

## ORIGINAL RESEARCH ARTICLE

**Bioautographic Profile of *Ziziphus mauritiana* Ethanolic Leaf Extract on *Escherichia coli* from Ready-to-Eat Foods Within Katsina Metropolis**Ahmad Dardau\*<sup>1</sup>, Badiyya Hassan Mashi<sup>2</sup> and Aminu Ado<sup>3</sup><sup>1</sup>Department of Microbiology, Umaru Musa Yar'adua University, Katsina, Nigeria<sup>3</sup>Department of Microbiology, Federal University Dutsin-ma, Katsina, Nigeria**ABSTRACT**

The current study aimed to detect the presence of *Escherichia coli* in ready-to-eat (RTE) foods sold in Katsina Metropolis and to assess the bioautographic profile of *Ziziphus mauritiana* ethanolic leaf extract against the isolates using culture and molecular-dependent techniques. A total of 50 food samples were collected randomly from 5 locations: Kofar Durbi, Kofar Marusa, Kofar Sauri, Kofar Guga, and Kofar Kwaya, including fried rice, jollof rice, boiled spaghetti, boiled yam, rice and beans, salad, moi moi, and jollof spaghetti. These samples were sent to the Microbiology Laboratory at Umaru Musa Yar'adua University for physico-chemical and microbiological analysis. The pH ranged from 6.7 to 7.2, and the temperature ranged from 28°C to 30.3°C, while the coliform count was variable, with the highest recorded in Kofar Sauri ( $1.26 \pm 0.99$  MPN). The level of *E. coli* ranged from  $3.0 \times 10^{-1}$  to  $3.6 \times 10^2$  CFU/g, with rice with salad having the highest contamination. The organism was found at four sites, with the highest prevalence in Kofar Marusa (40%). Biochemical tests detected Gram-negative rods. All isolates were identified as *Escherichia coli* with 100% identity by PCR amplification of the 16S rRNA gene and BLASTN sequence alignment against the *Escherichia coli* strain BNZ 03. The phylogenetic analysis also showed sequenced isolate clustered closely with known *Escherichia* species. Phytochemical screening of *Z. mauritiana* extract indicated the presence of alkaloids, flavonoids, tannins, glycosides and saponins. Sixty-six compounds, including benzoic acid (16.83%) and diisooctyl phthalate (16.13%), were identified by GC-MS and six functional groups were identified by FTIR. The TLC bioautography revealed inhibition zones, including those at R<sub>f</sub> 0.48 (7.2 mm). Based on the maximum inhibition zone observed in the TLC bioautography, isolate KMg was selected for further molecular characterization by PCR and for evaluation of its antibacterial activity, including MIC and MBC. The extract showed MIC and MBC values of 12,500 µg/mL and 50,000 µg/mL, respectively, suggesting that it was bacteriostatic at low concentration and bactericidal at high concentration. Lower MIC (6,250 µg/mL) and MBC (780 µg/mL) were observed with ciprofloxacin. The detection of *E. coli* in RTE foods indicates a food safety concern in Katsina Metropolis. *Z. mauritiana* demonstrated antibacterial properties, suggesting its potential as a natural agent for controlling foodborne pathogens.

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**INTRODUCTION**

Even though the global food poisoning problem has been solved and best hygienic practices have been instituted in developed countries, it remains a major public health problem in developing countries where best hygienic practices are not implemented. Approximately 70% of diarrheal disease cases are associated with food contamination (Zeru & Kumie, 2007). Food safety is now emerging as serious issues of public health several. Biological and chemical contamination pose risks to the processes of food production, storage, transportation, and distribution (Kafarstein & Abdussalam, 2009). Pathogenic microorganisms are mainly transmitted through the faeco-

oral route. Spread occurs via unclean food, water, hands and utensils.

For this purpose, the term Ready-to-Eat (RTE) food refers to foods that do not need to be cooked or heated before consumption. The growing demand for RTE food items has gained tremendous popularity in urban areas. The increasing popularity of ready-to-eat and instant foods is due to lifestyle changes, including increased participation of women in the workforce and less time for preparing food (Borch & Arinder, 2002; Gudbjornsdottir et al., 2004; Rodriguez et al., 2010). The lack of adequate sanitation, vendor training, access to safe

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water, and protection against microbial and chemical contamination can cause health problems when handling ready-to-eat foods. Studies carried out in Nigeria have shown the presence of pathogenic bacteria in the foods. *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* were detected in kunun-zaki sold in Zaria by Umoh *et al.* (2004). The study conducted by Ojo-Omoruyi *et al.* (2023) revealed that RTE foods from Lagos contained diverse microbial populations, including spoilage organisms and pathogens that pose serious health hazards to consumers.

Bacterial foodborne diseases impose a significant global disease burden. "Each year in the United States, they cause 76,000,000 illnesses, 128,000 to 325,000 hospitalizations, and 3,000 to 5,000 deaths." Meanwhile, such pests result in an estimated economic loss of \$83 billion (Mead *et al.*, 1999; Nyachuba, 2010; Ribot & Hise, 2016). In 2020, the EU reported 20 017 human cases and 3086 foodborne disease outbreaks. Salmonella was the most common bacterial agent, followed by *Campylobacter*, *Yersinia*, and Shiga toxin-producing *E. coli*. Every year, 4.1 million Australians become food-poisoned (Kirk *et al.*, 2014), and the most deadly food diseases are *E. coli* and listeria diseases (Authority, 2020). At the global level, however, 3 million cases of diarrhea are caused by microbial diseases (Ribot & Hise, 2016). Bintsiset *et al.* (2017) have reviewed the foodborne pathogens and the associated diseases.

Natural antimicrobial solutions which improve food safety are now under more scrutiny. Medicinal plants have been employed in traditional medicine systems for a long time due to their easy accessibility, lack of side effects, and low cost. Herbalists were among the earliest healers (El Ghazali *et al.*, 2003), and plant-based remedies remain crucial components of healthcare worldwide, particularly in low-resource setting. The World Health Organisation (WHO) recommends the incorporation of effective traditional medicines into national health programmes, as stated by Adzu & Haruna (2019).

Medicinal and aromatic plants are important from an economic perspective. They are used as raw materials for drugs, cosmetics and food preservatives according to Aiyelaagbe (2007). Crops have been proven to be antimicrobial and antifungal (Arora and Kaur, 1999). A variety of agents, such as antiseptics, coagulants, and wound-clearing agents, are widely used El Maaiden *et al.* (2020) reported that more than 70% of wound-healing products are derived from plants. The medicinal properties of plants result from their secondary metabolites, such as alkaloids, tannins, saponins, glycosides, and flavonoids, which are found to be useful against many diseases. Primary metabolites, such as sugars and proteins, in medicinal plants support plant growth, while secondary metabolites confer medicinal value.

*Ziziphus mauritiana* is a fruit tree belonging to the Rhamnaceae family. It is a fruit tree commonly known by various names, such as Chinese apple, Chinese date, Indian cherry, and jujube (Akhtar *et al.*, 2016). It is known as *Magarya* in Hausa, *Huhue* among the Yoruba, and *Uju* among the Igbo people in Nigeria. It is a warm temperate grower and is adaptable to less rigorous climates. *Ziziphus*

*mauritiana* is a shrub or tree with thorny branches, which was initially identified as *Z. jujube*. Two varieties are known in India: var. This part is called 'hysudrica', including wild and cultivated varieties with large fruits, and var. Fruitcosa is a small shrub found in the sub-Himalayas. A large number of cultivars have been developed for improved fruit production (Munawar, 2022).

