IPS Journal of Applied Microbiology and Biotechnology
IPS J Appl Microbiol Biotech, 4(3): 153-160 (2025)
DOI: https://doi.org/10.54117/ijamb.v4i2.57

Prophylactic Potential of the Most Potent Synergistic Biological Agent against Bacterial Infections from Smoked Fish and Chicken

C. C. Ekechukwu[1*], S. O. Umeh[2], I. H. Iheukwumere[3*] and C. M. Iheukwumere[2]

[1]Biology Education Department, Federal College of Education, Asaba, Delta State.
[2]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe, University, Awka, Nigeria.
[3]Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University.

*Corresponding authors e-mail:
oluebubechukwu2022@gmail.com; ik.iheukwumere@coou.edu.ng

Abstract

Bacterial infections from smoked fish and chicken pose significant risks to consumer health and also increase the spread of resistance strains. Therefore, the need for effective prophylactic strategies, and identifying and evaluating the prophylactic potential of potent synergistic biological agents could offer a promising solution. This study was undertaken to investigate the prophylactic potential of the most potent synergistic biological agent against bacterial infections from smoked fish and chicken, exploring its efficacy in preventing infections and promoting public health. A total of 280 samples that comprises 40 samples each of native chicken meat, layers chicken meat, broiler chicken meat, *Chupea havengus* (Herring/sawa), *Truchurus trachurus* (Horse Mackerel/Kote), *Scomber Scombrus* (Atlantic Mackerel/Titus) and *Sphyraema barracuda* (panla). The synergistic effect of the medicinal plants and *Lactobacillus* species was carried out using *in vitro* method. The prophylactic activity of the most potent synergistic agent was carried out using *in vivo* method. The data obtained were analyzed using one-way analysis of variance (ANOVA) and Turkey's test as a post hoc analysis. The study showed that the mixture of *Piper guineense* (PU), *Ocimum gratissimum* (OR) and *Gongronema latifolium* (GA) in 2:1:1 doses augmented with the mixture of *L. acidophilus* strain NC56 (LAN56) and *L. plantarum* stain 2359 (LP2359) in 2:1 doses significantly ($p \leq 0.05$) reduced the pathogenic features of *Escherichia coli* 0157:H7 strain ECP19-598 (ECEC1), *staphylococcus aureus* strain JP18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* serovar Enteritidis EC20110358 (SEEC2) isolated from smoked fish and chicken meat. Therefore, this study has shown the occurrences of ECEC1, SAJP1, LMLM1 and SEEC2 in the studied samples. The isolates showed significant pathological features that were reduced/prevented by the studied biological agents, of which OR+PU+GU augmented with LAN56 + LP2359 was the most effective.

Keywords: *Smoked-fish, Chicken-meat, Biological-agents, Extracts, Lactobacillus, In vivo*

Article History
Received: 05 May 2025
Accepted: 26 May 2025
Published: 31 May 2025

Scan QR code to view•

How to cite this paper: Ekechukwu, C. C., Umeh, S. O., Iheukwumere, I. H., & Iheukwumere, C. M. (2025). Prophylactic Potential of the Most Potent Synergistic Biological Agent against Bacterial Infections from Smoked Fish and Chicken. *IPS Journal of Applied Microbiology and Biotechnology*, *4*(2), 153–160. https://doi.org/10.54117/ijamb.v4i2.57.

Introduction

Bacterial infections occur when pathogenic bacteria invade a host through any of the portals of entry such as mouth, nose, eye, anus, urinary opening, genital area etc. (Bardoe *et al*., 2023). These infections are dangerous especially when the bacteria are highly pathogenic and the ability of the invaded bacteria to circumvent immune cells provides an avenue for proliferation, thereby releasing potent toxins, which damage the host epithelium tissues and vital organs (Bhaisare *et al*., 2014).

Pathogenic bacteria that are responsible for bacterial infections are widely distributed in the environment globally (Camargo *et al*., 2017). Most of these bacteria are introduced into ready-to-eat food such as smoked fish and chicken due to negligence and unsanitary practices (Bardoe *et al*., 2023). The presence of pathogenic bacteria in ready-to-eat food poses a huge threat to public health because an epidemic could ensue, which is capable of causing havoc in society through morbidity and mortality such as cholera from *Vibrio cholerae*, botulism from *Clostridium botulinum*, diarrahoea from enterotoxigenic

Escherichia coli (ETEC), typhoid fever from *Salmonella enterica* subspecies *enterica* serovar Typhimurium etc. (Amadi *et al*., 2016).

