

**PREVALENCE OF ENTERIC BACTERIA IN READY TO EAT SUYA AND ANTI  
BACTERIAL ACTIVITY OF *Tetrapleura tetraptera* (GUM TREE) – POD EXTRACT**

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**UNIVERSITY OF BENIN**

**BENIN CITY**

**SEPTEMBER 2023**

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**A RESEARCH SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY IN  
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DEGREE OF B.SC. (HONOUR) IN MICROBIOLOGY**

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**BENIN CITY**

**SEPTEMBER 2023**

## CERTIFICATION

This is to certify that the project work titled: “Prevalence of Enteric Bacteria in ready to eat Sua and Anti Bacteria Activity of *Tetrapleura tetraptera* (Gum Tree) – Pod Extract, was carried out by Blessing Adaeze ONYEUKWU with matriculation number LSC1906993 of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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Head of Department

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## **APPROVAL**

This is to certify that this project was accepted in partial fulfillment of the requirement of the award of Bachelor of Sciences (B.Sc.) degree in Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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**Prof. (Mrs) F.I Akinnibosun**  
**Head of Department**

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**Date**

## **DEDICATION**

I humbly dedicate this project work to Almighty God who has brought me this far. I also dedicate this work to my family, friends and supervisor who encouraged me every step of the way during my project work.

## **ACKNOWLEDGEMENT**

My utmost appreciation goes to God Almighty for His Infinite mercy and divine grace on my life, during the course of this research work, for the success of this work and His guidance.

I acknowledge gratitude to my supervisor PROF. (MRS) F.E. OVIASOGIE for her patience, guidance and supervision during this work process, and also I appreciate the Head of Department, Prof (Mrs) F.I. Akinnibosun, my Lecturers: Dr Abraham, Mr Afam and Dr Eribo, and the entire staff of the Department of Microbiology.

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## TABLE OF CONTENTS

CERTIFICATION .....	iii
DEDICATION .....	iv
ACKNOWLEDGEMENT .....	vi
TABLE OF CONTENTS .....	vii
LIST OF TABLES .....	x
LIST OF FIGURES .....	xi
ABSTRACT .....	xii
CHAPTER ONE .....	1
INTRODUCTION .....	1
1.1 BACKGROUND OF STUDY .....	1
1.2 AIM AND OBJECTIVES .....	3
1.2.2 AIM: .....	3
1.2.2 OBJECTIVES: .....	3
CHAPTER TWO .....	4
LITERATURE REVIEW .....	4
2.1 BOTANICAL DESCRIPTION OF PLANT .....	4
2.2 TRADITIONAL USES .....	5
2.3 NUTRITIONAL VALUE .....	6
2.4 BIOLOGICAL ACTIVITIES .....	7
2.4.1 ANTIOXIDANT ACTIVITY .....	7
2.4.2 ANTI-INFLAMMATORY ACTIVITY .....	8
2.5 SUYA .....	9
2.5.1 SOCIO-ECONOMIC CHARACTERISTICS OF SUYA .....	11
2.5.2 PROCESSING AND PACKAGING METHODS IMPACT ON SUYA .....	11
2.6 ENTERIC BACTERIA .....	13
2.6.1 MODES OF TRANSMISSION OF ENTERIC BACTERIA IN FOOD .....	14
2.6.2 SYMPTOMS AND RISK FACTORS .....	15
2.6.3 PREVENTION .....	16

CHAPTER THREE .....	17
MATERIALS AND METHODS .....	17
3.1 SAMPLE COLLECTION .....	17
3.2 SAMPLE PREPARATION .....	17
3.2.1 Pod Sample Preparation .....	17
3.2.2 Suya sample preparation .....	18
3.3 PREPARATION OF CULTURE MEDIA .....	18
3.3.1 Nutrient Agar .....	18
3.3.2 Muller Hinton Agar .....	18
3.3.3 Violet Red Bile Agar .....	18
3.3.4 Serial dilution .....	19
3.3.5 Microbial Plate Count .....	19
3.6 MATERIALS AND MEDIA USED .....	19
Phenotypic Identification of Bacteria from Samples .....	20
3.6.1 GRAM STAINING TEST .....	20
3.6.2 POTASSIUM HYDROXIDE (KOH) TEST .....	21
3.6.3 BIOCHEMICAL TEST .....	21
3.6.3.1 Indole Test .....	21
3.6.3.2 Oxidase Test .....	22
3.6.3.3 Catalase Test .....	22
3.6.3.4 Citrate Utilization Tests .....	23
3.6.3.5 Urease test .....	23
3.6.4 Mannitol Test .....	23
3.6.5 Triple Sugar Iron (TSI) test .....	24
3.7 GROWTH ON DIFFERENTIAL MEDIA .....	25
3.7.1 Bacillus Cereus Agar Base .....	25