The fruit of *Ziziphus mauritiana* is a good source of essential nutrients. Its iron content is 2.1-4.3 mg per 100 g dry weight, which has been shown to be significantly higher than that in apples, which are required for oxygen transport in the body (Nyanga *et al.*, 2013). The analysis of fruit pulp (parts of the fruit eaten as food) showed these were high in several nutrients and minerals such as iron (2.1–4.3 mg), zinc (0.6–0.9 mg), calcium (160–254 mg), sodium (185–223 mg), magnesium (83–150 mg) and vitamin C (15–43.8 mg 100 g dry weight) (Nyanga *et al.*, 2013). 100 g of pulp has been found to contain 15-43.8 mg of ascorbic acid (vitamin C) (Nyanga *et al.*, 2013). Similarly, its fruit also contains good amounts of vitamin A and the B-complex vitamins (Munawar, 2022). In addition, *Ziziphus mauritiana* fruit provides 363-376 kcal (1,516-1,575 kJ) in 100 g dry weight (Nyanga *et al.*, 2013).

## MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in Katsina Metropolis, the Administrative capital of Katsina Local Government Area, Katsina State, in North-Western Nigeria. The metropolis is located between latitudes 12°15'–12° 59' N and longitude 7°30'– 7°36' E, with an estimated population of 9,300,382, projected by the National Population Commission (NPC, 2020), and a land area of 24,192 km<sup>2</sup>.

### 2.2 Sample Collection and Handling

Fifty (50) Ready-to-Eat (RTE) foods were collected from the five locations in Katsina Metropolis, namely: Kofar Durbi (Jollof rice, spaghetti), Kofar Marusa (rice, yam and salad), Kofar Sauri (rice and beans), Kofar Guga (fried rice and salad) and Kofar Kwaya (Jollof, spaghetti and moi moi). *Z. mauritiana* leaves were collected from an irrigation farm in Katsina Metropolis with clean polythene bags and brought to the Department of Microbiology, Umaru Musa Yar'adua University, Katsina.

The number of samples was determined by the Cochran formula for simple random sampling:

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{e^2} = \frac{(1.96)^2 \cdot 0.5 \cdot 0.5}{(0.14)^2} \approx 49$$

### 2.3 Evaluation of Physico-Chemical Parameters

Physico-chemical factors such as pH, temperature, colour, taste, odour, and texture were assessed. The pH was determined by homogenising 2 g of the food sample in 20 mL of distilled water, reading it with a pH meter, and the temperature was determined with a food thermometer. Colour was evaluated visually and taste, odour and texture were evaluated by sensory analysis. All analyses were

performed in duplicate, and the results were presented as mean  $\pm$  standard deviation (Ngangyo-Heya *et al.*, 2020).

## 2.4 Bacteriological Examination of RTE Food Samples

The MPN method outlined by APHA (2004) was used for the bacteriological analysis of total coliforms and faecal coliforms (*E. coli*). Presumptive, confirmed and completed tests were analysed.

### 2.5 Presumptive Test

Each food sample was homogenised in buffered peptone water to produce a 225 mL solution containing 25 g of food. A lactose double- and single-strength broth tube was prepared following the manufacturer's instructions (Willey *et al.*, 2011). The 10mL homogenate was added to three double-strength tubes, and 1 mL and 0.1 mL to two other tubes, respectively (Rijal, 2021). The tubes were read after 24 hours of incubation at 44°C; those showing acid and gas production were recorded, and MPN values were calculated from the Macrady probability table (Cheesbrough, 2006).

### 2.6 Confirmed Test

Positive presumptive tubes were subcultured into lactose broth, nutrient agar slants and tryptone water (Cheesbrough, 2006). Lactose broth tubes were incubated at 37°C for 24–48 hours and checked for gas production. Nutrient agar growths were Gram-stained for identification of gram-negative non-spore-forming rods. The tubes were filled with tryptone water and incubated at 44°C for 24 hours, and Kovac's reagent was added to detect indole production, indicated by the appearance of a red ring (Rijal, 2021).

### 2.7 Completed Test

Isolates with positive cultures were streaked on Eosin Methylene Blue (EMB) agar and incubated at 37°C and 44°C for 24 hours. Colonies with a greenish metallic sheen were considered coliforms, and the presence of growth with a metallic sheen at 44°C was considered to indicate thermotolerant *E. coli* (Rijal, 2021).

## 2.8 Phytochemical Screening of *Z. mauritiana* Leaf

Qualitative determination of the phytochemical constituents of the ethanol extract of *Z. mauritiana* leaves was done for the presence of alkaloids, saponins, tannins, flavonoids and cardiac glycosides.

### 2.8.1 Test for Alkaloids

After acid-alcohol extraction, the extract was treated with Mayer's and Dragendorff's reagents. The presence of alkaloids was detected by the formation of a cream or reddish-brown precipitate (Yakubu *et al.*, 2020).

### 2.8.2 Test for Saponins

The extract was shaken up vigorously with distilled water and olive oil. The presence of saponins was determined by

the formation of stable froth and creamy emulsion (Yakubu *et al.*, 2020).

### 2.8.3 Test for Tannins

Ferric chloride solution was added to the boiled extract filtrate. The brownish-green or blue-black colour confirmed tannins (Yakubu *et al.*, 2020).

### 2.8.4 Test for Flavonoids

The extract was dissolved in diluted sodium hydroxide, and then hydrochloric acid was added. A yellow colour turning colourless indicated flavonoids (Yakubu *et al.*, 2020).

### 2.8.5 Test for cardiac glycosides:

Glacial acetic acid, ferric chloride and concentrated sulphuric acid were used for the Keller-Killiani test. Cardiac glycosides were confirmed by the formation of a brown ring at the interface (Yakubu *et al.*, 2020).

## 2.9 Preparation of Microbial Inoculum

The bacteria were grown in nutrient broth at 37°C overnight, and the concentration of the bacteria in the suspensions was adjusted to 0.5 McFarland equivalent to about  $1.5 \times 10^8$  CFU/mL in sterile saline (Abdallah *et al.*, 2022).

### 2.9.1 Direct Bioautography Assay

Using sterile forceps, TLC plates with the plant materials were dipped in bacterial suspension for 5–10 seconds. Excess inoculum was drained, and the plates were incubated at 37 °C for 18–24 hours to allow microbial growth on the chromatographic surface (Singh *et al.*, 2023; Smania *et al.*, 2021).

### 2.9.2 Visualisation of inhibition zones

After incubation, TLC plates were sprayed with 0.2% aqueous triphenyl tetrazolium chloride (TTC) and incubated for an additional 1–2 hours. The clear or colourless zones on a red background showed that the bacterial growth was inhibited and that antimicrobial compounds were present in the chromatogram (Gedefieet *al.*, 2021; Rahman *et al.*, 2023).

## 2.10 Antibacterial activity, MIC and MBC of *Z. mauritiana* Ethanolic leaf extract

The antibacterial activity of the ethanolic extract of *Ziziphus mauritiana* leaves was assessed against *Escherichia coli* isolated from ready-to-eat (RTE) foods using the Agar well diffusion method with modifications as described by CLSI (2021) and Balouiri *et al.* (2016). Of the five confirmed *E. coli* isolates, isolate KMg was selected for antibacterial activity, MIC, and MBC studies based on the most distinct inhibition zone in the preliminary bioautography bioactivity screening. A standardised bacterial suspension at 0.5 McFarland (approx.  $1.5 \times 10^8$  CFU/mL) was used to inoculate sterile Mueller-Hinton (MH) agar plates. The medium was aseptically bored with 6 mm diameter wells, and different concentrations of the

ethanolic extract (100,000, 50,000, 25,000, 12,500, and 6,250 µg/ml) were placed in the wells. Ciprofloxacin was used as the positive control, and sterile distilled water and dimethyl sulfoxide (DMSO) were used as the negative controls. Plates were left for 30 minutes for proper diffusion, followed by 24 hours of incubation at 37°C. The diameter of the zone of inhibition, in millimetres, was used to determine antibacterial activity, and the results were presented as mean ± standard deviation (Balouiriet al., 2016; Owuama, 2022).

