.20 <sup>b</sup>	11.80±0.20 <sup>b</sup>	13.70±0.20 <sup>b</sup>	15.20±0.20 <sup>b</sup>	16.80±0.20 <sup>b</sup>	19.70±0.20 <sup>c</sup>
PW2	7.30±0.20 <sup>b</sup>	9.20±0.20 <sup>b</sup>	11.00±0.20 <sup>b</sup>	13.80±0.20 <sup>b</sup>	14.60±0.20 <sup>b</sup>	17.30±0.20 <sup>b</sup>	21.50±0.20 <sup>c</sup>
PW3	7.30±0.20 <sup>b</sup>	8.90±0.20 <sup>b</sup>	11.00±0.20 <sup>b</sup>	12.90±0.20 <sup>b</sup>	14.20±0.20 <sup>b</sup>	17.30±0.20 <sup>b</sup>	21.80±0.20 <sup>c</sup>
PW4	8.70±0.20 <sup>b</sup>	9.40±0.30 <sup>b</sup>	11.60±0.30 <sup>b</sup>	13.40±0.30 <sup>b</sup>	17.20±0.30 <sup>b</sup>	18.90±0.30 <sup>c</sup>	21.80±0.20 <sup>c</sup>
CPX	18.20±0.81 <sup>c</sup>	18.80±0.90 <sup>c</sup>	17.60±0.70 <sup>c</sup>	18.40±0.58 <sup>c</sup>	19.00±0.20 <sup>c</sup>	18.40±0.82 <sup>b</sup>	17.90±0.73 <sup>b</sup>
WATER	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

PW1: Palm wine sample 1, PW2: Palm wine sample 2, PW3: Palm wine sample 3, PW4: Palm wine sample 4, CPX: Ciprofloxacin, Results were expressed in Mean±Standard deviation of three replicates, and means with same superscript in a column are not significantly different ( $p>0.05$ )

Streptomycin, Augmentin and Gentamycin on the test organisms (Table 1 compared with Table 2) but not as strong as the one mediated by ciprofloxacin (CPX) except for the growth inhibition observed with days 5 to 7 fermented palm wine samples on *E. coli* (Table 2) and day 7 fermented palm wine samples on *S. typhi* (Table 3).

Table 2 shows the antibacterial activity of palm wine samples (PW1-PW4) against *E. coli* across the 7 day fermentation period. The inhibition zones increased progressively with fermentation duration for all palm wine samples. PW1 showed zones of inhibition ranging from 8.50±0.20 mm on day 1 to 20.23±0.25 mm on day 7. PW2 exhibited similar patterns with zones from 8.20±0.20 mm (day 1) to 19.80±0.20 mm (day 7). PW3 demonstrated zones from 8.20±0.20 to 20.40±0.20 mm, while PW4 showed the highest activity, increasing from 8.70±0.20 to 23.10±0.30 mm. The positive control, Ciprofloxacin (CPX), maintained consistently high inhibition zones ranging from 15.60±0.98 mm to 17.00±0.66 mm throughout the study period. The negative control (sterile water) showed no inhibition (0.00±0.00 mm) across all days. Statistical analysis revealed significant differences (superscript letters a, b, c indicate significance groups) between treatments and across fermentation days. The consistent increase in antibacterial activity with fermentation suggests accumulation of antimicrobial compounds during the fermentation process.

The antibacterial activity of palm wine samples against *Salmonella typhi* is shown in Table 3. All palm wine samples demonstrated time-dependent increases in inhibition zones throughout the fermentation period. The PW1 exhibited zones ranging from 7.80±0.20 mm on day 1 to 19.70±0.20 mm on day 7. The PW2 showed inhibition zones from 7.30±0.20 mm to 21.50±0.20 mm, while PW3 displayed zones from 7.30±0.20 to 21.80±0.20 mm. The PW4 demonstrated zones ranging from 8.70±0.20 to 21.80±0.20 mm across the fermentation duration. The positive control, Ciprofloxacin, exhibited high and relatively stable inhibition zones throughout the study, ranging from 17.60±0.70 to 19.00±0.20 mm. Sterile water (negative control) showed no inhibitory activity (0.00±0.00 mm) at any time point. Statistically significant differences were observed between treatments and fermentation days, as indicated by the superscript letters. The progressive increase in antibacterial efficacy suggests enhanced production or concentration of antimicrobial compounds in the fermenting palm wine.

