



Biological Inhibition of Pathogenic Bacteria Isolated from Smoked Fish and Chicken: An *In Vitro* Study

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Abstract	Article History
The increasing prevalence of antibiotic-resistant pathogenic bacteria in smoked fish and chicken poses a significant threat to food safety and public health. Exploring alternative approaches to inhibit these pathogens is crucial for developing effective control measures. This study investigated the biological inhibition of pathogenic bacteria isolated from smoked fish and chicken, exploring potential natural alternatives for improving food safety and reducing the risk of foodborne illness. A total of 280 samples that comprises 40 samples each of native chicken meat, layers chicken meat, broiler chicken meat, <i>Chupea havengus</i> (Herring/sawa), <i>Trachurus trachurus</i> (Horse Mackerel/Kote), <i>Scomber Scombrus</i> (Atlantic Mackerel/Titus) and <i>Sphyræma barracuda</i> (panla). The <i>in vitro</i> activities of the biological agents against the pathogenic isolates were determined using the agar-welled diffusion method. The data obtained were analyzed using a one-way analysis of variance (ANOVA) and turkey's test as post hoc analysis. The pathogenic bacteria majorly encountered in the studied samples were <i>Escherichia coli</i> O157:H7 strain ECP19-598 (ECEC1), <i>Staphylococcus aureus</i> strain JP18269 (SAJP1), <i>Listeria monocytogenes</i> strain LM16 (LMLM1) and <i>Salmonella enterica</i> serovar Enteritidis EC20110358 (SEEC2). These bacteria recorded 60.40% resistance to conventional antibiotics majorly amoxicillin (AMX), septrin in (SXT), chloramphenicol (CN), streptomycin (S) and augmentin (AU). The plant extracts; <i>Piper guineense</i> (PU), <i>Ocimum gratissimum</i> (OR) and <i>Gongronema latifolium</i> (GA) which contained alkaloids, tannins, phenolics, saponins, flavonoid, glycosides and steroids, and <i>Lactobacillus</i> species; <i>L. acidophilus</i> strain NC56 (LAN56) and <i>T. plantarum</i> stain 2359 (LP2359) significantly ($p \leq 0.05$) inhibited the pathogenic isolates, and the activity was seen more on plant extracts, mostly against ECEC1. Therefore, the medicinal plant extracts and <i>Lactobacillus</i> species have shown pronounced activities against ECEC1, SAJP1, LMLM1 and SEEC2 isolated from smoked fish and chicken meat samples, and plant extracts were more pronounced mostly against ECEC1. Keywords: Smoked-fish, Chicken-meat, Pathogenic, Antibiotics, Extracts.	Received: 02 May 2025 Accepted: 10 May 2025 Published: 14 May 2025 Scan QR code to view*  License: CC BY 4.0*  Open Access article.
How to cite this paper: Ekechukwu, C. C., Umeh, S. O., Iheukwumere, I. H., & Iheukwumere, C. M. (2025). Biological Inhibition of Pathogenic Bacteria Isolated from Smoked Fish and Chicken: An In Vitro Study. <i>IPS Interdisciplinary Journal of Biological Sciences</i>, 4(2), 85–92. https://doi.org/10.54117/ijbs.v4i2.50.	

1. Introduction

Pathogenic bacteria are a group of bacteria that pose a threat to man in the environment due to their virulence (Amadi *et al.*, 2016). These bacteria comprise both Gram-negative and Gram-positive. Their pathogenic nature entails that they can cause debilitating illnesses to both man and animals when contracted (Adzitey *et al.*, 2013b). The ability of pathogenic bacteria to cause diseases in man and animals depends on factors such as microbial load, virulence, and state of the immune cells of the host (Bardoe *et al.*, 2023).

It is worthy to note that some pathogenic bacteria are innate while some bacteria acquire their virulence in their environment through transduction (Bhaisare *et al.*, 2014). Certain bacteria such as *Salmonella enterica* sub species *enterica* serovar Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus*, *Echerichia coli* O157:H7, *Shigella dysenteriae*, *Clostridium botulinum* etc. are known for their high pathogenic profile, which they exhibit by attacking cells, tissues, and organs of the host either directly or by producing potent toxins such as shiga toxin produced by *E. coli* O157:H7 (Bhaisare *et al.*, 2014; Camargo *et al.*, 2017). These

pathogenic bacteria are mostly found in ready to eat food such as smoked fish and chicken, and most people in the society depend on these type of food for survival on daily basis (Darsanki *et al.*, 2012).

Research has revealed that the impact of pathogenic bacteria in the host can be curtailed using antibiotics (Dec *et al.*, 2016). Antibiotics are substances that contain chemical compounds that can either inhibit or eliminate pathogens in the host. These antibiotics had been optimized for a long period of time, to an extent that the pathogens are no longer sensitive to most of them, as they continue to proliferate in the presence of the antibiotics, which used to eliminate or inhibit them (Dec *et al.*, 2014). This phenomenon is best described as antibacterial resistance, and it had been a major challenge in the health sector globally (Dhanasekaran *et al.*, 2010). Some researchers had attributed this ugly menace to excessive use of antibiotics, incomplete dosage, and self-medication (Bhaisare *et al.*, 2014; Camargo *et al.*, 2017). The effects of antibacterial resistance are burdensome to man as revealed in the high rate of morbidity and mortality, and overall depreciation in the standard of living of the infected individuals (Bardoe *et al.*, 2023).