### 2.10.1 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the ethanolic extract and ciprofloxacin against *E. coli* isolates was determined by the broth dilution method according to CLSI guidelines (CLSI, 2021). The extract was serially diluted twofold into sterile nutrient broth, and the standardised bacterial suspension was used to inoculate. After incubation at 37°C for 24 hours, tubes were observed for turbidity. The lowest concentration of the extract which did not produce any visible bacterial growth was considered as the MIC (Wiegand et al., 2008; Elshikh et al., 2016).

### 2.10.2 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined by transferring the samples from the MIC tubes, with no visible growth, onto sterile Mueller-Hinton agar plates and incubating them at 37°C for 24 hours. The minimum concentration of the extract which did not produce any colony on the plate was considered as the MBC against the tested bacterium (Balouiriet al., 2016; CLSI, 2021).

## RESULTS

### 3.1 Physico-Chemical Parameters of the Food Samples

The physico-chemical properties of the foods analysed, such as pH, temperature, colour, taste, odour, and texture, vary. The variations depend on the type of food material. The pH values in food samples varied between 7.2 and 6.8, while the temperature ranged from 28 to 30 °C. The food colours were light brown, brownish-green and yellowish-green. When tasted, all food samples give rich, delicate flavour after eating and possess no stink, with rough grease and soft texture after feeling. All the food samples were cooked or fried (Table 3.1).

**Table 3.1: Physico-Chemical Parameters of the Food Samples**

Location of Sample	Food Types	PH	Temp <sup>0</sup> C	Color	Taste	Odor	Texture	Status
KD	Jollof rice, boiled spaghetti	6.8±0.05	29.2±0.1	Brownish	Bland	No off odour	Rough	Cooked
KM	Rice and boiled yam with salad	7.2±0.0	28.1±0.0	Brownish	Bland	Odorless	Greasy	Cooked
KS	Rice and beans	6.7±0.0	30.3±0.1	Brownish	Bland	Odorless	Soft	Cooked
KG	Fried rice with salad	6.8±0.1	29±0.0	Brownish-green	Bland	Odorless	Rough	Fried
KK	Jollof spaghetti with moi moi	6.9±0.1	30.1±0.2	Yellowish-green	Bland	Odorless	Rough	Cooked

Legends; Mean±S.D

KD= Kofar Durbi

KM= Kofar Marusa

KS= Kofar Sauri

KG= Kofar Guga

KK= Kofar Kwaya

**Table 3.2: Mean Coliform Count of RTE Foods Sold in Five Sampling Sites Using the Most Probable Number (MPN) Technique**

Sampling Sites	No. of Samples	MPN Index (Mean)	Coliforms (Mean ±SD)
KD	10	0.54	0.54 ± 0.54
KM	10	1.08	1.08 ± 1.42
KS	10	1.26	1.26 ± 0.99
KG	10	0.40	0.40 ± 0.16
KK	10	0.51	0.51 ± 0.18

Legends: Mean±S.D

KD=Kofar Durbi

KM=Kofar Marusa

KS=Kofar Sauri

KK=Kofar Kwaya

KG=Kofar Guga

### 3.2 Mean value of coliform count index of RTE food sold in five sampling sites.

The level of coliform contamination in the food samples collected from 5 sites is indicated below (Table 3.2). Kofar Sauri recorded the highest mean coliform count of

1.26 ± 0.99 MPN, indicating constant heavy contamination. The mean MPN for Kofar Marusa was 1.8 ± 1.42, indicating a moderate contamination level. The mean of Kofar Durbi was 0.54 ± 0.54, Kofar Kwaya recorded 0.51 ± 0.18 MPN, while Kofar Guga recorded

the least mean with  $0.40 \pm 0.16$  MPN. The mode of the number was around at each site, and each case of the

number was very stable and was used to calculate the most probable number (MPN) index.



**Figure 1: Electrophoresis showing DNA Amplification of Bacterial Isolate**

Key: Lane Bp: Base pair, Lane M: Molecular marker, Lane 1: Positive control, Lan 2: KMg

**Table 3.3: Coliform Count For the Number of Positive Tubes From RTE Foods**

Sample ID	No. of Tubes Showing Positive Reactions	MPN/g
KDa	1-0-0	0.3
KDc	0-1-0	0.3
KDh	0-1-0	0.3
KDi	0-0-1	0.3
KDj	0-2-1	1.5
KMa	0-1-0	0.3
KMb	1-0-1	0.61
KMc	0-1-0	0.3
KMf	0-1-1	0.61
KMg	1-2-1	3.6
KSa	1-1-1	2.4
KSb	0-0-1	0.3
KSf	1-1-0	0.92
KSh	1-1-1	2.4
KSi	0-1-0	0.3
KGc	0-1-0	0.3
KGd	1-0-1	0.61
KGe	0-1-0	0.3
KGg	0-1-0	0.3
KGi	0-0-1	0.3
KGj	0-1-1	0.61
KKa	0-1-1	0.61
KKb	1-0-1	0.61
KKj	0-0-1	0.3

**3.3: Coliform Count For the Number of Positive Tubes from RTE Foods**

The readings for coliforms contamination were not the same. There were differences in the levels of <https://publications.umyu.edu.ng/scientifica>

contamination between RTE food samples collected from five different places (as shown in Table 3.3). The highest MPN/g of coliform cfu was observed in KM, with a single sample (KMg) having an MPN/g of 3.6/g. This high number indicated significant contamination. The samples from Kofar Sauri (KSa, KSh) had MPN values of 2.4/g, indicating moderate to high faecal contamination. The MPN values of 0.3 to 0.61/g were obtained for low levels of contamination in KDj (1.5/g), KSf (0.92/g), and many of the Kofar Durbi (KD), Kofar Guga (KG), and Kofar Kwaya (KK) options.

**3.4 Biochemical tests-based identification of the bacteria**

Based on biochemical test reactions, colonial morphology and gram's reaction, a total of 5 bacterial isolates were identified in this research (Table 3.4).

**3.5 Molecular Identification of Isolate**

PCR amplification of the 16S rRNA gene from isolate KMg produced a clear amplicon of approximately 1000 bp as observed on agarose gel electrophoresis (Figure 1). Sequence analysis and BLASTn comparison with sequences in the NCBI GenBank database identified the isolate as *Escherichia coli* strain BNZ 03 (Accession Number PX482740.1), showing 100% sequence similarity with the reference sequence (Table 3.5). This result confirmed that the bacterial isolate KMg belonged to the species *Escherichia coli*.

**3.6 Phytochemical profile of *Z. Mauritiana* leaf extract**

Phytochemical screening of the *Z. mauritiana* leaf extract revealed the presence of the following phytochemicals: Dardau et al., /USci, 5(2): 183 – 197, June 2026 187

alkaloids, tannins, flavonoids, glycosides and saponins, but the presence of terpenoids was not detected, as shown in Table 3.6 below.