3.7.2	Mannitol Salt Agar (MSA).....	25
3.7.3	Pseudomonas Agar .....	25
3.7.4	Eosin Methylene Blue (EMB) agar .....	26
3.8	PHYTOCHEMICAL SCREENING .....	26
3.8.1	Detection of Flavonoids.....	26
3.8.2	Detection of Terpenoids .....	26
3.8.3	Detection of Alkaloids .....	26
3.8.4	Detection of Phenols .....	26
3.8.6	Detection of Tannins .....	27
3.8.7	Detection of Glycosides .....	27
3.8.8	Detection of Steroids .....	27
3.9.1	Antibiotic Susceptibility Testing .....	28
3.9.2	Determination of minimum inhibitory concentrations (MIC).....	29
CHAPTER FOUR	.....	31
RESULTS	.....	31
4.1	Results .....	31
CHAPTER FIVE	.....	41
DISCUSSION AND CONCLUSION	.....	41
5.1	Discussion.....	41
5.2	Conclusion: .....	42
REFERENCES	.....	43

## **LIST OF TABLES**

Table 4.1: Morphological and Biochemical Characteristics of cultured isolates. 37

Table 4.2. The quantitative phytochemical characteristics of the ethanolic extracts. 38

## LIST OF FIGURES

Figure 2.1: Pods of Gum Tree ( <i>Tetrapleura tetraptera</i> )	9
Figure 4.1: Heterotrophic bacterial count for unsmoked meat.	32
Figure 4.2: Coliform count for unsmoked meat.	33
Figure 4.3: Heterotrophic bacterial count for smoked meat.	34
Figure 4.4: Coliform count for smoked meat.	35
Figure 4.5: Zones of diameter/zones of inhibition (mm).	39
Figure 4.6: MIC of the pod extract of gum tree.	40

## ABSTRACT

In this study, prevalence of enteric bacteria in ready to eat suya and antibacterial activity of Tetrapleura tetraptera pod extract was carried out. The aim of this research was to isolate enteric bacteria from suya meats and determine the antibacterial potential of T. tetraptera against enteric bacteria. Standard microbiological methods were used to determine the total heterotrophic bacteria and coliform count of the suya samples using pour plate methods. Ethanolic extraction of pod of Tetrapleura tetraptera was done. The result of this study showed the total heterotrophic bacteria count (Log<sub>10</sub> Cf<sub>u</sub>/g) ranged between 3.00±3.00 to 5.455606±2.50 (Log<sub>10</sub> Cf<sub>u</sub>/g) while Total Coliform Count ranged from 3.00±0.50 to 4.921686±1.00 (Log<sub>10</sub> Cf<sub>u</sub>/g). The bacterial isolates isolated were *Morgenella* sp, *Enterobacter* sp, *Pseudomonas* sp, *E. coli*, *Klebsiella* sp and *Bacillus* sp. Phytochemicals screening showed the presence of saponin, terpenoids, phenol, glycoside and alkanoids. Antibacterial screening showed that Tetrapleura tetraptera pod extracts possessed antibacterial activities against the test isolates with *Klebsiella* sp. Having 33.67mm for 100mg/ml while the minimum zone was recorded in *Escherichia coli* with 6.33mm for 75mg/ml concentration of extract. This finding also suggests good antibacterial potential of the pod extracts of Gum tree.

# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND OF STUDY

*Tetrapleura tetraptera*, a deciduous tree that annually sheds its leaves and reaches a height of around 20-25 meters, is characterized by a relatively small, round crown that flattens with age. *Tetrapleura tetraptera*'s fruit emits a fragrant, notably pungent odor, which may explain its potential insecticidal properties. Plant extracts have been reported to be toxic to tadpoles and the larvae of *Anopheles gambiae* (Seiyaboh *et al.*, 2020). Additionally, *Tetrapleura tetraptera* is used as a spice in the preparation of traditional dishes in rural areas of countries like Ghana and Nigeria. Similar to many plants, it contains valuable phytochemicals and bioactive compounds, contributing to its therapeutic potential (Kigigha *et al.*, 2015).