Table 4: Growth inhibitory effect of fermenting palm wine on *Shigella dysenteriae*

Treatment	Diameter zones of inhibition (mm)/duration of fermentation (days)						
	1	2	3	4	5	6	7
PW1	7.20±0.20 <sup>b</sup>	9.50±0.20 <sup>b</sup>	11.80±0.20 <sup>b</sup>	15.20±0.20 <sup>b</sup>	17.70±0.20 <sup>b</sup>	19.80±0.20 <sup>b</sup>	22.50±0.20 <sup>b</sup>
PW2	8.80±0.20 <sup>b</sup>	10.30±0.20 <sup>b</sup>	13.40±0.20 <sup>b</sup>	15.70±0.20 <sup>b</sup>	17.30±0.20 <sup>b</sup>	18.40±0.20 <sup>b</sup>	20.10±0.20 <sup>b</sup>
PW3	8.20±0.20 <sup>b</sup>	10.70±0.20 <sup>b</sup>	13.30±0.20 <sup>b</sup>	15.20±0.20 <sup>b</sup>	18.80±0.20 <sup>b</sup>	21.90±0.20 <sup>b</sup>	23.60±0.20 <sup>b</sup>
PW4	9.00±0.20 <sup>b</sup>	11.00±0.30 <sup>b</sup>	13.70±0.30 <sup>b</sup>	16.10±0.30 <sup>b</sup>	19.60±0.30 <sup>b</sup>	21.70±0.30 <sup>b</sup>	23.60±0.30 <sup>b</sup>
CPX	26.80±0.45 <sup>c</sup>	27.90±0.87 <sup>c</sup>	26.60±0.75 <sup>c</sup>	27.30±0.90 <sup>c</sup>	26.40±0.70 <sup>c</sup>	27.80±0.83 <sup>c</sup>	27.20±0.85 <sup>c</sup>
WATER	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>

PW1: Palm wine sample 1, PW2: Palm wine sample 2, PW3: Palm wine sample 3, PW4: Palm wine sample 4, CPX: Ciprofloxacin, Results were expressed in Mean±Standard deviation of three replicates, and means with same superscript in a column are not significantly different ( $p > 0.05$ )

Table 5: Bacterial and fungal loads of the palmwine samples fermented for 7 days (Sample A)

Fermentation duration (days)	Bacterial load CFU/mL	Fungal load SFU/mL
1	$1.1 \times 10^7$	$2.5 \times 10^4$
2	$1.1 \times 10^7$	$2.9 \times 10^4$
3	$8.2 \times 10^6$	$3.7 \times 10^4$
4	$7.4 \times 10^6$	$4.2 \times 10^4$
5	$7.9 \times 10^6$	$1.7 \times 10^4$
6	$7.1 \times 10^6$	$2.3 \times 10^4$
7	$6.3 \times 10^6$	$2.6 \times 10^4$

CFU: Colony forming units and SFU: Spore forming units

Table 6: Bacterial and fungal loads of the palm wine samples fermented for 7 days (Sample B)

Fermentation duration (days)	Bacterial load CFU/mL	Fungal load SFU/mL
1	$1.2 \times 10^7$	$1.9 \times 10^4$
2	$1.1 \times 10^7$	$3.1 \times 10^4$
3	$7.7 \times 10^6$	$3.6 \times 10^4$
4	$7.4 \times 10^6$	$4.5 \times 10^4$
5	$8.1 \times 10^6$	$1.5 \times 10^4$
6	$7.8 \times 10^6$	$2.1 \times 10^4$
7	$6.5 \times 10^6$	$2.9 \times 10^4$

Table 4 indicates the antibacterial activity of palm wine samples against *Shigella dysenteriae* over the 7-day fermentation period. All palm wine samples showed progressive increases in inhibition zones as fermentation advanced. The PW1 exhibited zones from 7.20±0.20 mm (day 1) to 22.50±0.20 mm (day 7). The PW2 demonstrated zones ranging from 8.80±0.20 to 20.10±0.20 mm, while PW3 showed zones from 8.20±0.20 to 23.60±0.20 mm. The PW4 displayed zones from 9.00±0.20 to 23.60±0.30 mm. Notably, *Shigella dysenteriae* showed the highest susceptibility to the palm wine samples among the three test organisms, with the largest inhibition zones recorded. Ciprofloxacin (positive control) exhibited very high antibacterial activity with inhibition zones ranging from 26.40±0.70 to 27.90±0.87 mm throughout the study period. The negative control showed no activity (0.00±0.00 mm) across all days. Statistical analysis revealed significant differences (indicated by superscript letters a, b, c) among treatments and fermentation days. The enhanced activity against *S. dysenteriae* suggests possible organism-specific susceptibility to the antimicrobial compounds produced during palm wine fermentation.

**Total microbial load of palm wine samples:** The total bacterial and fungal loads obtained from the palm wine samples (A, B, C, and D) monitored over a period of 7 days of fermentation duration ranged from  $5.7 \times 10^6$  to  $1.2 \times 10^7$  CFU/mL, while the fungal counts ranged from  $1.2 \times 10^4$  to  $4.5 \times 10^4$  SFU/mL (Table 5-8). The bacterial loads of all the palm wine samples gradually reduced from day 1 to day 7, while the fungal loads peaked by day 4 before it starts to decline.