### 3.7 Gas Chromatography Mass Spectrometric Profile of *Z. mauritiana* leaf extract

The total ion chromatogram (TIC) indicated the characteristic of sixty-six (66) compounds of *Ziziphus*

*mauritiana* leaf (Figure 2 and Table 3.7). The major compounds found were 8.67 RT (Diisooctyl phthalate with 16.13% peak area), 5.40 RT (Benzene, 1,2,3-trimethyl- with 6.58% peak area), and 16.83.25 RT (Benzoic acid with 16.83% peak area). The lowest level of the mixture was 0.19% 2,4-Di-tert-butylphenol

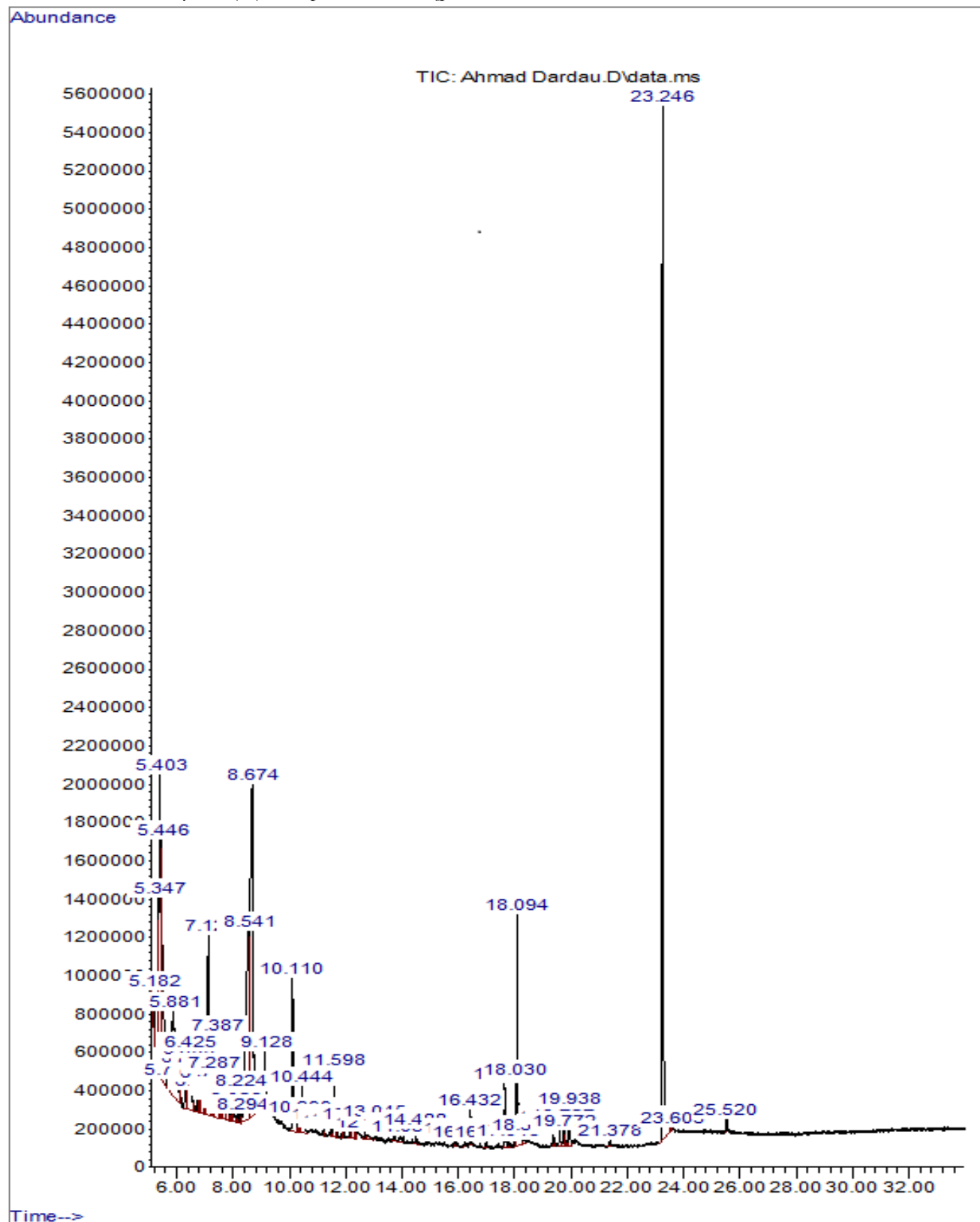


Figure 2: Total Ion Chromatogram (TIC) of *Z. mauritiana* Ethanolic Leaf Extract

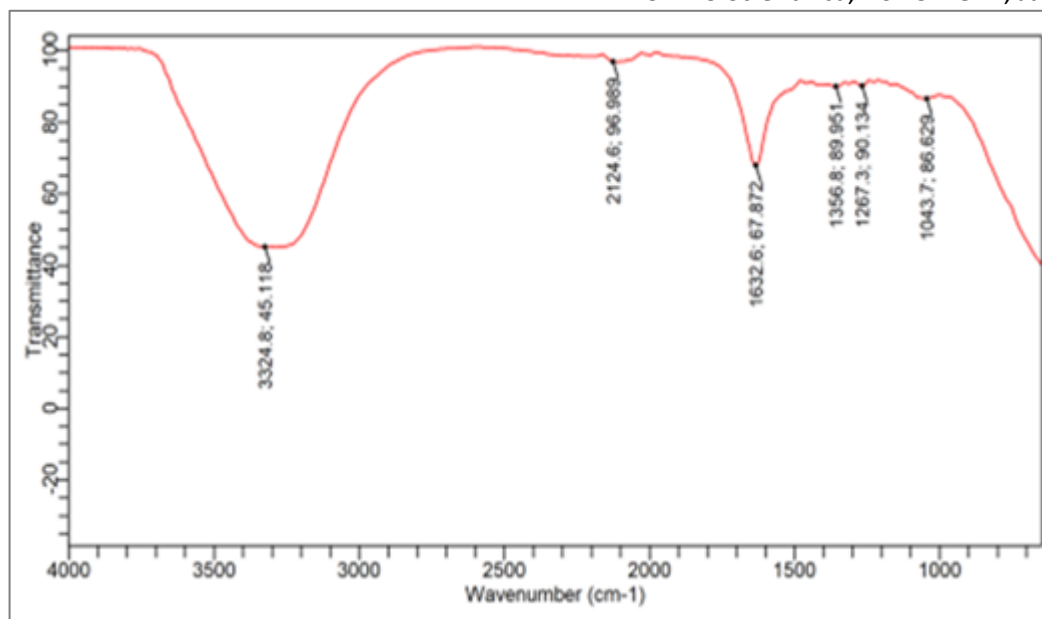


Figure 3 FTIR spectrum of *Z. mauritiana* Leaf Extract Showing Characteristic Peaks Corresponding to Functional Groups

Table 3.4 Biochemical Profile of bacteria Isolated from RTE food

Isolates	GS	Cat	Cit	Ind	L&M	Man	Mot	TSIA	MR	VP	Probable Identity
KD <sub>c</sub>	-	+	-	+	+	-	+	+	+	-	<i>E.coli</i>
KM <sub>b</sub>	-	+	-	+	+	-	+	+	+	-	<i>E.coli</i>
KM <sub>g</sub>	-	+	-	+	+	-	+	+	+	-	<i>E.coli</i>
KS <sub>a</sub>	-	+	-	+	+	-	+	+	+	-	<i>E.coli</i>
KK <sub>j</sub>	-	+	-	+	+	-	+	+	+	-	<i>E.coli</i>