Research has shown that there is an extensive study on alternative sources of antibacterial agents, which could serve as an antidote to the threat posed by the resistant bacterial species (Bhaisare *et al.*, 2014). Certain bacteria such as *Lactobacillus* species are generally known for their probiotics potential (Dec *et al.*, 2014). These bacteria are capable of producing secondary metabolites, which pose a threat to pathogenic bacteria, and have been termed GRAS; meaning generally regarded as safe. The optimization of potentials in these bacteria could be invaluable to curbing the threat of antibiotic resistance in society (Camargo *et al.*, 2017).

Several researchers have worked on the effect of probiotic bacteria in the control of pathogenic bacteria such as Dhanasekaran *et al.* (2010), Darsanki *et al.* (2012), Dec *et al.* (2014), and Dec *et al.* (2016) but few studies are available on the *in vitro* biological inhibition of pathogenic bacteria isolated from smoked fish and chicken. Hence, the aim of this study is to evaluate an *in vitro* biological inhibition of pathogenic bacteria isolated from smoked fish and chicken.

2. Materials and Methods

Sample Collection: A total of 280 samples which comprises 120 roasted chicken meat samples and 160 smoked fish were used for this study (Iheukwumere *et al.*, 2018a). The roasted chicken meat samples included 40 samples of each of Native chicken meat samples, old layer meat samples and broiler chicken meat samples. The smoked fish samples included 40 samples of each of *Clupea harengus* (Sawa/Herring), *Trachurus trachurus* (Kote/Horse Mackerel), *Scomber scombrus* (Titus/Atlantic Mackerel) and *Sphyrna barracuda* (Panla). Ready-to-eat samples were aseptically separated using a sterile stainless spoon (Hamada) and collected into a sterile aluminium foil through hand picking (Iheukwumere *et al.*, 2018b). Before the sampling, the hands were washed thoroughly with soap and cleaned water and then rinsed with 70 % ethanol. Sampling was done at different

selling locations in different towns in Anambra State. The samples were placed into a cooler containing ice blocks wrapped in a sterile polythene bag and were used for sample transportation. The temperature of the cooler was checked and adjusted to 28°C-30°C by reducing the quantity of ice inside the cooler to reduce or prevent microbial shock. The samples were carefully and aseptically arranged inside the cooler without direct contact with the ice bag. The cooler was then covered and the drain plug was securely taped with packing tape to prevent accidental opening of the cooler. The cooler was then sagely carried to the Laboratory for analysis within 2 hours of sample collection. The same procedure was repeated for other collection times (Iheukwumere *et al.*, 2018c).

Sample Preparation: The samples were prepared using the routine laboratory technique. The meat samples were ground using a sterile blender (LXB 242). Then 1.0 g of each of the ground samples was aseptically weighed into a 10 mL test tube (Pyrex) each respectively. Three milliliter of sterile peptone water was aseptically added into each test tube and these were shaken thoroughly and then made up to 10.0 mL using the sterile peptone water for each test tube as described in Chesbrough (2010)

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Iheukwumere *et al.* (2017), Ejike *et al.* (2017), Iheukwumere and Ejike, (2016) and Chesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35± 2°C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% BaCl₂ 2H₂O and 99.5 mL of 1% Conc. H₂SO₄. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

In vitro activity of conventional antibiotics against the isolates using disc diffusion method: The susceptibility of the isolates to the conventional antibiotics was done using disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate equally. It was then left to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37°C for 24 h. Meter rule was used underside of the plates to determine the diameter zones of inhibition in millimeter as described in the study published by Meshaal *et al.* (2021).

In Vitro Antibacterial Activities of the Plant Extracts and Lactobacillus Species

Preparation of plant materials: The fresh seeds *Piper guineense*, leaves of *Ocimum gratissimum* and leaves of *Gongronema latifolium* were collected and they were appropriately authenticated. The fresh seeds and the leaves were dried under shade at room temperature for 14 days. The

dried samples were ground to powdered form using sterile electric grinder (LE Max/ LXB 242). Twenty grams of the ground samples each was macerated with ethanol for 72 h. Whatman No 1 filter paper was used to filter the mixture. The extracts were concentrated by evaporating to dryness at room temperature in a steady air current (Iheukwumere and Ejike 2016, Chukwurah and Iheukwumere, 2013 and Iheukwumere *et al.*, 2020).

Phytochemical analysis of the plant extracts: The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by Ashraf *et al.* (2018) and Iheukwumere *et al.* (2021).

Alkaloids: Five milliliters of the sample were mixed with 96 % ethanol and 20% tetraoxosulphate (VI) acid (1:1). One milliliter of the filtrate from the mixture was added to 5 mL of 60 % tetraoxosulphate (VI) acid and allowed for 5 minutes. Then, 5 mL of 0.5 % formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 550 nm.

Glycosides: This was carried out using Buljet's reagent. One gram of the fine powder of the sample was soaked in 10 mL of 70 % alcohol for 2 h and then filtered with Whatman No. 1 filter paper. The extract was then purified using lead acetate solution and disodium hydrogen tetraoxosulphate (VI) solution before the addition of freshly prepared Buljet's reagent. The absorbance was read at 550 nm.

Flavonoids: Five milliliters of the extract were mixed with 5 mL of dilute hydrochloric acid and allowed to boil for 30 minutes. The boiled extract was left to cool and then filtered with Whatman No. 1 filter paper. One milliliter of the filtrate was added to 5 mL of ethyl acetate and 5 mL of 1 % ammonia solution. The absorbance was taken at 420 nm.

Phenolics: Ten milliliters of the sample were boiled with 50 mL acetone for 15 minutes. Five milliliters of the solution were pipetted into 50 mL flask. Then, 10 mL of distilled water was added. This was followed by addition of 2 M ammonium hydroxide solution and 5 mL of concentrated amyl alcohol solution. The mixture was left for 30 minutes and absorbance was taken at 550 nm.