Research has shown that medicinal plants contain phytochemical compounds, which can be extracted and optimized in crude form or as essential oils for tackling bacterial pathogens (Ajiboye *et al*., 2022). Phytochemical compounds in the medicinal plants' extracts such as flavonoids, saponins, taninins, glycosides, etc. had been revealed to exhibit antibacterial potentials. These extracts are potent to extent that metabolic processes and structural development in pathogenic bacteria are inhibited and eliminated (Ahmed *et al*., 2014).

According to Bisi-Johnson *et al*. (2017), extracts from medicinal plants are best optimized when combined with another extract or conventional antibiotic otherwise known as synergism optimization. The combined efficacy of two or more extracts produces effects that could be difficult to be resisted by pathogenic bacteria. Similarly, *Lactobacillus* species are known for their probiotic potentials, and the ability of these species to produce inhibitory substances had been documented (Dahiya and Purkaryastha, 2012). This shows that combining extracts from medicinal plants with *Lactobacillus* species could result to enhancement or acceleration of the bioactive compounds produced by the bacteria as reported by Holkem *et al*. (2022).

It is worthy to note that combination of extracts from medicinal plants and *Lactobacillus* species could provide protective potential against pathogenic bacterial isolated from smoked fish and chicken (Ziarno *et al*., 2021). This indicates that when the biological agent is administered to a susceptible host, the pathogen is neutralized or its toxins are rendered impotent, resulting to immunity of the host. Prophylactic and therapeutic potential of extracts from medicinal plants and *Lactobacillus* in a combined state are yet to be fully explored globally (Holkem *et al*., 2022).

Several researchers have studied the synergistic effects of medicinal plants' extracts and *Lactobacillus* species against common pathogens such as Ziarno *et al*. (2021), Holkem *et al*. (2022), and Sookkhee *et al*. (2024) but little study is available on the prophylactic potential of the most potent synergistic biological agent against bacterial infections from smoked fish and chicken. Hence, the aim of this study is to evaluate the prophylactic potential of the most potent synergistic biological agent against bacterial infections from smoked fish and chicken.

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), *Truchurus trachurus* (Kote/Horse Mackerel), *Scomber scombrus* (Titus/Atlantic Mackerel) and *Sphyraema 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^{o}C-30^{o}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)

***In Vitro* Synergistic Antibacterial Activities of the Plant Extracts and *Lactobacillus* Species**

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010), Iheukwumere *et al*. (2018d), Okpalla *et al*. (2012) and Iheukwumere *et al*. (2017). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^{0}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% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using a UV/visible spectrophotometer.

Preparation of plant extracts: The weight obtained was weighed using an electronic weighing balance, 10 mL of peptone water (diluents) 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*. (2017). Also, 50 mL mixtures of U (OG+PU+GA) at optimum concentration (250 mg/mL) were prepared as followed: U (1:1:1), U (1:1:2), U (1:2:1) and U (2:1:1), and tested for the best activity (Iheukwumere and Ejike, 2016; Chukwura and Iheukwumere, 2013 and Ejike *et al*., 2017).

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+ 2^0C for 24 h. Then 50 mL of different mixtures of LAN56 and LP2359 were prepared as followed: V (1:1), V (1:2) and V (2:1) and tested for the best activity.

***In vitro* susceptibility study of the the biological agents using agar well diffusion method:** This was carried out by the modified method described by Iheukwumere *et al*. (2020) and Iheukwumere and Ejike, (2016). 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. The procedure was repeated using the mixture of *Lactobacillus* species broth culture

***In Vivo* Activities of the Most Effective Biological Agents against the Pathogenic Bacterial Isolates**

Experimented animal: In this study, the laboratory animal used was albino Wistar rats purchased from the animal house at University of Nigeria, Nsukka (UNN). The rats were transported to the animal house for study. The rats were randomly examined for suitability and viability for the study. Those that were not suitable were excluded in the study. The rats were also selected and grouped according to their weights and experimental design.

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010) and Iheukwumere *et al*. (2018c). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^0C 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% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. 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 for *in vivo* study: The weight obtained was weighed using electronic weighing balance, 10 mL of Phosphate Buffer Saline (PBS) was added and made up to 50 mL using the diluents. Then 50 mL mixtures of U (OG+PU+GA) were prepared as U (2:1:1).