Legends:

Cat= Catalase                      Cit- Citrate                      Ind= Indole                      L&M= Lactose & Maltose  
 Man= Mannitol                      MR= Methyl red                      Mot= Motility                      TSIA= Triple sugar iron agar  
 VP= Voges proske

Table 3.5: Identification of Isolate using NCBI BLASTn Search

Sample ID	Identified Species Name of Isolate	Accession Number	% similarity
KM <sub>g</sub>	<i>Escherichia coli</i> strain BNZ 03 ribosomal RNA gene, partial sequence	PX482740.1	100

Table 3.6 Phytochemical Constituents of Ethanolic Extract of *Ziziphus mauritiana* Leaf

Plant	Part	Alkaloid	Glycoside	Flavonoid	Saponin	Tannin	Terpenoid
<i>Ziziphus mauritiana</i>	Leaf extract	+	+	+	+	+	-

Legends: +: Indicates presence of the compound

-: Indicates absence of the compound

### 3.8 Fourier Transform Infrared Spectroscopy Constituents of *Z. mauritiana* Leaf Extract

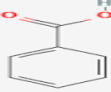
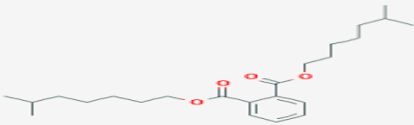
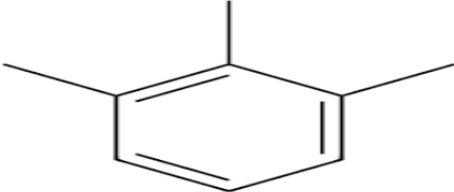
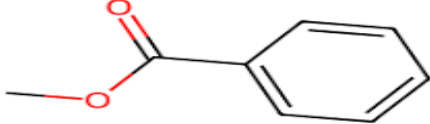
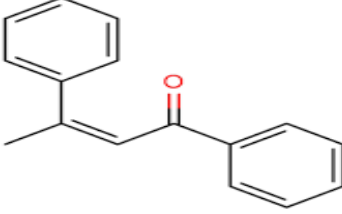
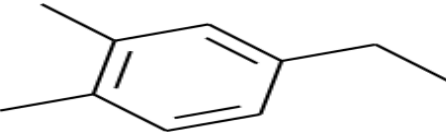
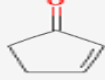

The FTIR spectrum of the ethanolic extract of *Ziziphus mauritiana* leaves showed six absorption peaks (Figure 3 and Table 3.8) which signify that there are six major functional groups present in the extract namely; alcohols and phenols (O–H) with Strong broad Peak around 3324 cm<sup>-1</sup>, alkenes or amines (C=C or N–H) with Moderate Peak near 1632 cm<sup>-1</sup>, esters or ethers (C–O) with peaks near 1043 and 1267 cm<sup>-1</sup>, alkynes/Nitriles (C≡C or C≡N) with Peak at 2124 cm<sup>-1</sup> suggests presence of triple bonds or nitrile group and methyl/alkane groups with bending vibration near 1356 cm<sup>-1</sup>.

### 3.9 Inhibition Zones and RF Values of Bioautograph

The extract showed significant antibacterial activity, with the largest and clearest inhibition zones at Rf values 0.18, <https://publications.umyu.edu.ng/scientifica>

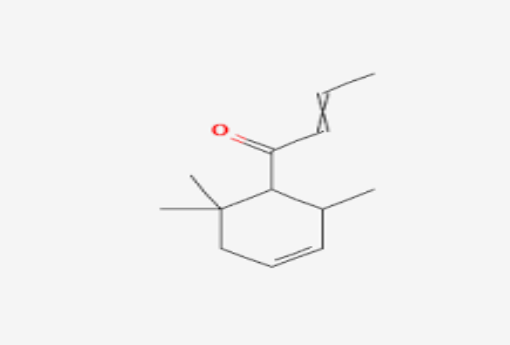

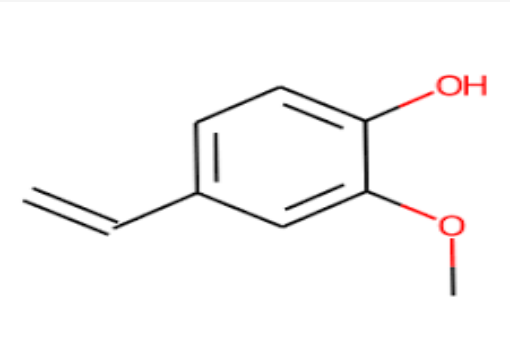



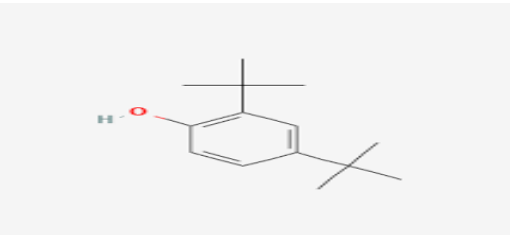
0.48, and 0.78 (Table 3.9). The most effective activity is KM<sub>g</sub>, with the largest inhibition zone (7.2 mm), followed by KS<sub>a</sub> (0.65), which has a smaller, less sharp zone. The differences in Rf values indicate that the extract contains different types of phytochemicals, with varying polarities, capable of inhibiting microbes. Clear inhibition zones at specific Rf values during TLC bioautography indicate the presence and localisation of antimicrobial compounds, with larger, clearer zones suggesting stronger antibacterial activity (Choma & Jesionek, 2015). In comparison, ciprofloxacin demonstrated significantly higher antibacterial activity across all concentrations tested, with the highest zone of inhibition at 100,000 µg/mL (29.4 ± 0.42 mm), followed by 23.65 ± 0.21 mm at 50,000 µg/mL, 22.05 ± 0.07 mm at 25,000 µg/mL, 19.15 ± 0.21 mm at 12,500 µg/mL, and 17.65 ± 0.07 mm at 6,250 µg/mL.

**Table 3.7 Gas Chromatography Mass Spectrometric Constituents of *Z. mauritiana* leaf**

S/N	Compound Name	Retention Time (min)	Peak Area (%)	Molecular Formula	Molecular structure
1	Benzoic acid	8.6743	16.8335	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	
2	Diisooctyl phthalate	23.2461	16.1356	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	
3	Benzene, 1,2,3-trimethyl-	5.4026	6.5815	C <sub>9</sub> H <sub>12</sub>	
4	Benzoic acid, methyl ester	7.1224	4.9480	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>	
5	2-Buten-1-one, 1,2-diphenyl-	18.0943	3.8372	C <sub>16</sub> H <sub>14</sub> O	
6	Benzene, 4-ethyl-1,2-dimethyl-	6.4246	3.0101	C <sub>10</sub> H <sub>14</sub>	
7	2-Cyclopenten-1-one, 3,4,5-trimethyl-	7.3869	2.3061	C <sub>8</sub> H <sub>12</sub> O <sub>2</sub>	
8	Hexadecanoic acid, methyl ester	17.6448	0.9615	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	

*To be continued next page*

Table 3.7 continued

S/N	Compound Name	Retention Time (min)	Peak Area (%)	Molecular Formula	Molecular structure
9	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydroclopenta[c]pyran-1-yl)ethanone	11.5978	0.9283	C <sub>13</sub> H <sub>18</sub> O <sub>2</sub>	
10	n-Hexadecanoic acid (Palmitic acid)	18.0297	0.8635	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	
11	2-Methoxy-4-vinylphenol	10.4445	0.8619	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	
12	Octadecanoic acid (Stearic acid)	19.9379	0.6500	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	
13	Methyl stearate	19.5974	0.3938	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	
14	Octadec-9-enoic acid (Oleic acid)	19.7274	0.3690	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	
15	2,4-Di-tert-butylphenol	13.0445	0.1940	C <sub>14</sub> H <sub>22</sub> O	