Tannins: Ten milliliters were pipetted into 50 mL plastic containing 50 mL of distilled water. This was mixed for 1 h on a sterile mechanical shaker. The mixture was filtered with Whatman No. 1 filter paper, and 5 mL of the filtrate was mixed with 2 mL of iron (III) chloride solution in 0.1 N hydrochloric acid. The absorbance was read at 550 nm.

Steroids: The extract was eluted with normal ammonium hydroxide solution. Two milliliters of eluate were mixed with 2 mL of chloroform in a test tube. Three milliliters of ice-cold acetic anhydride were added to the mixture and allowed to cool. The absorbance was taken at 420 nm.

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and

incubated at 35± 2°C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% BaCl₂ 2H₂O and 99.5 mL of 1% Conc. H₂SO₄. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

Preparation of plant extracts: The weight obtained was weighed using electronic weighing balance, 10 mL of peptone water (diluent) was added and made up to 50 mL using the diluents. Then 50 mL mixture (1:1) of OR+PU, OR+GA, PU+GA and 1:1:1 mixture of OR+PU+GA were prepared. All these were tested for the best activity as described in the study published by Iheukwumere *et al.* (2017a). Also, 50 mL mixtures of U (OG+PU+GA) at optimum concentration (250 mg/mL) were prepared

In vitro susceptibility study of the plant extracts using agar well diffusion method: This was carried out by the modified method described by Dahiya and Purkayastha (2012). The test organisms were seeded in Muller Hinton Agar (MHA/BIOTECH) plates. The plates were appropriately labelled. A sterile cork borer of 5 mm diameter was used to make the wells on the medium. A one-tenth millilitre of various extracts was dropped into each labelled well and then placed vertically in the Bacteriological incubator and incubated at 37±2°C for 24 h. The susceptibility patterns were determined by measuring the diameter of the zones of inhibition (mm) produced after incubation

Isolation of *Lactobacillus* species: One milliliter (1.0 ml) sample (Yoghurt and banana) was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline). One milliliter of the diluted sample (10⁻¹) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing deMann Rogosa Sharpe medium (MRSBiotech) using pour plate method. All the plates in triplicates were incubated inverted in microaerophilic conditions for 48 h (Cheesbrough, 2010). The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described in the study published by Okpalla *et al.* (2012), Cheesbrough (2010) and Frank and Robert (2015).

Preparation of *Lactobacillus* broth culture: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35± 2°C for 24 h. Then 50 mL of different mixtures of LAN56 and LP2359 were prepared

In vitro susceptibility study of *Lactobacillus* broth cultures using agar well diffusion method: This was carried out by the modified method described by Dahiya and Purkayastha (2012). The test organisms were seeded in Muller Hinton Agar (MHA/BIOTECH) plates. The plates were appropriately labeled. A sterile cork borer of 5 mm diameter was used to make the wells on the medium. One tenth millilitre of 24 h broth culture of the isolate was dropped into each labeled wells and then placed vertically in the Bacteriological incubator and

incubated at 37±2°C for 24 h. The susceptibility patterns were determined by measuring the diameter of the zones of inhibition (mm) produced after incubation

Statistical Analysis

The data obtained from this study were represented in Tables, Figures and as mean ± standard deviation. The significance of the study was carried out using a one-way Analysis of Variance (ANOVA) at 95 % confidence level. The pair-wise comparison was done using the Turkey test as described by Iheukwumere *et al.* (2021).

3. Results

The molecular characteristics revealed the presence of *Escherichia coli* O157:H7 strain ECP19-598 (ECEC1), *Staphylococcus aureus* strain JP 18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain EC20110358 (SEEC2) as shown in Table 1.

Susceptibility of the Pathogenic Bacterial Isolates to Conventional Antibiotics

The study showed that 60.04% of the total isolates were resistant to conventional antibiotics mainly amoxicillin (AMX), septrin (SXT), chloramphenicol (CN), streptomycin (S) and augmentin (AU). The study also revealed that LMLM1 showed more resistance to conventional antibiotics than other implicated isolates. The data obtained from this study also revealed that there is an association between the strain of the bacterial isolates and susceptibility patterns to conventional antibiotics as the data were statistically significant ($P < 0.05$) as shown in Table 2.

In vitro Antibacterial Activities of the Plant Extracts and Lactobacillus species

The phytochemical constituents of *Piper guineense* (seeds), *Ocimum gratissimum* (leaves) and *Gongronema latifolium* (leaves) extracts revealed the presence of glycosides, alkaloids, steroids, tannins, flavonoids, phenolics and saponins (Table 4). *Piper guineense* (PU) recorded the highest content of glycosides, steroids, flavonoids and phenolics. *Ocimum gratissimum* (OR) recorded the highest content of saponins whereas *Gongronema latifolium* (GA) recorded the highest alkaloids and tannins as shown in Table 3.

The plant extracts (PU, OR and GA) showed pronounced activities against the test isolates (ECEC1d, SAJP1, LMLM1 and SEEC2) as shown in Figures 1, 2 and 3. It was observed that the plant extracts inhibited ECEC1 the most, followed by LMLM1, SEEC2 and SAJP1 showed the least inhibition. The activities of the plant extracts were statistically significant ($p < 0.05$) when compared to one another. OR recorded the highest activities against the test isolates when compared to PU and GA but the variation in their activities was statistically non-significant ($p > 0.05$).

The study also revealed a progressive increase in the activities of the plant extracts as the concentrations of the plant extracts increased but recorded retardation after the concentration reached 250mg/ml as shown in Figures 1, 2 and 3.

The molecular identities of the *Lactobacillus* species revealed the presence of *Lactobacillus acidophilus* strain NC56 (LAN56) and *Lactobacillus plantarum* strain 2359 (LP2359) as shown in Table 4.