Preparation of *Lactobacillus* broth culture for *in vivo* study: 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+ 2^0C for 24 h. Then 50 mL of different mixtures of LAN56 and LP2359 were prepared as V (2:1)

Experimental design: A total of 160 Wistar rats was used for this study. The rats were grouped into four groups (1, 2, 3 and 4). Group 1, 2, 3 and 4 represented ECEC1, SAJP1, LMLm1 AND SEEC2 respectively. Each group was grouped into five sets (A, B, C, D and E). Each sets contained eight (8) rats. In group 1, sets A were rats administered 0.5 mL/ 100g of ECEC1 (10^8 cells/mL); set B were rats administered 0.5mL/100g of U (PU+OR+GA) daily for 7 days; set C were rats administered 0.5mL/100g of V (LAN56+LP2359) daily for 7 days; set D were administered 0.5mL/100g of U together with 0.5mL/100g of V for 7 days and set E were the normal control group that were fed with normal feed and water. The set-ups were repeated for groups 2, 3 and 4 in which the sets were replaced with SAJP1, LMLm1 and SEEC2 respectively. The experimented rats were monitored for three weeks during which the obvious pathological signs were observed and recorded. The body and organ weights of the rats were determined at week 0, 1, 2, 3 and 4 using electronic weighing balance (LXB 200C), and the morphologies of the viscera organs were determined as described by Nwobodo *et al*. (2018). Also, Portions of the viscera organs harvested were ground and weighed (1.0 g), 3 mL of sterile peptone water was added; this was shake and then made up to 10 mL using peptone water. Then 1.0 mL of the solution was aseptically plated on Nutrient Agar (NA/BIOTECH) and PALCAM Agar using pour plate technique, and this was incubated at inverted position at 35±2 °C for 24 h. Viable counts of the test organism was carried out and recorded.

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 one-way Analysis of Variance (ANOVA) at 95 % confidence level. Pair wise comparison was done using Turkey test as described by Iheukwumere *et al*. (2018a).

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. 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 2

The study further revealed that the dose mixtures of the biological agents showed pronounced activities against the test isolates (Table 3). The dose mixture U (2:1:1) of the plant extract (OR+PU+GU) and V (1:1) of the *Lactobacillus* species (LAN56 + LP2359) showed the highest activities against the test isolates. Their activities were statistically significant ($p<0.05$) whereas the activities among the dose mixtures were statistically non-significant ($p>0.05$).

***In vivo* Activities of the Most Effective Biological Agents**

The most effective biological agent used for this study were OR + PU + GA designated as "U", LAN56 + LP2359 designated as "V" and their combination designated as "U + V" as shown in Tables 4, 5, 6, 7, 8, 9 10 and 11. The study revealed that the obvious pathological profiles of the bacterial

pathogens such as isolation, diarrhoea, bloody diarrhoea, anorexia, weakness, stiff neck, loss of balance, rashes, dehydration and death were significantly ($p<0.05$) reduced to one/zero these were most effective among those rats protected by U and also fed with V

The study also showed progressive retardation in the body and organs weight of the infected rats and these were statistically significant ($p<0.05$) when compared to the body and organ weights of normal control rats a shown in Tables 8 and 9. There were progressive increase in the body and organ weight of the protected rats, and these were most effective among those rats protected with U and also fed with V as shown in Tables 8 and 9. The study revealed that the increase in the weights of the rats were statistically non-significant ($p>0.05$) when compared with the weights of the normal control rats.

The study revealed that postmortem examination of the visceral organs of the experimented rats revealed significant ($p<0.05$) reduction / elimination of some pathological features such as liver congestion, hepatomegaly, hydronephrosis and cardiomegaly among those protected with U and also fed with V as shown in Table 10. There was significant ($p<0.05$) isolation of the test isolates from the visceral organs of the infected rats whereas the protected rats recorded zero bacterial count as shown in Table 11.