The microbial population dynamics during the fermentation of palm wine sample 1 (PW1) are presented in Table 5. The bacterial load showed a declining trend from day 1 ( $1.1 \times 10^7$  CFU/mL) to day 7 ( $6.3 \times 10^6$  CFU/mL). A notable decrease was observed between day 2 ( $1.1 \times 10^7$  CFU/mL) and day 3 ( $8.2 \times 10^6$  CFU/mL), after which the bacterial count remained relatively stable with slight fluctuations

Table 7: Bacterial and fungal loads of the palm wine samples fermented for 7 days (Sample C)

Fermentation duration (day)	Bacterial load CFU/mL	Fungal load SFU/mL
1	$1.2 \times 10^7$	$2.1 \times 10^4$
2	$9.8 \times 10^6$	$2.8 \times 10^4$
3	$7.2 \times 10^6$	$3.3 \times 10^4$
4	$6.8 \times 10^6$	$3.9 \times 10^4$
5	$7.7 \times 10^6$	$1.2 \times 10^4$
6	$7.2 \times 10^6$	$2.4 \times 10^4$
7	$5.7 \times 10^6$	$2.5 \times 10^4$

CFU: Colony forming units and SFU: Spore forming units

Table 8: Bacterial and fungal loads of the palm wine samples fermented for 7 days (Sample D)

Fermentation duration (day)	Bacterial load CFU/mL	Fungal load SFU/mL
1	$1.19 \times 10^7$	$2.2 \times 10^4$
2	$1.03 \times 10^7$	$3.0 \times 10^4$
3	$7.9 \times 10^6$	$3.8 \times 10^4$
4	$7.4 \times 10^6$	$4.2 \times 10^4$
5	$8.1 \times 10^6$	$1.2 \times 10^4$
6	$7.5 \times 10^6$	$2.3 \times 10^4$
7	$6.3 \times 10^6$	$2.8 \times 10^4$

CFU: Colony forming units and SFU: Spore forming units

between  $6.3 \times 10^6$  and  $7.9 \times 10^6$  CFU/mL through day 7. The fungal load exhibited a different pattern, with an initial increase from  $2.5 \times 10^4$  SFU/mL (day 1) to a peak of  $4.2 \times 10^4$  SFU/mL on day 4, followed by a decline to  $1.7 \times 10^4$  SFU/mL on day 5, and subsequent fluctuations to  $2.6 \times 10^4$  SFU/mL by day 7. The overall reduction in bacterial population may be attributed to the acidic conditions and production of antimicrobial metabolites during fermentation, while the fungal population dynamics reflect the changing biochemical environment of the fermenting palm wine.

Table 6 shows the microbial dynamics during the fermentation of palm wine sample 2 (PW2). The bacterial load exhibited a decreasing trend from an initial count of  $1.2 \times 10^7$  CFU/mL on day 1 to  $6.5 \times 10^6$  CFU/mL on day 7. A significant reduction occurred between day 2 ( $1.1 \times 10^7$  CFU/mL) and day 3 ( $7.7 \times 10^6$  CFU/mL), after which the bacterial population remained relatively stable with minor variations between  $6.5 \times 10^6$  and  $8.1 \times 10^6$  CFU/mL. The fungal load showed variable patterns, starting at  $1.9 \times 10^4$  SFU/mL on day 1, reaching a maximum of  $4.5 \times 10^4$  SFU/mL on day 4, declining to  $1.5 \times 10^4$  SFU/mL on day 5, and ending at  $2.9 \times 10^4$  SFU/mL on day 7. Similar to PW1, the decline in bacterial population in PW2 likely resulted from accumulation of organic acids and other antimicrobial metabolites, while fungal population fluctuations reflect adaptation to the changing fermentation environment.

The microbial population changes during fermentation of palm wine sample 3 (PW3) are presented in Table 7. The bacterial count decreased from  $1.2 \times 10^7$  CFU/mL on day 1 to  $5.7 \times 10^6$  CFU/mL on day 7, representing the lowest final bacterial count among all samples. A sharp decline was observed between day 1 ( $1.2 \times 10^7$  CFU/mL) and day 2 ( $9.8 \times 10^6$  CFU/mL), with further reduction to  $7.2 \times 10^6$  CFU/mL by day 3. The bacterial load remained relatively stable between days 3 and 7, fluctuating between  $5.7 \times 10^6$  and  $7.7 \times 10^6$  CFU/mL. The fungal population started at  $2.1 \times 10^4$  SFU/mL on day 1, peaked at  $3.9 \times 10^4$  SFU/mL on day 4, decreased to its lowest level of  $1.2 \times 10^4$  SFU/mL on day 5, and stabilized at approximately  $2.4$ - $2.5 \times 10^4$  SFU/mL by day 7. The pronounced bacterial reduction in PW3 suggests possibly higher antimicrobial activity or more rapid acidification compared to other samples.