The pronounced activities of LAN56 and LP2359 against ECEC1, SAJP1, LMLM1 and SEEC2 are shown in Table 5. It was observed that the *Lactobacillus* species inhibited ECEC1 most when compared to other pathogenic bacteria involved in this study but the variations in the diameter zone of inhibition of LAN56 and LP2359 among the bacterial isolates were statistically not significant ($p < 0.05$). Also, the activities of the LP2359 against the test isolates were non-significantly ($p > 0.05$) higher than that of LAN56, and significantly ($p < 0.05$) lower than that of ciprofloxacin (CPX) as shown in Table 5.

The *in vitro* biotreatment of the pathogenic bacterial isolates are shown in Table 6. The study revealed the pronounce activities of the plant extracts and *Lactobacillus* species. The activities of the biological agents were most against ECECE1, and OR showed the highest activities against the test isolates. The activities of CPX were significantly ($p < 0.05$) higher than the activities of GA, LAN56 and LP2359 against ECEC1, and non-significantly ($p > 0.05$) higher than the activities of the biological agents against SAJP1 and SEEC2 except that but its activities were lower than the activities of OR and PU against LMLM1 as shown in Table 6. The activities of the biological agents against the test isolates were statistically significant ($p < 0.05$) whereas the variations among the activities of the biological agents were statistically non-significant ($p > 0.05$).

Table 1: Molecular characteristics of the bacterial isolates

Parameter	Isolate P	Isolate Q	Isolate R	Isolate S
Max score	38284	34485	25078	26613
Total Score	38284	34485	60734	26613
Query cover (%)	100	100	100	100
E-value	0.0	0.0	0.0	0.0
Identity (%)	100	100	100	100
Accession Number	CP066753.1	CP097114.1	CP027029.1	CP007260.1
Description	<i>Escherichia coli</i> strain ECP19-598 (ECEC1)	<i>Staphylococcus aureus</i> strain JP18269 complete genome (SAJP1)	<i>Listeria monocytogenes</i> strain LM16 chromosome (LMLM1)	<i>Salmonella enterica</i> subsp <i>enterica</i> serovar Enteritidis strain EC 20110358 complete genome (SEEC2)

Table 2: Susceptibility of the Isolates to Conventional Antibiotics

Isolate	Number (%)	Resistant Strain (%)	Susceptible Strain (%)	Resistant Antibiotic
ECEC 1	29(18.83)	17(58.62)	12(41.38)	AMX, AU, S, PN, AMA, SXT, CN
SAJP 1	76(49.35)	46(60.53)	30(39.47)	AMX, AU, S, CN, SXT, ORF, CPX
LMLM 1	19(12.34)	13(68.42)	6(31.58)	AMX, AU, S, CN, SXT, ORF, CPX
SEEC 2	30(19.48)	18(60.00)	12(40.00)	AU, S, PN, SXT
Total	154(100.00)	94(61.04)	60(38.96)	

Table 3: Phytochemical constituents of the medicinal plant extracts used for the study

Parameters	<i>Piper guineense</i>	<i>Ocimum gratissimum</i>	<i>Gongronema latifolium</i>
Glycosides	1.22 ± 0.03	1.02 ± 0.01	0.44 ± 0.04
Steroids	0.84 ± 0.01	0.27 ± 0.01	0.08 ± 0.00
Alkaloids	1.88 ± 0.11	1.21 ± 0.04	9.88 ± 0.13
Tannins	3.14 ± 0.21	2.49 ± 0.11	7.26 ± 0.12
Flavonoids	6.02 ± 0.11	1.77 ± 0.03	1.07 ± 0.21
Phenolics	4.08 ± 0.14	0.81 ± 0.01	1.22 ± 0.11
Saponins	1.68 ± 0.07	3.18 ± 0.17	3.09 ± 0.12

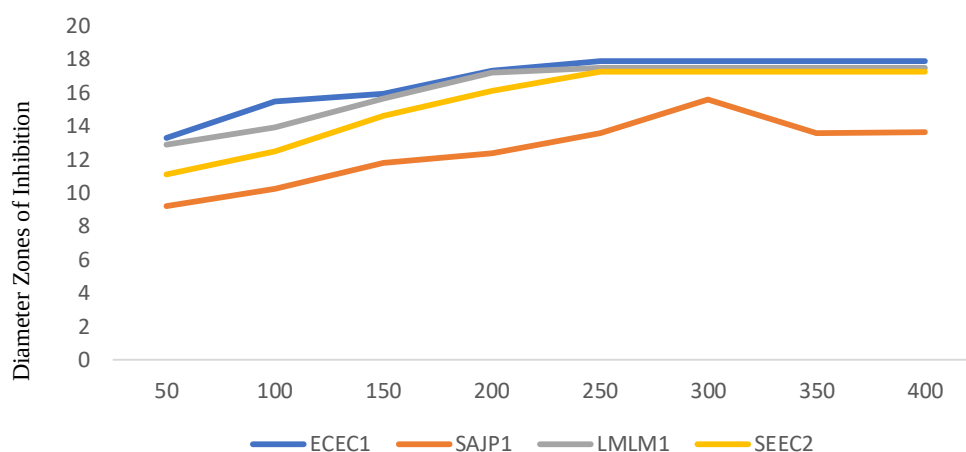


Figure 1: Diameter zones of inhibition of *Ocimum gratissimum* against the test isolates.