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	*Escherichia coli* 0157:H7 strain ECP19-598 C.G (ECEC1)	*Staphylococcus aureus* strain JP18269 complete genome (SAJP1)	*Listeria monocytogenes* strain LM16 chromosome (LMLM16)	*Salmonella enterica* subsp *enterica* serovar Enteritidis strain EC 20110358 complete genome (SEEC2)

Table 2: 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	*Lactobacillus acidophilus* strain NC56 chromosome complete genome (LAN56)	*Lactobacillus plantarum* strain 2359 chromosome complete genome (LP2359)

Table 3: *In vitro* activities of different dose mixture of the biological agents against the test isolates

Dose Mixture	**Mean Zone Diameter (mm) of 5mm corkborer**			
	ECEC1	**SAJP1**	**LMLM1**	**SEEC2**
U (1:1:1)	21.86 ± 0.82	18.21 ± 0.11	23.14 ± 0.12	18.33 ± 0.17
U (1:1:2)	21.33 ± 0.12	18.02 ± 0.12	22.88 ± 0.42	18.21 ± 0.11
U (1:2:1)	21.72 ± 0.11	18.12 ± 0.11	23.72 ± 0.33	18.86 ± 0.17
U (2:1:1)	22.08 ± 0.22	18.76 ± 0.11	23.72 ± 0.33	18.86 ± 0.17
V (1:1)	16.84 ± 0.14	16.22 ± 0.81	15.11 ± 0.11	17.86 ± 033
V (1:2)	16.38 ± 0.11	16.01 ± 0.21	14.87 ± 0.19	17.33 ± 0.17
V (2:1)	16.92 ± 0.81	16.74 ± 0.33	15.42 ± 0.12	18.08 ± 0.11
CPX	24.56 ± 0.12	14.22 ± 0.11	15.48 ± 0.14	19.36 ± 0.12

Table 4: Obvious pathological features of ECEC1 in each experiment group (n=8)

Parameter	ECEC1	ECEC1+ U	ECEC1+ V	ECEC1 + U+V	Control
Isolation	8	2	2	0	0
Diarrhoea	8	1	0	0	0
Bloody diarrhoea	5	0	0	0	0
Anorexia	8	1	1	0	0
Weakness	8	1	1	0	0
Stiff neck	0	0	0	0	0
Loss of balance	1	0	0	0	0
Rashes	0	0	0	0	0
Dehydration	8	0	0	0	0
Death	3	0	0	0	0

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongrnema latifolium*
U = PU+OR+GA; V = LAN56 +LP2359

Table 5: Obvious pathological features of SAJP1 in each experimented group (n=8)

Parameter	**SAJP1**	**SAJP1+ U**	**SAJP1+ V**	**SAJP1 + U+V**	**Control**
Isolation	4	0	0	0	0
Diarrhoea	4	0	0	0	0
Bloody diarrhoea	0	0	0	0	0
Anorexia	4	0	0	0	0
Weakness	4	1	0	0	0
Stiff neck	0	0	0	0	0
Loss of balance	0	0	0	0	
Rashes	7	0	2	0	0
Dehydration	4	0	0	0	0
Death	2	0	0	0	0

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongronema latifolium*
U = PU+OR+GA; V = LAN56 +LP2359

Table 6: Obvious pathological features of LMLM1 in each experimented group (n=8)

Parameter	**LMLM1**	**LMLM1+ U**	**LMLM1+ V**	**LMLM1 + U+V**	**Control**
Isolation	7	1	1	0	0
Diarrhoea	8	2	1	0	0
Bloody diarrhoea	0	0	0	0	0
Anorexia	7	1	1	0	0
Weakness	8	1	1	0	0
Stiff neck	7	0	0	0	0
Loss of balance	7	0	0	0	
Rashes	0	0	0	0	0
Dehydration	8	0	0	0	0
Death	6	0	0	0	0

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongrnema latifolium*
U = PU+OR+GA; V = LAN56 +LP2359

Table 7: Obvious pathological features of SEEC2 in each experimented group (n=8)

Parameter	SEEC2	SEEC2+ U	SEEC2+ V	SEEC2 + U+V	Control
Isolation	8	2	2	0	0
Diarrhoea	8	1	0	0	0
Bloody diarrhoea	3	0	0	0	0
Anorexia	8	1	1	0	0
Weakness	8	1	1	0	0
Stiff neck	0	0	0	0	0
Loss of balance	0	0	0	0	
Rashes	0	0	0	0	0
Dehydration	8	0	0	0	0
Death	4	0	0	0	0

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongronema latifolium*
U = PU+OR+GA; V = LAN56 +LP2359

Table 8: Mean body weight of each experimented group (n=8)