**Table 3.8 Fourier Transform Infrared Profile of *Z. mauritiana* leaf extract**

Peak Number	Wave number (cm <sup>-1</sup> )	Intensity	Functional Group	Assignment
1	1043.65	86.63	C-O	Alcohols, ethers, esters, or carboxylic acids
2	1267.29	90.13	C-N or C-O	Aliphatic amines or esters
3	1356.75	89.95	C-H	Methyl groups (alkanes
4	1632.57	67.87	C=C or N-H	Alkenes or primary amines
5	2124.58	96.99	C≡C or C≡N	Alkynes or nitriles
6	3324.79	45.12	O-H or N-H	Alcohol, phenols or amines

**Table 3.9 Inhibition Zones and RF Values of Bioautograph**

Sample ID	Rf Value	Zone Diameter (mm)	Color Observation	Activity
KDc	0.18	6.0	Clear zone	A
KMb	0.33	4.5	Clear zone	A
KMg	0.48	7.2	Clear zone	S.A
KSa	0.65	3.5	Faint zone	W.A
KKj	0.78	5.0	Clear zone	A

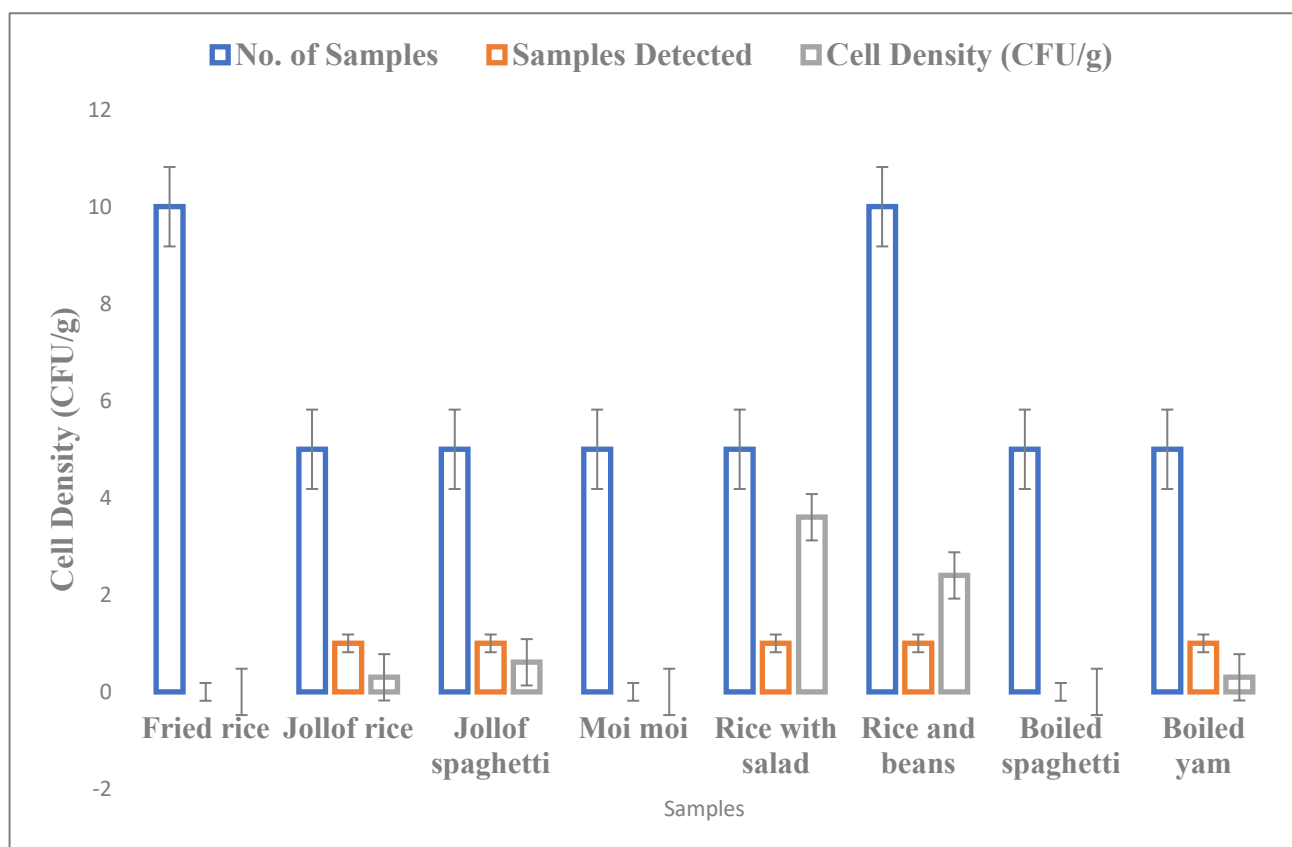
Legends; Rf= Retention factor    A= Active    S.A= Strongly Active    W.A=Weakly Active

**Table 3.10: Antibacterial Activity of Ethanolic Extract of *Z. mauritiana* against *Escherichia coli* strain BNZ 03 Isolate from RTE Foods**

Extract	Zone of Inhibition (mm) at different concentrations				
	100,000 µg/ml	50,000 µg/ml	25,000 µg/ml	12,500 µg/ml	6,250 µg/ml
<i>Z. mauritiana</i>	16.1 ± 0.14	13.5 ± 0.00	9.15 ± 0.21	8.05 ± 0.07	0.00 ± 0.00
Ciprofloxacin	29.4 ± 0.42	23.65 ± 0.21	22.05 ± 0.07	19.15 ± 0.21	17.65 ± 0.07

**Table 3.11: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of *Z. mauritiana* Ethanolic Extract against *Escherichia coli* strain BNZ 03 Isolate from RTE Foods**

Extracts	MIC (µg/ml)	MBC (µg/ml)
<i>Z. mauritiana</i>	12,500	50,000
Ciproflaxacin	6,250	780