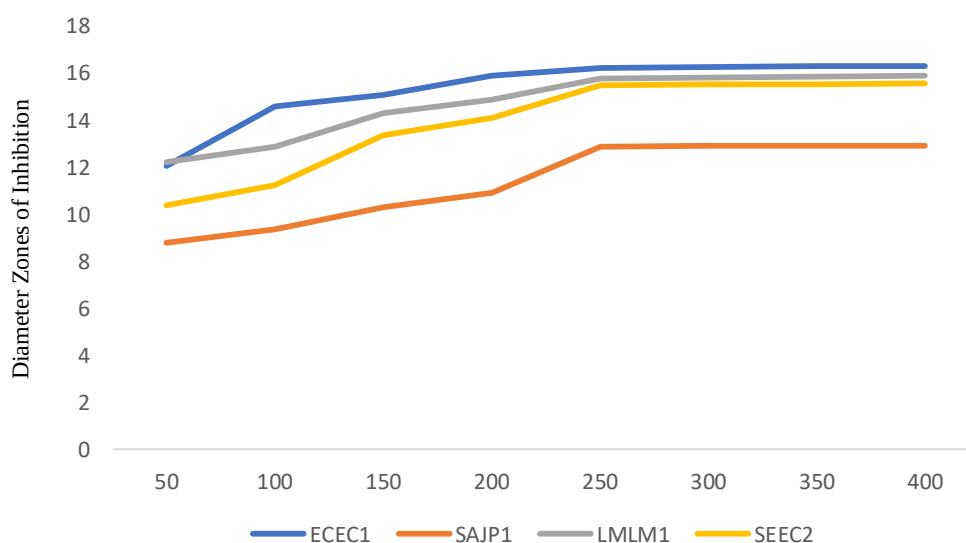


Figure 2: Diameter zones of inhibition of *Piper guineense* extract against the test isolates.

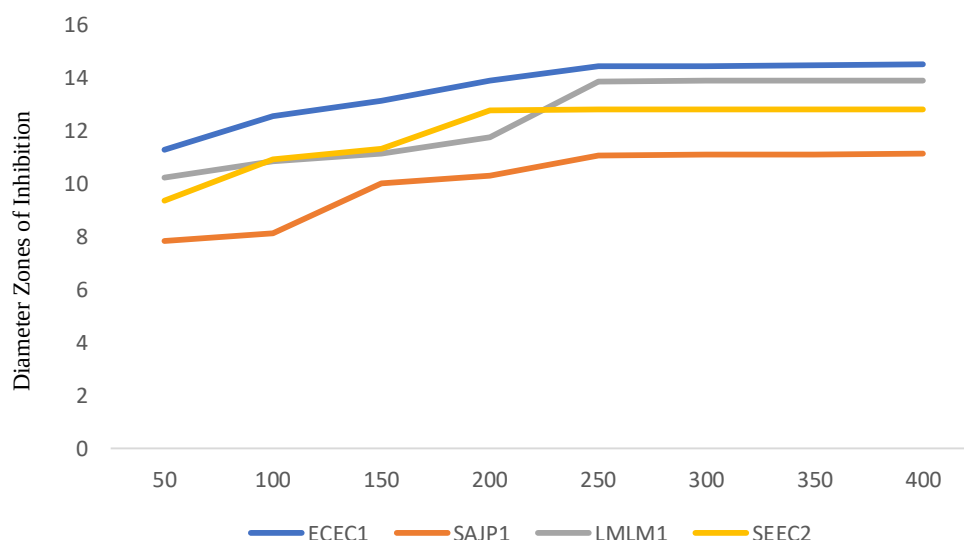


Figure 3: Diameter zones of inhibition of *Gongronema latifolium* extract against the test isolates.

Table 4: Molecular characteristics of the *Lactobacillus* species

Parameter	Isolate K	Isolate M
Max score	40501	35179
Total score	40501	68428
Query cover (%)	100	100
E – value	0.0	0.0
Percent identity	100	100
Accession number	CP106868.1	CP145812.1
Description	<i>Lactobacillus acidophilus</i> strain NC56 chromosome complete genome (LAN56)	<i>Lactobacillus plantarum</i> strain 2359 chromosome complete genome (LP2359)

Table 5: Diameter Zones of inhibition of the *Lactobacillus* species against the test isolates.

Isolate	Mean Diameter ($\bar{x} \pm SD$) mm		
	LAN56	LP2359	CPX
ECEC1	14.26 $\pm$ 0.11	15.84 $\pm$ 0.81	24.56 $\pm$ 0.12
SAJP1	10.84 $\pm$ 0.21	15.83 $\pm$ 0.41	14.22 $\pm$ 0.11
LMLM1	12.36 $\pm$ 0.33	14.28 $\pm$ 0.17	15.48 $\pm$ 0.14
SEEC2	12.92 $\pm$ 0.22	16.22 $\pm$ 0.11	19.36 $\pm$ 0.12

Table 5: *In vitro* biotreatment of pathogenic bacterial isolates

Biotreatment	Mean Diameter (mm) using 5mm corkborer			
	ECEC1	SAJP1	LMLM1	SEEC2
OR (250mg/ml)	17.86 $\pm$ 0.11	13.57 $\pm$ 0.12	17.48 $\pm$ 0.11	17.22 $\pm$ 0.17
PU (250mg/ml)	16.22 $\pm$ 0.11	12.86 $\pm$ 0.22	15.77 $\pm$ 0.33	15.48 $\pm$ 0.11
GA (250mg/ml)	14.44 $\pm$ 0.11	11.08 $\pm$ 0.17	13.86 $\pm$ 0.14	12.78 $\pm$ 0.11
LAN56 (10 ⁸ cells/ml)	14.26 $\pm$ 0.11	10.84 $\pm$ 0.21	12.36 $\pm$ 0.33	12.92 $\pm$ 0.22
LP2359 (10 ⁸ cells/ml)	15.84 $\pm$ 0.81	15.83 $\pm$ 0.41	14.28 $\pm$ 0.17	16.22 $\pm$ 0.11
CPX (5mg/ml)	24.56 $\pm$ 0.12	14.22 $\pm$ 0.11	15.48 $\pm$ 0.14	19.36 $\pm$ 0.12

4. Discussion

The significant mean bacterial counts, majorly total heterotrophic bacterial counts (THBC), total coliform counts (TCC), total faecal coliform counts (TFCC), total *Salmonella* counts (TSC), total *Shigella* counts (TSHC), total *Staphylococcus aureus* counts (TSAC) and total *Listeria monocytogenes* counts (TLMC) supported The findings of many researchers (Adeyanju and Ishola, 2014; Mashak, 2018; Ishihava *et al.*, 2020; Karisma *et al.*, 2021).