Group	Week 0	Week 1	Week 2	Week 3	Week 4
ECEC1	1244.12 ± 1.18	126.44 ± 1.13	129.12 ± 1.01	131.21 ± 1.61	132.46 ± 1.22
ECEC1 + U	124.86 ± 1.18	126.44 ± 1.13	140.22 ± 1.07	147.33 ± 1.33	155.26 ± 1.12
ECEC1 + V	124.36 ± 1.41	130.11 ± 1.31	141.11 ± 1.22	149.22 ± 1.23	156.17 ± 1.11
ECECE1 + U+V	125.08 ± 1.02	133.45 ± 1.33	146.08 ± 1.33	152.11 ± 1.08	158.62 ± 1.33
SAJP1	124.47 ± 1.07	127.08 ± 1.41	128.22 ± 1.22	129.92 ± 1.11	130.44 ± 1.21
SAJP1 + U	125.02 ± 1.22	131.04 ± 1.51	138.56 ± 1.51	146.64 ± 1.02	153.57 ± 1.17
SAJP1 + V	125.07 ± 1.17	130.81 ± 1.08	139.31 ± 1.42	146.87 ± 1.22	153.68 ± 1.12
SAJP1 + U + V	124.92 ± 1.08	132..92 ± 1.22	144.18 ± 1.41	150.86 ± 1.33	156.12 ± 1.13
LMLM1	124.56 ± 1.27	126.18 ± 1.33	129.33 ± 1.08	130.11 ± 1.02	131.22 ± 1.27
LMLM1 + U	124.44 ± 1.21	129.84 ± 1.33	140.19 ± 1.02	146.87 ± 1.07	150.36 ± 1.12
LMLM1 + U + V	124.72 ± 1.02	132.44 ± 1.22	146.27 ± 1.37	150.33 ± 1.41	155.81 ± 1.22
SEEC2	124.68 ± 1.03	126.33 ± 1.27	129.11 ± 1.02	131.02 ± 1.11	131.86 ± 1.08
SEEC2 + U	124.66 ± 1.22	130.26 ± 1.42	140.02 ± 1.21	147.03 ± 1.33	151.02 ± 1.02
SEEC2 + V	124.52 ± 1.12	130.02 ± 1.12	141.86 ± 1.31	147.83 ± 1.17	151.76 ± 1.33
SEEC2 + U + V	125.04 ± 1.12	132.56 ± 1.21	144.68 ± 1.17	151.64 ± 1.08	157.22 ± 1.21
CONTROL	124.38 ± 1.03	133.58 ± 1.27	146.86 ± 1.11	152.12 ± 1.02	158.77 ± 1.08

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongronema latifolium*
U = PU+OR+GA; V = LAN56 +LP2359

Table 9: Mean organ weights of each experimented group

Group	Liver (g)	Kidney (g)	Heart (g)	Lungs (g)
ECEC1	5.56 ± 0.01	0.52 ± 0.00	0.41 ± 0.00	0.88 ± 0.00
ECEC1 + U	6.18 ± 0.01	0.55 ± 0.00	0.43 ± 0.00	1.06 ± 0.00
ECEC1 + V	6.14 ± 0.01	0.56 ± 0.00	0.43 ± 0.00	1.06 ± 0.00
ECEC1 + U + V	6.28 ± 0.01	0.58 ± 0.00	0.44 ± 0.00	1.09 ± 0.00
SAJP1	5.86 ± 0.01	0.55 ± 0.00	0.43 ± 0.00	1.04 ± 0.00
SAJP1 + U	6.21 ± 0.01	0.57 ± 0.00	0.43 ± 0.00	1.06 ± 0.00
SAJP1 + V	6.22 ± 0.01	0.57 ± 0.00	0.44 ± 0.00	1.07 ± 0.00
SAJP1 + U + V	6.32 ± 0.01	0.58 ± 0.00	0.44 ± 0.00	1.08 ± 0.00
LMLM1	6.01 ± 0.01	0.54 ± 0.00	0.42 ± 0.00	0.84 ± 0.00
LMLM1 + U	6.21 ± 0.01	0.56 ± 0.00	0.43 ± 0.00	1.04 ± 0.00
LMLM1 + V	6.26 ± 0.01	0.57 ± 0.00	0.44 ± 0.00	1.10 ± 0.00
LMLM1 + U + V	6.30 ± 0.01	0.59 ± 0.00	0.44 ± 0.00	1.06 ± 0.00
SEEC2	5.92 ± 0.01	0.53 ± 0.00	0.40 ± 0.00	1.01 ± 0.00
SEEC2 + U	6.16 ± 0.01	0.56 ± 0.00	0.43 ± 0.00	1.07 ± 0.00
SEEC2 + V	6.16 ± 0.01	0.56 ± 0.00	0.43 ± 0.00	1.09 ± 0.00
SEEC2 + U + V	6.27 ± 0.01	0.59 ± 0.00	0.44 ± 0.00	1.09 ± 0.00
CONTROL	6.34 ± 0.01	0.59 ± 0.00	0.44 ± 0.00	1.10 ± 0.00