**Figure 4: Percentage Frequency of *E. coli* from RTE foods**

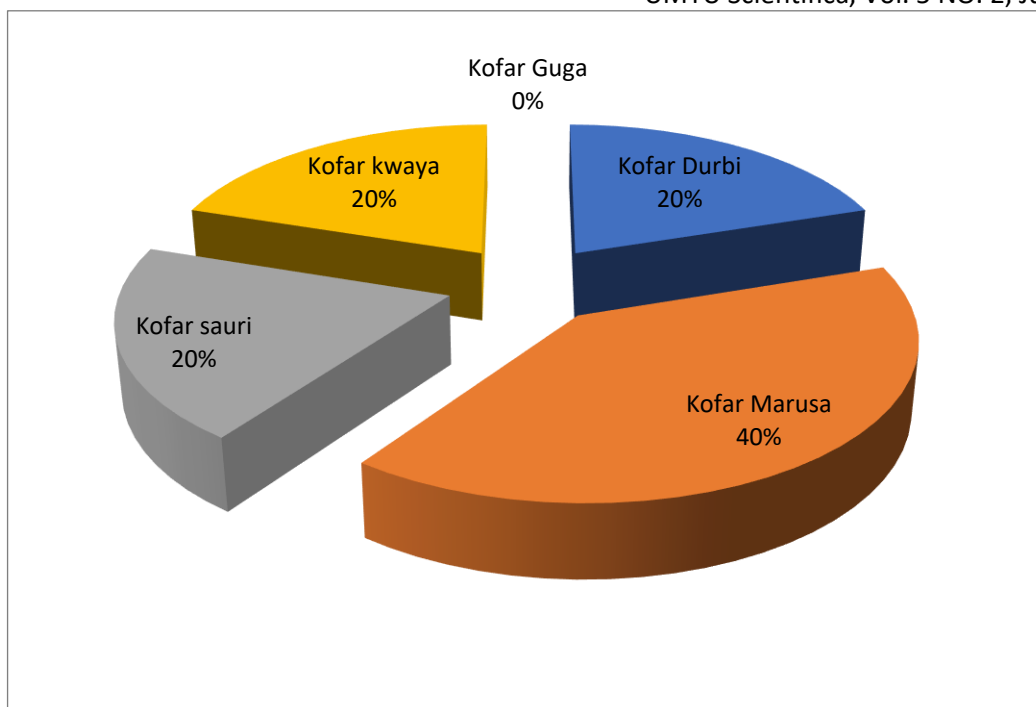


Figure 5: Percentage Frequency of Occurrence (Prevalence) of *E. coli* from Five Different Sampling Sites

Table 3.12: Percentage Frequency of Occurrence of *E. coli* from Five Different Sampling Sites

Site	No. of Samples	No. +ve for <i>E. coli</i>	% Frequency
KD	10	1	20
KM	10	2	40
KS	10	1	20
KG	10	0	0
KK	10	1	20

Legends: %: Percentage

### 3.10 Antibacterial Activity of Ethanolic Extract of *Z. mauritiana* against *Escherichia coli* strain BNZ 03 Isolate from RTE Foods

The antibacterial activity of ethanolic extract of *Ziziphus mauritiana* and ciprofloxacin against *Escherichia coli* strain BNZ 03 isolates at different concentrations is presented in Table 3.10. It was observed that both the plant extract and the standard antibiotic inhibited growth in direct proportion to their concentrations.

The ethanolic extract of *Ziziphus mauritiana* showed significant antibacterial activity at high concentrations; the largest zone of inhibition was observed at 100,000 µg/mL (16.1 ± 0.14 mm). This decreased progressively to 13.5 ± 0.00 mm at 50,000 µg/mL, 9.15 ± 0.21 mm at 25,000 µg/mL, and 8.05 ± 0.07 mm at 12,500 µg/mL, while no inhibition (0.00 ± 0.00 mm) was observed at the lowest concentration of 6,250 µg/mL. This shows an antibacterial effect of the extract, dependent on concentration.

#### 3.10.1 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of *Z. mauritiana* Ethanolic Extract against *Escherichia coli* strain BNZ 03 Isolate from RTE Foods

The ethanolic extract of *Ziziphus mauritiana* exhibited a minimum inhibitory concentration (MIC) of 12,500

µg/mL and a minimum bactericidal concentration (MBC) of 50,000 µg/mL against the growth of *Escherichia coli* strain BNZ 03, which was isolated from ready-to-eat foods (Table 3.11).

Compared to this, ciprofloxacin was more effective, with an MIC of 6,250 µg/mL and an MBC of 780 µg/mL, indicating that the plant extract had a moderate antibacterial effect.

### 3.11 Prevalence of *E. coli* in Ready-to-Eat Foods from Five Different Sampling Sites

The prevalence of *Escherichia coli* in all 50 ready-to-eat (RTE) food samples screened is 10% as shown in Figure 4 and Table 3.12. *E. coli* was not present in any of the fried rice, moi moi and boiled spaghetti. A total of 1 positive sample was obtained from different food types, including jollof rice, jollof spaghetti, rice with salad, rice and beans, and boiled yam. In the samples that tested positive, the presence of *Escherichia coli* in the jollof rice and boiled yam was 3.0 × 10<sup>-1</sup> CFU/g and 3.6 × 10<sup>0</sup> CFU/g, respectively, with the rice with salad being the most contaminated. The prevalence of *Escherichia coli* varied across the study locations. The highest prevalence (40%) was recorded in Kofar Marusa, while Kofar Guga had a prevalence of 0%. Moderate prevalence rates of 20% each were observed in Kofar Kwaya, Kofar Durbi, and Kofar Sauri (Figure 5).

## DISCUSSION

The current study investigated the presence of *Escherichia coli* in Ready-to-Eat (RTE) foods collected from 5 sampling sites in Katsina Metropolis, and the bioautographic profile and phytochemical composition of *Ziziphus mauritiana* leaf extract against the isolates.

Physico-chemical parameters (pH, temperature, colour, taste, odour, and texture) of the food samples showed mild variation across the different testing sites and food types. The pH ranged from 6.7 to 7.2, indicating near-neutral conditions, which are favourable for microbial growth, particularly that of coliforms. The values are comparable to those reported by Ogunbanwo *et al.* (2022), who reported a similar pH value for RTE food products in urban Nigeria. The temperature of the foods ranged from 28–30.3°C, which is the ambient temperature at which the foods are displayed and vended, and which creates a conducive environment for microbial growth.

Texture was rated rough to greasy to soft and generally aligned with the type of food and preparation (fried vs. cooked). There was no detectable odour of these foods, and the bland taste was not affected, but microbial contamination was detected by the coliform index.

The distribution of coliform counts obtained using the Most Probable Number (MPN) technique showed varying counts, ranging from 1.26 MPN/g in Kofar Sauri to 0.40 MPN/g in Kofar Guga. Coliforms in cooked foods indicate post-processing contamination, which is most likely due to handling or storage. These observations indicate moderate-to-strong contamination and may pose serious public health risks. An observation by Abakpa *et al.* (2021) found high levels of coliforms in RTE foods in northern Nigeria, where unsanitary conditions during food preparation and vending contributed to the contamination.

Four of the tested locations also tested positive for *E. coli*, further underscoring the risk. The highest incidence of *E. coli* was observed at Kofar Marusa (40%); the remaining four sites, Kofar Durbi, Kofar Kwaya, and Kofar Sauri, had an incidence of 20% each, while Kofar Guga showed no presence of *E. coli*. The results indicate that there is a local problem regarding cleanliness or sanitation behaviour at Kofar Marusa, which may be due to pollution of the environment, water quality or human behaviour. This is in line with the findings of Iroha *et al.* (2020), who reported *E. coli* contamination of RTE foods from Nigerian markets, with most contamination attributed to poor water sources and improper food handling.

All the isolates were identified as *E. coli* by biochemical tests, which showed consistently positive catalase, indole, and methyl red tests, and negative citrate and Voges–Proskauer tests (Table 3.4). The morphology of the isolates was also verified by Gram staining, which revealed the organisms as Gram-negative rods characteristic of *E. coli*. Furthermore, the 16S rRNA gene sequencing and BLASTn analysis of one representative isolate revealed 100% similarity with *Escherichia coli* strain BNZ 03 (Accession No. PX482740.1) with query cover of 97% and

e-value of 2e-159, which further supported the identification of the isolates as *Escherichia coli*.

The phytochemical screening of the leaf extract of *Z. mauritiana* showed the presence of alkaloids, tannins, flavonoids, saponins, and glycosides, but no terpenoids were detected. These phytoconstituents have been reported to exhibit antimicrobial and antioxidant properties. Certain components of *Z. mauritiana*, for example, were associated with reductions in clinical and foodborne pathogens by Shehu *et al.* (2023). These compounds indicate the potential use of the plant as a therapeutic agent against foodborne *E. coli*.