The significant resistance of the conventional antibiotics mainly amoxicillin (AMX), septrin (SXT), chloramphenicol (CN), streptomycin (S) and augmentin (AU) against the studied pathogenic bacterial isolates supported the findings of many researchers (Adzitey, 2011; Adzitey *et al.*, 2012a; Adzitey *et al.*, 2012b; Singh *et al.*, 2013). The resistance of these antibiotics against the organisms could be attributed to the presence of resistance plasmid, antibiotic rethix CV mutation. Similar deductions were made by Adzitey *et al.* (2013a) and Nobile *et al.*

(2013). Several researchers such as Adzitey, (2011) and Adzitey *et al.* (2013a) detected resistant *E. coli* from their studied samples, Adzitey *et al.* (2012b) and Singh *et al.* (2013) detected resistant *Salmonella* species in their studied samples, Adzitey *et al.* (2013b) detected resistant *Listeria monocytogenes* in their studied samples.

The study further highlighted the presence of glycosides, steroids, alkaloids, tannins, flavonoids, phenolics and saponins in the *Piper guineense* (PU) *Ocimum gratissimum* (OR) and *Gongronema latifolium* (GA) extracts. The presence of these phytochemicals could be attributed to the antibacterial activities of the plant extracts. Similar deductions were made by Iheukwumere *et al.* (2017). The pronounced activities of the plant extracts against the pathogenic bacterial isolates supported the findings of many researchers (Ahmed *et al.*, 2014; Radaelli *et al.*, 2016; Ugur *et al.*, 2016; Heer *et al.*, 2017; Iheukwumere *et al.*, 2017; Mostafa *et al.*, 2018; Hetta *et al.*, 2020; Ghavam *et al.*, 2020; Oliveira *et al.*, 2024; Meshaal *et al.*, 2021). The most significant inhibition of the extracts against ECEC1 agrees with the findings of Iheukwumere *et al.* (2017) and Ghavam *et al.* (2020). The highest activities of OR against the studied pathogenic bacterial isolates could be attributed to the potency of the extract. Similar deductions were made by Iheukwumere *et al.* (2017). The pronounced activities of *Lactobacillus acidophilus* strain NC56 (LAN56) and *Lactobacillus plantarum* strain 2359 (LP2359) against the studied pathogenic bacterial isolates corroborated with the findings of many researchers (Nouri *et al.*, 2010; Darsanaki *et al.*, 2012; Tonekabon, 2013; Dec *et al.*, 2014; Bratz *et al.*, 2015; Dec *et al.*, 2016). The slight increase in the activities of LP2359 when compared to that of LAN56 could be attributed to the potency and level of bacteriocins produced by the *Lactobacillus* species.

5. Conclusion

Therefore, the plant extracts; *Piper guineense* (PU), *Ocimum gratissimum* (OR) and *Gongronema latifolium* (GA) and *Lactobacillus* species; *L. acidophilus* strain NC56 (LAN56) and *T. plantarum* stain 2359 (LP2359) have shown pronounced activities against *Escherichia coli* 0157:H7 strain ECP19-598 (ECEC1), *Saphylococcus aureus* strain JP18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* serovar Enteritidis EC20110358 (SEEC2) isolated from smoked fish and chicken meat samples, and plant extracts were more pronounced mostly against ECEC1.

Acknowledgments

We are grateful to all our study participants who join the study voluntarily. We are grateful to ZAHARM Analytical and Research Laboratory, Amawbia, Awka Anambra State, Nigeria for providing enabling environment, resources and techniques for this study. We really salute their wonderful efforts.

Conflict of interests: The authors declare that they have no conflict of interests.

Funding: This research did not receive specific grant from any funding agencies.