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongrnema latifolium*
U = PU+OR+GA, V = LAN56 +LP235

Table 10: Postmorten examination of the visceral organs of the experimented group (n=8)

Group	Liver congestion	Hepatomegaly	Hydr0-nephrosis	Cardiomegaly
ECEC1	8	8	4	4
ECEC1 + U	2	1	1	0
ECEC1 + V	3	1	2	1
ECEC1 + U + V	0	0	0	0
SAJP1	2	2	2	1
SAJP1 + U	0	0	0	0
SAJP1 + V	0	1	0	0
SAJP1 + U + V	0	0	0	0
LMLM1	8	8	6	6
LMLM1 + U	2	1	1	0
LMLM1 + V	2	0	3	1
LMLM + U + V	0	0	0	0
SEEC2	7	7	3	3
SEEC2 + U	1	1	1	0
SEEC2 + V	1	1	0	0
SEEC2 + U + V	0	0	0	0
CONTROL	0	0	0	0

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongrnema latifolium*
U = PU+OR+GA; V = LAN56 +LP2359

Table 11: Invasive potentials of the test isolates in each experimented group (n=8)

Group	Liver (g)	Kidney (g)	Heart (g)	Lungs (g)
ECEC1	21.18 ± 0.11	24.33 ± 0.33	17.14 ± 0.07	17.21 ± 0.21
ECEC1 + U	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ECEC1 + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ECEC1 + U + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SAJP1	8.00 ± 0.00	4.00 ± 0.00	0.00 ± 0.00	6.00 ± 0.00
SAJP1 + U	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SAJP1 + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SAJP1 + U + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LMLM1	19.82 ± 0.11	29.72 ± 0.12	15.86 ± 0.14	19.21 ± 0.11

LMLM1 + U	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LMLM1 + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LMLM1 + U + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SEEC2	13.76 ± 0.07	9.00 ± 0.01	6.00 ± 0.00	4.00 ± 0.00
SEEC2 + U	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SEEC2 + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SEEC2 + U + V	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

PU – *Piper guineense*; OR – *Ocimum gratissimum*; GA – *Gongrnema latifolium*
U = PU+OR+GA

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 pronounced activities of the mixture of the biological agents 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 *in vivo* activities of the biological agents against the test isolates revealed a significant reduction /complete prevention of the clinical manifestations of the pathogenic bacterial isolates in albino Wistar rats and this is in line with the findings of Bratz *et al*. (2015). The most significant activities of the mixture of the three extracts (U) supported with the consortium of the *Lactobacillus* species (v) in the present study showed the level of synergism among the extracts, among the *Lactobacillus* species, and between the extracts and *Lactobacillus* species.

Conclusion

This study has shown the occurrences *Escherichia coli* 0157:H7 strain ECP19-598 (ECEC1), S*taphylococcus aureus* strain JP18269 (SAJP1), *Listeria monocytogenes* strain LM16 (LMLM1) and *Salmonella enterica* serovar Enteritidis EC20110358 (SEEC2) in smoked fish and chicken meat samples. The isolates showed significant pathological features that were reduced/prevented by the studied biological agents, and the mixture of *Piper guineense* (PU), *Ocimum gratissimum* (OR) and *Gongronema latifolium* (GA) in 2:1:1 doses augmented with the mixture of *L. acidophilus* strain NC56 (LAN56) and *L. plantarum* stain 2359 (LP2359) in 2:1 doses was the most effective.

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

All authors hereby declare that "Principles animal care" (NCARE with Ref No FPSRA/UNN/24/0113), certified on 4th November, 2024 at University of Nigeria, Nsukka, were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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.

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