"Suya," a popular West African street food, has been linked to outbreaks of foodborne illnesses. *Tetrapleura tetraptera*, renowned for its medicinal qualities, has been investigated as a natural antimicrobial agent in this context.

Ready-to-eat suya is a well-known street food in several African nations, appreciated for its distinct flavor and aroma. However, its consumption has been associated with foodborne illnesses due to the presence of enteric bacteria like *Escherichia coli*, *Salmonella*, and *Campylobacter* (Adeleke, *et al.*, 2019; Ahmed, *et al.*, 2020). These bacteria can contaminate suya during various stages of its preparation, handling, and storage, posing significant health risks to consumers.

In recent years, concerns about food safety have grown, prompting the need for effective strategies to control and reduce microbial risks in ready-to-eat suya. This study aims to investigate the prevalence of enteric bacteria in suya samples collected from different vendors

and locations. By understanding the extent of microbial contamination, appropriate measures can be taken to enhance suya's safety and quality for consumers.

"Suya" is a traditional barbecue, smoke, or roast made from thinly sliced boneless meat marinated with various spices, including clove, ginger, pepper, salt, peanut cake, vegetable oil, as well as food additives and flavorings. It is a popular street food providing an affordable, convenient, and often nutritious option in urban and rural areas. It also serves as a significant source of income for many individuals and creates opportunities for self-employment. However, food preparation can lead to inadvertent contamination, especially if mishandled.

Reports from various sources have identified a wide range of microbes isolated from ready-to-eat suya in Nigeria, including *Bacillus* sp, *Clostridium* sp, *Enterobacter* sp, *Escherichia* sp, *Klebsiella* sp, *Micrococcus* sp, *Proteus* sp, *Pseudomonas* sp, *Salmonella* sp, *Shigella* sp, *Staphylococcus* sp, and *Streptococcus* species. Many of these genera are of public health concern and have been linked to gastroenteritis and other foodborne diseases (Moshood *et al.*, 2012}. This research involved collecting suya samples, identifying enteric bacteria, and assessing the antimicrobial properties of *Tetrapleura tetraptera* pod extract against these bacteria.

## **1.2 AIM AND OBJECTIVES**

### **1.2.1 AIM:**

To determine the prevalence of enteric bacteria in ready to eat suya and anti-bacterial activity of *tetrapleura tetraptera* (gum tree) – pod extract.

### **1.2.2 OBJECTIVES:**

The objectives of this study were:

1. To determine the prevalence of enteric bacteria in ready-to-eat suya samples collected from various sources.
2. To assess the effectiveness of *Tetrapleura tetraptera* pod extract as an antibacterial agent against identified enteric bacteria, commonly associated with foodborne illnesses.
3. To determine the effect of smoking on the ready- to- eat suya meat.
4. Determine the effect of selective antibiotics on isolates.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 BOTANICAL DESCRIPTION OF PLANT

*Tetrapleura tetraptera* taub is classified as a deciduous tree species within the Fabaceae family. It typically attains a height ranging from 20 to 25 meters, with a girth that can span from 1.2 to 3 meters (Adesina *et al.*, 2016). This tree is commonly encountered in diverse ecosystems, including high forest regions, riparian forests, southern savannas, and African forests.

The bark of *Tetrapleura tetraptera* is notable for its smooth, gray-brown appearance, and it emits a distinct reddish aroma. Twigs and young foliage on this tree are either nearly hairless or bear fine hairs. Its leaves are sessile, adorned with hair, and share a common stem, typically measuring 15 to 30 cm in length and displaying a slight channel on the upper surface.

In terms of its foliage, *Tetrapleura tetraptera* typically exhibits 5 to 9 pairs of pinnae, each ranging from 5 to 10 cm in length. These pinnae are usually opposite in arrangement but may at times alternate. On each side of the pinna stem, there are 6 to 12 leaflets, always arranged alternately. These leaflets measure approximately 12 to 25 mm in length and 6 to 12 mm in width, and they are supported by slender stems approximately 2 mm long.

The flowers of *Tetrapleura tetraptera* are characterized by their creamy pink hue, which transitions to orange. They are densely clustered on branches measuring 5 to 20 cm in length. The tree's fruit is notably persistent, hanging from the tips of branches on robust stems. These fruits are glossy, hairless, and dark brown-purple in color, typically reaching diameters of around 5 cm. They possess four longitudinal veins, each measuring roughly 3 cm in width. Among these veins, two are woody, while the other two contain sweet, sugary, oily, and aromatic pulp.