Table 8 shows the microbial dynamics during fermentation of palm wine sample 4 (PW4). The bacterial load declined from  $1.19 \times 10^7$  CFU/mL on day 1 to  $6.3 \times 10^6$  CFU/mL on day 7. The reduction followed a pattern similar to other samples, with a notable decrease between day 1 and day 2 ( $1.03 \times 10^7$  CFU/mL), followed by continued decline to  $7.9 \times 10^6$  CFU/mL on day 3. The bacterial count then stabilized, fluctuating

Table 9: Types of microorganisms isolated from each palm wine samples

Microorganisms	Sample			
	A	B	C	D
<i>Leuconostoc mesenteroides</i>	+	-	-	-
<i>Lactobacillus plantarum</i>	+	-	+	+
<i>Bacillus subtilis</i>	+	+	-	-
<i>Lactobacillus lactis</i>	-	+	-	-
<i>Proteus vulgaris</i>	-	+	-	+
<i>Pseudomonas aeruginosa</i>	-	-	-	-
<i>Micrococcus sp.</i>	+	+	-	-
<i>Aspergillus fumigatus</i>	+	-	-	+
<i>Saccharomyces cerevisiae</i>	+	+	+	+
<i>Penicillium sp.</i>	-	+	+	-
<i>Neurospora crassa</i>	-	+	-	+
<i>Aspergillus niger</i>	+	-	-	-
<i>Aspergillus flavus</i>	-	-	+	-
Total	6	6	6	5

+: Positive and -: Negative

Table 10: Microbial succession in fermenting palm wine (Sample A)

Microorganisms	Fermentation (days)						
	1	2	3	4	5	6	7
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	-	+	-	-	-	-	-
<i>Penicillium sp.</i>	+	-	-	-	-	-	-
<i>Lactobacillus lactis</i>	-	-	+	+	-	+	-
<i>Micrococcus sp.</i>	-	+	-	-	-	-	-
<i>Aspergillus flavus</i>	-	-	+	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-
<i>Neurospora crassa</i>	-	-	-	-	+	-	-
<i>Lactobacillus plantarum</i>	-	-	-	-	-	+	+
<i>Leuconostoc mesenteroides</i>	-	-	-	-	-	+	-
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	+
<i>Bacillus subtilis</i>	-	-	+	+	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-
Total = 13							

+: Positive and -: Negative

between  $6.3 \times 10^6$  and  $8.1 \times 10^6$  CFU/mL from days 4 to 7. The fungal load showed an initial value of  $2.2 \times 10^4$  SFU/mL on day 1, increased to peak at  $4.2 \times 10^4$  SFU/mL on day 4, dropped sharply to  $1.2 \times 10^4$  SFU/mL on day 5, and then increased to  $2.8 \times 10^4$  SFU/mL by day 7. The bacterial and fungal population trends in PW4 were consistent with observations in other samples, confirming the reproducibility of fermentation dynamics across different palm wine batches.

**Types of microorganisms isolated from fermenting palm wine samples:** A total of 7 bacterial species and 6 fungal species were isolated. The bacterial species include: *Micrococcus sp.*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus lactis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The fungal species on the other hand, include: *Saccharomyces cerevisiae*, *Aspergillus niger*, *Penicillium notatum*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Neurospora crassa*. The types of microorganisms present in each palm wine sample are shown in Table 9. Only *Saccharomyces cerevisiae* was found in all the palm wine samples.

The diversity of microorganisms isolated from the four palm wine samples at the onset of fermentation is indicated in Table 9. A total of 13 different microbial species were identified across the four samples, comprising both bacteria and fungi. All four samples contained *Saccharomyces cerevisiae* and showed

Table 11: Microbial succession in fermenting palm wine (Sample B)

Microorganisms	Fermentation (days)						
	1	2	3	4	5	6	7
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+
<i>Penicillium</i> sp.	-	-	-	+	-	-	-
<i>Lactobacillus lactis</i>	-	-	+	+	-	+	-
<i>Micrococcus</i> sp.	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	-	-	+	-	-	-	-
<i>Aspergillus niger</i>	+	+	+	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-
<i>Neurospora crassa</i>	-	-	+	-	-	-	-
<i>Lactobacillus plantarum</i>	-	-	-	-	-	+	+
<i>Leuconostoc mesenteroides</i>	-	-	-	-	-	+	-
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	+
<i>Bacillus subtilis</i>	-	-	-	+	+	-	-
<i>Proteus vulgaris</i>	+	-	-	-	-	-	-
Total=	13						