Sixty-six compounds were identified by GC–MS, and the most prominent compound was benzoic acid (16.83%). One of the well-known food preservatives is benzoic acid exhibits inhibitory activity against Gram-negative and Gram-positive bacteria (Synowiec *et al.*, 2021). Diisooctyl phthalate at 16.13% is possibly a contaminant from plasticisers and has been reported in plant extracts, possibly due to environmental exposure.

The functional groups identified by FTIR spectroscopy included C–O/C–N stretches (1043 and 1267 cm<sup>-1</sup>), C=C/N–H stretches (1632 cm<sup>-1</sup>) and broad O–H stretches (3324 cm<sup>-1</sup>), indicative of phenolic, alcoholic, ester and alkyne/nitrile substances. The presence of hydroxylated and unsaturated bioactive compounds may account for these peaks, contributing to the antimicrobial activity. Yakubu *et al.* (2021) reported a similar FTIR spectrum for phytotherapeutic plants used for their effects on enteric pathogens.

Bioautography of the thin-layer chromatography (TLC) showed distinct inhibition zones at various R<sub>f</sub> values. For further antibacterial activity, MIC and MBC analysis, one *E. coli* isolate, KMg, was chosen because it showed the largest and clearest inhibition zone in the bioautographic screening. Especially on R<sub>f</sub> 0.48, where sample KMg showed the largest inhibition zone of 7.2 mm. This means that there are very potent antimicrobial compounds at that chromatographic position. Clear inhibition zones were also observed for other fractions, namely KDc (R<sub>f</sub> 0.18) and KKj (R<sub>f</sub> 0.78), indicating that more than one bioactive compound may be present in the extract. On the other hand, the weak inhibition zone observed at R<sub>f</sub> 0.65 (KSa) could be due to a lower concentration or to a lower antimicrobial activity of the compound. The presence of inhibition zones at multiple R<sub>f</sub> values indicates the complexity of phytochemical interactions within the extract and suggests the potential of bioautography to identify active antimicrobial phytochemicals.

The antibacterial activity of the ethanolic leaf extract of *Ziziphus mauritiana* was also validated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for *Escherichia coli*. The extract had an MIC of 12,500 µg/mL and an MBC of 50,000 µg/mL, indicating that the extract inhibited bacterial growth at relatively moderate concentrations but required a higher concentration to kill the bacterial cells. This correlation between MIC and

MBC indicates that the extract exhibits bacteriostatic effect at lower concentrations and bactericidal effect at higher concentrations. This inhibitory effect is probably due to the combined action of the antimicrobial fractions obtained by TLC bioautography, as plant extracts typically contain several phytochemicals that act synergistically. Comparable results have been observed in studies on plant antimicrobial agents, which have shown that crude extracts exhibit antimicrobial activity at moderate concentrations against Gram-negative bacteria such as *E. coli*, as described by Balouiri *et al.*, 2016, and Abubakar & Haque, 2020. In addition, the ciprofloxacin control showed the lowest MIC (6,250 µg/mL) and MBC (780 µg/mL), confirming the higher potency of synthetic antibiotics compared to the crude plant extracts. However, the inhibitory activity observed in *Z. mauritiana* justifies its use in ethnomedicine and provides evidence that phytochemical such as flavonoids, tannins, and alkaloids have the potential to affect cell membrane integrity or disrupt microbial metabolic pathways.

The presence of *E. coli* and coliforms in RTE foods is a continuous food safety risk in Katsina metropolis. Given the increasing spread of multidrug-resistant *E. coli* worldwide (Agusi *et al.*, 2024), there is an urgent need for regular monitoring, enforcement of hygienic standards among street vendors, and public education on safe food handling. The promising antimicrobial properties of *Ziziphus mauritiana* could be used as an adjunctive strategy for controlling foodborne bacteria, particularly in low-resource settings.

## CONCLUSION

In this study, the microbiological quality of ready-to-eat (RTE) foods sold in Katsina Metropolis was assessed, and the antimicrobial potential of the ethanolic leaf extract of *Ziziphus mauritiana* against *Escherichia coli* isolated from the RTE foods was also evaluated. Physico-chemical parameters determined indicated that RTE foods ranged in pH from 6.7 to 7.2 and in temperature from 28 to 30.3 °C, which might be favourable for the survival and growth of microorganisms. Foods possessed acceptable sensory properties after these environmental conditions, but these conditions may promote microbial growth if foods are not handled or stored properly from a hygienic perspective. The Most Probable Number (MPN) technique used to enumerate coliforms indicated varying levels of contamination at the sampling locations. The highest mean coliform count was recorded in Kofar Sauri, while the lowest was recorded in Kofar Guga. Coliform organisms are indicators of potential post-processing contamination by the handling, storage or vending of ready-to-eat foods. Cultural and biochemical tests identified and confirmed the presence of *Escherichia coli* in some sampling locations. The biochemical reactions of the isolates were characteristic of *E. coli*. Based on the findings, some of the ready-to-eat foods sold in Katsina metropolis could be potential vehicles for foodborne pathogens. The phytochemical screening of the ethanolic leaf extract of *Ziziphus mauritiana* revealed the presence of significant bioactive phytochemicals, including alkaloids, tannins, flavonoids, glycosides, and saponins, which are

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well documented to have antimicrobial properties. Additional characterisation of the extract by Gas Chromatography–Mass Spectrometry (GC–MS) revealed the presence of several chemical compounds, of which benzoic acid was the most prominent. Fourier Transform Infrared (FTIR) spectroscopy showed functional groups associated with biologically active phytochemicals, including hydroxyl, ester, and aromatic groups. The results of these analysis techniques validated the presence of bioactive compounds that might contribute to the plant extract's antimicrobial activity.

The antimicrobial activity of the extract, assessed by thin-layer chromatography (TLC) bioautography, showed good inhibition zones at various Rf values, indicating that the extract contains components with various Rf values that inhibit *E. coli*. The highest activity was observed with Rf 0.48, indicating the presence of the most potent antimicrobial constituents in the fraction. Due to this high activity, isolate KMg was selected for further antibacterial susceptibility testing, MIC, and MBC evaluation. Furthermore, the extract exhibited significant antibacterial activity, with minimum inhibitory concentration (MIC) of 12,500 µg/ml and minimum bactericidal concentration (MBC) of 50,000 µg/ml, demonstrating that the extract inhibits bacterial growth and exerts bactericidal activity at higher concentrations. The results of this study showed that some of the ready-to-eat foods sold in Katsina metropolis were contaminated with coliforms, and *Escherichia coli*, which can pose food safety challenges to consumers due to poor food handling and inadequate hygiene practices. In addition, the ethanolic extract of *Ziziphus mauritiana* leaves exhibited significant antibacterial activity against the isolates and thus has potential to be used as a natural antimicrobial agent in the control of food borne pathogens.

## RECOMMENDATIONS

The following recommendations are based on the significant results of the present study:

1. It is recommended that strict hygiene practices among the food vendors in Katsina metropolis be put in place to minimise food contamination risk among the people in the state.
2. Street foodstuffs sold in the state should be monitored regularly to ensure compliance with safety standards.
3. Encouragement of further research on *Z. mauritiana* leaf extract as a natural preservative and antibacterial agent for the benefit of the masses.
4. Food handlers and vendors should be trained in the importance of food safety and to avoid contamination.
5. Policies should be developed and enforced, thereby carrying out regular inspections and testing of street-vended foods.

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