Ethical approval: Not applicable

Authors Contributions: All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

References

- Adeyanju, G. T., and Ishola, O. (2014). *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springer Plus*, 3, 139.
- Adzitey, F., Rusul, G., Huda, N., Cogan, T., & Corry, J. (2013b). Prevalence, antibiotic resistance and genetic diversity of *Listeria monocytogenes* isolated from ducks, their rearing and processing environments in Penang, Malaysia. *Food Control*, 32, 607–614.
- Adzitey, F. (2011). *Escherichia coli*, its prevalence and antibiotic resistance in Malaysia—a mini review. *Microbiology Journal*, 1, 47–53.
- Adzitey, F., Huda, N., & Ali, G. R. R. (2013a). Molecular techniques for detecting and typing of bacteria, advantages, and application to foodborne pathogens isolated from ducks. 3 *Biotech*, 3, 97–107.
- Adzitey, F., Liew, C. Y., Aronal, A. P., & Huda, N. (2012a). Isolation of *Escherichia coli* from ducks and duck-related samples. *Asian Journal of Animal and Veterinary Advances*, 7(4), 351–355.
- Adzitey, F., Rusul, G., Huda, N., Cogan, T., & Corry, J. (2012b). Prevalence, antibiotic resistance, and RAPD typing of *Campylobacter* species isolated from ducks, duck rearing and processing environments in Penang, Malaysia. *International Journal of Food Microbiology*, 154, 197–205.
- Ahmed, A. M., Rabii, N. S., Garbaj, A. M., & Abolghait, S. K. (2014). Antibacterial effect of olive (*Olea europaea* L.) leaves extract in raw peeled undeveined shrimp (*Penaeus semisulcatus*). *International Journal of Veterinary Science and Medicine*, 2(1), 53–56.
- Ajiboye, B. O., Iwaloye, O., Owolabi, O. V., Ejeje, J. N., Okerewa, A., Johnson, O. O., Udebor, A. E., and Oyinloye, B. E. (2022). Screening of potential antidiabetic phytochemicals from *Gongronema latifolium* leaf against therapeutic targets of type 2 diabetes mellitus: Multi-target drug design. *SN Applied Sciences*, 4(1), 1–13.
- Amadi, O., Faith, O., Ruth, E., & Nathaniel, N. (2016). Bacterial status and microbial susceptibility profile of selected pathogens associated with suya meat samples purchased in Bori metropolis, River State, Nigeria. *International Research Journal on Public Environmental Health*, 3(2), 14–16.
- Ashraf, A. M., Abdulaziz, A. A., Khalid, S. A., Turki, M. D., Essan, N. S., & Marwah, M. B. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, 25(2), 361–366.
- Bardoe, D., Gyabeng, J., Hayford, D., and Ibrahim, I. (2023). Evaluation of bacteria composition in smoked fish processed in Yeji-Pru East District, Ghana. *Journal of Advances in Microbiology*, 23(4), 50–65.
- Bhaisare, D. B., Thyagarajan, D., Churchil, R. R., and Punnamurthy, N. (2014). Bacterial pathogens in chicken: A review. *International Journal of Life Science and Resources*, 2(3), 1–7.
- Bratz, K., Gözl, G., Janczyk, P., Nöckler, K., & Alter, T. (2015). Analysis of in vitro and in vivo effects of probiotics against *Campylobacter* spp. *Berl. Münch. Tierärztl. Wochenschr*, 128(3-4), 155–162.
- Camargo, A. C., Woodward, J. J., Call, D. R., & Nero, L. A. (2017). *Listeria monocytogenes* in food-processing facilities, food contamination, and human listeriosis: the Brazilian scenario. *Foodborne Pathogens and Disease*, 14(11), 623–636.
- Cheesbrough, M. (2010). *Microbiological test: District laboratory practice in tropical countries*. In Cremer, A., & Evan, G. (Eds.), Cambridge University Press, U.K, pp. 211–226.
- Chukwura, E.I and Iheukwumere, I. (2013). Larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves on *Anopheles gambiae*. *Journal of Scientific & Industrial Research* 72: 577 – 580.
- Dahiya, P., & Purkaryastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multidrug-resistant bacteria from clinical isolates. *Indian Journal of Pharmaceutical Sciences*, 125, 443–450.
- Darsanaki, R. K., Rokhi, M. L., Aliabadi, M. A., & Issazadeh, K. (2012). Antimicrobial activities of *Lactobacillus* strains isolated from fresh vegetables. *Middle-East Journal of Scientific Research*, 11(9), 1216–1219.
- Dec, M., Puchalski, A., Nowaczek, A., & Wernicki, A. (2016). Antimicrobial activity of *Lactobacillus* strains of chicken origin against bacterial pathogens. *International Journal of Microbiology*, 19(1), 57–67.
- Dec, M., Puchalski, A., Urban-Chmiel, R., & Wernicki, A. (2014). Screening of *Lactobacillus* strains of domestic goose origin against bacterial poultry pathogens for use as probiotics. *Poultry Science*, 93(10), 2464–2472.