+: Positive and -: Negative

similar total microbial counts (5-6 species per sample). Sample A yielded 6 species including *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Aspergillus fumigatus*, *S. cerevisiae*, and *Aspergillus niger*. Sample B also contained 6 species, including *Bacillus subtilis*, *Lactobacillus lactis*, *Proteus vulgaris*, *S. cerevisiae*, *Penicillium* sp., and *Neurospora crassa*. Sample C contained 6 species including *Lactobacillus plantarum*, *Pseudomonas aeruginosa*, *Micrococcus* sp., *S. cerevisiae*, *Penicillium* sp., and *Aspergillus flavus*. Sample D yielded 5 species: *Lactobacillus plantarum*, *Proteus vulgaris*, *Aspergillus fumigatus*, *S. cerevisiae*, and *Neurospora crassa*. The presence of *S. cerevisiae* in all samples confirms its central role in palm wine fermentation, while the variety of other microorganisms suggests a complex microbial ecosystem contributing to fermentation and metabolite production.

**Succession of the isolated microorganisms in the palm wine samples:** The succession pattern of isolated microorganisms in fermenting palmwine samples over a 168 hrs (7 day) fermentation period revealed a dynamic microbial community (Table 10-13). *Saccharomyces cerevisiae* emerged as a key player in the fermentation process, being consistently present from day 1 throughout the 7 day fermentation period in all the samples. This underscores its crucial role in palm wine fermentation. *Lactobacilli*, particularly *Lactobacillus lactis* and *L. plantarum*, became apparent by day 3 and day 6, respectively during palmwine fermentation, although in Sample D, they appeared later (day 5).

The temporal distribution of microorganisms during the fermentation of palm wine sample 1 (PW1) is shown in Table 10. *Saccharomyces cerevisiae* was present throughout the entire fermentation period (days 1-7), confirming its dominance as the primary fermenting organism. *Penicillium* sp. was detected only on day 1, while *Aspergillus niger* appeared briefly on day 2. *Aspergillus flavus* was present on day 3, and *Neurospora crassa* appeared on day 5. *Lactobacillus lactis* showed intermittent presence on days 3, 4, and 6. *Lactobacillus plantarum* emerged late in fermentation (days 6-7), as did *Leuconostoc mesenteroides* (day 6) and *Aspergillus fumigatus* (day 7). *Bacillus subtilis* was present on days 3 and 4. *Micrococcus* sp. appeared only on day 2, while *Pseudomonas aeruginosa* and *Proteus vulgaris* were not detected at any time point during fermentation. The succession pattern indicates early dominance by yeasts and fungi, with lactic acid bacteria appearing later, likely as the environment became more acidic.

Table 11 shows the microbial succession pattern during fermentation of palm wine sample 2 (PW2). *Saccharomyces cerevisiae* remained consistently present throughout all seven days of fermentation, underscoring its essential role in the fermentation process. *Aspergillus niger* was detected during the early fermentation phase (days 1-3), while *Proteus vulgaris* appeared only on day 1. *Penicillium* sp. was present on day 4, and *Aspergillus flavus* appeared on day 3. *Lactobacillus lactis* showed presence on days 3, 4,

Table 12: Microbial succession in fermenting palm wine (Sample C)

Microorganisms	Fermentation (days)						
	1	2	3	4	5	6	7
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	-	+	-	-	-	-	-
<i>Penicillium</i> sp.	-	+	-	-	-	+	-
<i>Lactobacillus lactis</i>	-	-	+	-	-	+	+
<i>Micrococcus</i> sp.	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	-	+	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	+	+	-	-	-
<i>Neurospora crassa</i>	-	-	+	-	+	-	-
<i>Lactobacillus plantarum</i>	-	-	-	-	-	+	+
<i>Leuconostoc mesenteroides</i>	-	-	-	-	+	+	-
<i>Aspergillus fumigatus</i>	-	-	-	-	-	+	+
<i>Bacillus subtilis</i>	-	+	-	+	-	-	-
<i>Proteus vulgaris</i>	-	+	-	-	-	-	-
Total=	13						

+: Positive and -: Negative

Table 13: Microbial succession in fermenting palm wine (Sample D)

Microorganisms	Fermentation (days)						
	1	2	3	4	5	6	7
<i>Saccharomyces cerevisiae</i>	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	-	-	-	-	-	-	-
<i>Penicillium</i> sp.	+	-	+	-	-	-	-
<i>Lactobacillus lactis</i>	-	-	-	-	+	+	-
<i>Micrococcus</i> sp.	-	+	-	-	-	-	-
<i>Aspergillus flavus</i>	-	-	+	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-
<i>Neurospora crassa</i>	-	-	-	-	+	-	-
<i>Lactobacillus plantarum</i>	-	-	-	-	+	+	+
<i>Leuconostoc mesenteroides</i>	-	-	-	-	-	+	-
<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	+
<i>Bacillus subtilis</i>	-	-	-	+	+	-	-
<i>Proteus vulgaris</i>	+	-	-	-	-	-	-
Total=	13						

+: Positive and -: Negative

and 6, similar to the pattern observed in PW1. *Neurospora crassa* was detected on day 3, while *Bacillus subtilis* appeared on days 4 and 5. *Lactobacillus plantarum* emerged late in fermentation (days 6-7), consistent with observations in PW1. *Leuconostoc mesenteroides* appeared on day 6, and *Aspergillus fumigatus* was detected on day 7. *Micrococcus* sp. and *Pseudomonas aeruginosa* were not detected during the fermentation of PW2. The succession pattern in PW2 was generally similar to PW1, indicating reproducible microbial dynamics across different palm wine batches.

The microbial succession during the fermentation of palm wine sample 3 (PW3) is demonstrated in Table 12. *Saccharomyces cerevisiae* maintained a continuous presence throughout the 7-day fermentation period, consistent with observations in PW1 and PW2. Several organisms showed distinct temporal patterns: *Aspergillus niger* and *Aspergillus flavus* appeared on day 2, while *Proteus vulgaris* was also detected on day 2. *Penicillium* sp. was present on days 2 and 6, showing an interrupted distribution pattern. *Pseudomonas aeruginosa* appeared on days 3 and 4, unique to this sample. *Lactobacillus lactis* was detected on days 3, 6, and 7. *Neurospora crassa* showed presence on days 3 and 5, while *Bacillus subtilis* appeared on days 2 and 4. *Leuconostoc mesenteroides* was detected on days 5 and 6. *Aspergillus fumigatus* appeared late in fermentation on days 6 and 7, as did *Lactobacillus plantarum* (days 6-7). *Micrococcus* sp. was not detected in PW3. The presence of *P. aeruginosa* distinguishes this sample's microbial community from PW1 and PW2, suggesting some variability in the initial microbial ecosystem.

Table 14: pH and titratable acidity profiles of palm wine samples (Sample A)

Fermentation duration (Day)	pH	Titratable acidity (%)
1	5.55	0.95
2	4.93	1.58
3	4.29	2.66
4	3.96	3.38
5	3.70	4.09
6	3.44	4.82
7	3.17	5.54

Table 15: pH and titratable acidity profiles of palm wine samples (Sample B)

Fermentation duration (Day)	pH	Titratable acidity (%)
1	5.51	0.99
2	4.89	1.62
3	4.25	2.70
4	3.92	3.42
5	3.67	4.14
6	3.41	4.86
7	3.14	5.58

Table 13 shows the temporal distribution of microorganisms during fermentation of palm wine sample 4 (PW4). Similar to all previous samples, *Saccharomyces cerevisiae* was present continuously throughout the fermentation period, confirming its universal and consistent role in palm wine fermentation. *Proteus vulgaris* and *Penicillium* sp. appeared early on day 1, with *Penicillium* sp. reappearing on day 3. *Micrococcus* sp. was briefly detected on day 2. *Aspergillus flavus* appeared on day 3, while *Bacillus subtilis* was present on days 4 and 5. *Lactobacillus lactis* and *Neurospora crassa* both appeared on day 5. *Lactobacillus plantarum* showed late-stage emergence (days 5-7), consistent with patterns observed in other samples. *Leuconostoc mesenteroides* was detected on day 6, and *Aspergillus fumigatus* appeared on day 7. *Aspergillus niger* and *Pseudomonas aeruginosa* were not detected in PW4 at any fermentation stage. The microbial succession in PW4 followed similar general patterns to other samples, with early yeast dominance followed by bacterial colonization, though with some sample-specific variations in the timing and presence of particular species.

**Physicochemical properties of palm wine samples during fermentation:** The pH of the palm wine samples ranged from 3.08 to 5.58 over the seven-day fermentation period. The pH of sample A decreased from 5.55 by day 1 to 3.17 by day 7, while the TTA increased from 0.95% by day 1 to 5.54% by day 7 (Table 14). Similar trends were also observed in the pH and TTA of all the other palm wine samples (Table 15-17).

The physicochemical changes during the fermentation of palm wine sample 1 (PW1) are accessible in Table 14. The pH showed a progressive decline throughout the fermentation period, decreasing from 5.55 on day 1 to 3.17 on day 7. The most significant pH drop occurred during the first three days (5.55 to 4.29), after which the decline continued at a slower rate. Titratable acidity exhibited an inverse relationship with pH, increasing progressively from 0.95% on day 1 to 5.54% on day 7. The acidity increase was most pronounced between days 1 and 3 (0.95% to 2.66%), corresponding to the period of rapid pH decline. The steady increase in acidity and concurrent pH reduction are characteristic of fermentation processes, resulting from the production of organic acids (primarily lactic acid and acetic acid) by fermenting microorganisms. These changes create an increasingly acidic environment that contributes to the antimicrobial properties of the fermenting palm wine and helps preserve the beverage.