- Dhanasekaran, D., Saha, S., Thajuddin, N., Rajalakshmi, M., & Panneerselvam, A. (2010). Probiotic effect of *Lactobacillus* isolates against bacterial pathogens in freshwater fish. *Journal of Coastal Development*, 13(2), 103–112.
- Ejike, C.C., Iheukwumere, I. H. and Amadi, R. E. (2017). The effects of *Allium sativum* and *Zingiber officinale* extracts on *Shigella dysenteriae* isolated from ready-to-eat fried chicken sold in Ihiala L.G.A, Anambra State. *Journal of Natural Sciences Research* 7: 1–5.
- Frank, E. B., & Robert, C. J. (2015). *Practical Medical Microbiology for Clinicians* (1st ed.). Wiley & Sons Inc., Hoboken, New Jersey, USA, pp. 13–40.
- Ghavam, M., Manca, M. L., Manconi, M., & Bacchetta, G. (2020). Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of *Salvia hydrangea* DC. ex Benth. *Scientific Reports*, 10(1), 15647.
- Heer, A., Guleria, S., & Razdan, V. K. (2017). Chemical composition, antioxidant and antimicrobial activities and characterization of bioactive compounds from essential oil of *Cinnamomum tamala* grown in north-western Himalaya. *Journal of Plant Biochemistry and Biotechnology*, 26(2), 191–198.
- Hetta, H. F., Kh Meshaal, A., Algammal, A. M., Yahia, R., Makharita, R. R., Marraiki, N., ... & Batiha, G. E. S. (2020). In-vitro antimicrobial activity of essential oils and spices powder of some medicinal plants against *Bacillus* species isolated from raw and processed meat. *Infection and Drug Resistance*, 4367–4378.
- Iheukwumere, I. H., Amadi, R. E. and Unaeye, B. C. (2017). Enterotoxigenicity profile of *Salmonella enterica* serovar Typhimurium in suckling albino mice. *Journal of Natural Sciences Research* 7 (14):16–20.
- Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018a). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research* 8: 21–33.
- Iheukwumere, I.H., Chude, C. and Unaeye, B.C. (2018b). Toxicological study and antibacterial activities of effectively validated medicinal plants against enteric bacteria isolated from chicken feeds. *Journal of Health, Medicine and Nursing* 7: 19–34.
- Iheukwumere, I.H., Chukwura, E.I and Chude, C. (2018c). *In vivo* activities of some selected antimicrobial agents against enteric bacteria isolated from chicken feeds on broiler layers. *Journal of Biology, Agriculture and Healthcare* 9: 21–36.
- Iheukwumere, I.H., Dimejesi, S.A., Iheukwumere, C.M., Chude, C.O., Egbe, P.A., Nwaolisa, C.N., Amutaigwe, E.U., Nwakoby, N.E., Egbuna, C., Olisah, M.C. and Ifemeje, J.C. (2020). Plasmid curing potentials of some medicinal plants against citrate-negative motile *Salmonella* species. *European Journal of Biomedical and Pharmaceutical Sciences* 7(5); 40 – 47.
- Iheukwumere, I. H., Opara, G. R., Iheukwumere, M. C., Okafor, . C. F., & Nwakoby, N. E. (2021). The prophylactic potential of Essential cream produced from *Chromolaena odorata* leaf extract against *Cladosphialophora bantiana* strain D12E. *IPS Journal of Applied Microbiology and Biotechnology*, 1(1), 1–11
- Iheukwumere, I.H. and Ejike, C.E. (2016). Comparative study of the inhibitory activities of *Ocimum gratissimum* and *Nepeta cataria* against *Salmonella enterica* serovar Typhi and their larvicidal effect against *Anopheles gambiae*. *African Journal of Education, Science and Technology* 5(22): 112 – 118.
- Iheukwumere, I.H., Amadi, E.R. and Chude, C. (2018d). Synergistic effects of probiotics and autogenous bacterin against inositol negative motile *Salmonella* Species. *Journal of Biology, Agriculture and Healthcare* 9: 37–49.
- Ishihara, K., Nakazawa, C., Nomura, S. S., Elah, S., Yamashita, M., and Fujikawa, H. (2020). Effects of climatic elements on *Salmonella* contamination in broiler chicken meat in Japan. *Journal of Veterinary Medical Science*, 82(5), 646–652.
- Karisma, U., Wiqoyah, N., and Pusarawati, S. (2021). Prevalence of *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus* bacteria in chicken meat of traditional markets in Surabaya City. *Journal of Science and Technology*, 8(2), 193–204.
- Mashak, Z. (2018). Prevalence and antibiotic resistance of *Escherichia coli* O157: H7 isolated from raw meat samples of ruminants and poultry. *Journal of Food Nutrition and Research*, 6(2), 96–102.
- Meshaal, A. K., Hetta, H. F., Yahia, R., Abualanja, K. M., Mansour, A. T., Al-Kadmy, I. M., Alghamdi, S., Dabool, A. S., Emran, T. B., Sedky, H., Batiha, G. E., & El-Kazzaz, W. (2021). In vitro antimicrobial activity of medicinal plant extract against some bacterial pathogens isolated from raw and processed meat. *Life*, 4(11), 1178–1186.
- Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, 25(2), 361–366.
- Nouri, M., Rahbarizadeh, F., Ahmadvand, D., Mousakhani, F., Sadeghzadeh, E., Lavasani, S., & Khodami, V. V. (2010). Inhibitory effects of *Lactobacillus salivarius* and *Lactobacillus crispatus* isolated from chicken gastrointestinal
- Okpalla, J., Ubajekwe, C.C., Agu, K.C. and Iheukwumere, I. (2012). Biochemical changes of melon seeds (*Citrullus vulgaris*) fermented by pure cultures of *Bacillus licheniformis*. *International Journal of Agriculture and Biosciences* 1(1): 42 – 45.
- Oliveira, R. D., Araújo, C., & Almeida-Aguiar, C. (2024). In vitro antimicrobial potential of Portuguese propolis extracts from Gerês against pathogenic microorganisms. *Antibiotics*, 13(7), 655.
- Radaelli, M., Silva, B. P. D., Weidlich, L., Hoehne, L., Flach, A., Costa, L. A. M. A. D., & Ethur, E. M. (2016). Antimicrobial activities of six essential oils commonly used as condiments in Brazil against *Clostridium perfringens*. *Brazilian Journal of Microbiology*, 47, 424–430.
- Singh, V. K., Jain, U., Yadav, J. K., & Bist, B. (2014). Bacterial quality of raw meat samples from retail meat outlets and local slaughter houses. *Journal of Foodborne and Zoonotic Diseases*, 2(1), 15–18.
- Tonekabon, I. (2013). Evaluation of antimicrobial activity of three *Lactobacillus* spp. against antibiotic resistance *Salmonella typhimurium*. *Adv Studies Biol*, 5(2), 61–70.
- Ugur, A. R., Dagi, H. T., Ozturk, B., Tekin, G., & Findik, D. (2016). Assessment of in vitro antibacterial activity and cytotoxicity effect of *Nigella sativa* oil. *Pharmacognosy Magazine*, 12(Suppl 4), S471.



FEATURED PUBLICATIONS

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DOI: <https://doi.org/10.54117/ijjbs.v4i2.24>

Cite as: Oguntuyinbo, O. O., Ogunmurewa, J. A. V., & Ormoba, O. S. (2023). Antioxidant and Dietary Fibre Content of Noodles Produced From Wheat and Banana Peel Flour. *IPS Journal of Nutrition and Food Science*, 2(2), 46–51.

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