The pH and titratable acidity changes during fermentation of palm wine sample 2 (PW2) as indicated in Table 15. The pH decreased steadily from an initial value of 5.51 on day 1 to 3.14 on day 7. The rate of pH decline was most rapid during the early fermentation phase (days 1-3), decreasing from 5.51 to 4.25, then continued to decline more gradually to 3.14 by day 7. Titratable acidity showed a corresponding increase

Table 16: pH and titratable acidity profiles of palm wine samples (Sample C)

Fermentation duration (Day)	pH	Titratable acidity (%)
1	5.58	0.90
2	4.95	1.58
3	4.29	2.61
4	3.98	3.33
5	3.72	4.05
6	3.46	4.77
7	3.19	5.49

Table 17: pH and titratable acidity profiles of palm wine samples (Sample D)

Fermentation duration (Day)	pH	Titratable acidity (%)
1	5.43	1.08
2	4.76	1.80
3	4.12	2.88
4	3.85	3.60
5	3.59	4.32
6	3.32	5.04
7	3.08	5.76

from 0.99% on day 1 to 5.58% on day 7. The acidity increase was most pronounced during the first three days (0.99 to 2.70%), paralleling the rapid pH decline. The trends observed in PW2 were very similar to those in PW1, with only slight numerical differences, indicating consistent fermentation biochemistry across samples. The acidification pattern reflects active microbial metabolism and organic acid production, which are essential for the characteristic taste and antimicrobial properties of palm wine.

The physicochemical parameters during fermentation of palm wine sample 3 (PW3) are shown in Table 16. The pH declined from 5.58 on day 1 to 3.19 on day 7, following a pattern consistent with PW1 and PW2. The most rapid pH decrease occurred between days 1 and 3 (5.58 to 4.29), after which the decline continued more gradually. Titratable acidity increased correspondingly from 0.90% on day 1 to 5.49% on day 7. The steepest increase in acidity occurred during the first three days (0.90 to 2.61%), coinciding with the rapid pH decline phase. The pH and acidity trends in PW3 closely mirrored those observed in PW1 and PW2, with minimal variation in absolute values. This consistency across all three samples confirms the reproducibility of the fermentation process and suggests similar microbial metabolic activities regardless of minor variations in initial microbial composition. The final pH of approximately 3.2 and titratable acidity of approximately 5.5% represent typical values for fermented palm wine.

Table 17 shows the pH and titratable acidity changes during fermentation of palm wine sample 4 (PW4). The pH decreased from an initial value of 5.43 on day 1 to 3.08 on day 7, representing the lowest final pH among all four samples. The pH decline followed the characteristic pattern observed in other samples, with rapid decrease during days 1-3 (5.43 to 4.12), followed by gradual reduction to 3.08 by day 7. Titratable acidity increased from 1.08% on day 1 to 5.76% on day 7, the highest final acidity recorded among all samples. The acidity increase was most significant during the first three days (1.08 to 2.88%), consistent with observations in other samples. The slightly higher initial acidity (1.08% vs. 0.90-0.99% in other samples) and the lower final pH (3.08 vs. 3.14-3.19) in PW4 may reflect subtle differences in the initial microbial composition or tapping conditions. Nevertheless, all four samples demonstrated similar overall fermentation patterns, confirming the consistency and reproducibility of palm wine fermentation dynamics.

## DISCUSSION

The antibacterial activity of palm wine against common diarrhoeagenic bacteria was investigated in this study. Findings revealed that the palm wine samples evaluated exerted antibacterial activity against *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae*. These results are in line with the work

of Efendi *et al.*<sup>26</sup>, who reported significant antibacterial activity of lactic acid bacteria from fermented palm sap against *E. coli* and *S. aureus* growth. The observation that the growth inhibition mediated by the palm wine samples in this study was highest with the 7-day fermented palm wine sample on these test bacteria shows that the antibacterial activity of palm wine was influenced by the fermentation duration of the palm wine. The longer the duration of fermentation of palm wine, the greater the antibacterial potency of the wine on the test organisms.

There were significant variations in the microbial loads and types of palm wine samples during the 7-day fermentation period. The total bacterial counts ranged from  $5.7 \times 10^6$  to  $1.20 \times 10^7$  CFU/mL, while fungal counts ranged from  $1.2 \times 10^4$  to  $4.5 \times 10^4$  SFU/mL. These findings align with previous studies on fermented beverages, where diverse microbial populations have been observed<sup>27</sup>. The gradual reduction in bacterial loads and the peak in fungal counts around day 4 suggest a dynamic microbial succession during fermentation. This pattern is consistent with the observations of Das and Tamang<sup>28</sup>, who reported similar trends in palm wine fermentation. The consistent presence of *Saccharomyces cerevisiae* throughout the fermentation process in all the palm wine samples underscores its crucial role in palm wine fermentation, as previously noted by Oluwole *et al.*<sup>23</sup>. The isolation of various bacterial and fungal species, including *Lactobacillus plantarum*, *Bacillus subtilis*, and *Aspergillus niger* is in agreement with earlier studies on palm wine microbiota<sup>14</sup>. The presence of these microorganisms could be attributed to the natural fermentation process, environmental factors, or handling practices during palm wine collection and storage<sup>28</sup>. The physicochemical changes observed during fermentation, particularly the decrease in pH from around 5.5 to 3.1-3.2 and the increase in titratable acidity (TTA) from about 0.9-1.1 to 5.5-5.8% over 7 days, are indicative of the metabolic activities of the fermenting microorganisms. These changes are similar to those reported by Amoah-Awua *et al.*<sup>29</sup> in their study of palm wine fermentation kinetics. The low pH might have been responsible for the greater inhibition of the test diarrhoeagenic organisms observed in this study by the 5th day of fermentation of the palm wine samples. The antibacterial properties of palm wine are thought to arise from a combination of factors, including its alcohol content, organic acids, and phenolic compounds<sup>18</sup>. Ethanol, even at relatively low concentrations, can disrupt bacterial cell membranes and denature proteins, leading to cell lysis. Organic acids such as lactic and acetic acid can lower the pH of the environment, creating unfavorable conditions for bacterial growth, and may also have specific antimicrobial actions<sup>30</sup>. Phenolic compounds in palm wine may also contribute to its antibacterial activity through various mechanisms, including disruption of cell membranes, inhibition of essential enzymes, and chelation of metals required for bacterial metabolism<sup>31</sup>. For example, gallic acid and its derivatives<sup>31</sup> have been shown to have broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria<sup>32</sup>.

This study has shown that palm wine exerted significant antibacterial activity against the test diarrhoeagenic bacteria that is comparable and, in some cases, superior to that of ciprofloxacin a very potent antibiotic belonging to the fluoroquinolones at day 5 fermentation duration of the wine. It is conceivable, therefore that palm wine can be exploited for the treatment of bacterial diarrhoea in cases of emergency and no quick access to conventional antibiotics. However, relevant *in vivo* assays are required to determine the effectiveness in the human body, the dosage and safety, more so that excessive alcohol consumption, regardless of the source, can have detrimental health effects such as increased risk of cardiovascular disease, liver damage, diabetes, and many more<sup>33,34</sup>.

## CONCLUSION

This research work has provided valuable insights into the potential antibacterial properties of palm wine samples studied and the microbial ecology. The study revealed a complex and dynamic microbial community within the palm wine samples, characterized by high bacterial and fungal counts that evolved over the course of the 7-day fermentation period. Bacterial loads ranged from  $5.7 \times 10^6$  to  $1.2 \times 10^7$  CFU/mL,

while fungal counts varied between  $1.2 \times 10^4$  and  $4.5 \times 10^4$  SFU/mL, with both generally showing a declining trend as fermentation progressed. The isolation and identification of microorganisms revealed a diverse array of species, including beneficial bacteria such as *Lactobacillus plantarum* and yeasts like *Saccharomyces cerevisiae*, which can be exploited as probiotics. However, the presence of potential pathogens such as *Proteus vulgaris* and *Pseudomonas aeruginosa* raises concerns about possible contamination and highlights the need for stringent hygiene practices during palm wine production and handling. The succession pattern of microorganisms throughout the fermentation process provided valuable information on the changing microbial landscape of palmwine over time. This knowledge could be instrumental in optimizing the fermentation process to enhance the growth of beneficial microorganisms while suppressing potential pathogens. Physicochemical analysis demonstrated a consistent decrease in pH (from 5.58 to 3.08) and a corresponding increase in titratable acidity (from 0.90% to 5.76%) over the fermentation period. These changes reflect the metabolic activities of the microorganisms present and are indicative of the ongoing fermentation process. However, the most significant findings of this study were the observed antibacterial activity of the palm wine samples against common diarrhoeagenic bacteria, including *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae*, which increased with fermentation duration. It is therefore conceivable that palm wine can be utilized as an alternate therapy for the management of diarrhoea caused by the test diarrhoeagenic bacteria in infected individuals due to its antibacterial activity on the test organisms.

#### SIGNIFICANCE STATEMENT

This study highlights the potential of palm wine as a natural antibacterial agent against key diarrhoeagenic bacteria, including *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella typhi*. With rising antibiotic resistance, the significant inhibitory activity observed suggests that locally fermented palm wine could offer an accessible, cost-effective alternative for managing bacterial diarrhoea. Understanding its microbial composition and bioactive compounds may inform development of novel, plant-based therapeutics for vulnerable populations in developing regions.

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