Enclosed within the pods are small, black, hard, flat seeds, each approximately 8 mm in length, and these seeds contain oil (Ojewole and Adewunmi, 2004).

## 2.2 TRADITIONAL USES

Traditional practices often involve the use of plants and herbs for medicinal and culinary purposes. *Tetrapleura tetraptera*, recognized by various names including "Esesè," "djetk," "Sepan," and "Sasâs" among different Cameroonian ethnic groups, "Aridan" in western Nigeria, or "Prekese" within the Akan community of Ghana, holds significant traditional value. This tree is employed in traditional medicine, with various parts such as leaves, bark, roots, and seeds used to address a wide range of health issues, including gastric ulcers, rheumatism, fevers, rash, convulsions, smallpox, malaria, dysentery, inflammation, hypertension, jaundice, leprosy, diabetes, arthritis, coughs, and hemorrhoids (Nwaichi *et al.*, 2013).

In the culinary realm, *Tetrapleura tetraptera* fruits serve as a spice, enhancing the flavor of soups and sauces, particularly in dishes like "sauce jaune" and "nkondrè" in Cameroon. In Nigeria, they also find application in the production of seasoning spices, pomades, and soaps, largely due to their pleasing aroma (Adetunji, 2007). In Ghana, these fruits are incorporated into soups consumed by nursing mothers to prevent post-partum contractions and are valued as a source of vitamins. Additionally, they are employed in perfumes, and the pleasant aroma of dried fruit acts as a natural insect repellent (Aladesanmi, 2007).

Convalescents often use an infusion of the whole fruit for bathing to alleviate feverish conditions and constipation (Akintola *et al.*, 2015). Furthermore, a combination of stem barks from both *T. tetrapleura* Taub. and *R. heudoletti* Bail serves as a diuretic and is employed for managing obesity in local practices (Dongmo *et al.*, 2019).

## 2.3 NUTRITIONAL VALUE

Macronutrients and micronutrients play essential roles in maintaining overall health and metabolic functions (Ironi *et al.*, 2013). Proteins contribute to enzyme, hormone, and antibody synthesis, while carbohydrates and lipids serve as energy sources and participate in various physiological and metabolic processes.

*Tetrapleura tetraptera* has been scrutinized for its nutritional content. In terms of macronutrients, the fruit of *T. tetraptera* features protein content ranging from 5.61% to 6.69%, moisture content ranging from 5.06% to 8.22%, fat content ranging from 11.19% to 24.71%, carbohydrate content ranging from 58.48% to 63.86%, ash content ranging from 2.65% to 4.02%, and fiber content ranging from 3.14% to 4.11% (Akintola *et al.*, 2015).

The micro-nutrient composition of this fruit encompasses minerals such as Manganese (ranging from 322.00 mg/100g to 342.00 mg/100g), potassium (ranging from 251.22 mg/100g to 288.62 mg/100g), calcium (ranging from 182.11 mg/100g to 200.02 mg/100g), magnesium (ranging from 92.56 mg/100g to 98.66 mg/100g), and phosphorus (ranging from 36.22 mg/100g to 43.11 mg/100g). Additionally, it contains vitamins, including vitamin A (ranging from 3.22% to 4.69%), vitamin E (ranging from 2.66% to 3.69%), and vitamin C (ranging from 0.88% to 1.20%) (Akintola *et al.*, 2015).

Moreover, a study conducted by Ironi *et al.* in 2013 revealed that the maturity stage of the fruit affects both macro and micro-nutrient levels. Ripe-brown pods displayed the highest carbohydrate content at 87.49%, while ripe-brown seeds had the highest crude fat (14.46%), protein (28.72%), ash (5.59%), and food energy (424.60 cal/g). These stages also contained minerals such as Ca (15508.67 mg/Kg), Mg (403.54 mg/Kg), and P (1494.74 mg/Kg). The proximate composition from the dry fruit of *T. tetraptera* included ash content ranging from

3.17% to 3.48%, crude protein content ranging from 5.13% to 8.65%, sugar content ranging from 3.29% to 39.63%, and starch content ranging from 7.56% to 29.10% (Akin-idowu et al. in 2011).

Mineral content in this dry fruit included Fe content ranging from 29.69 mg/Kg to 65.06 mg/Kg, Mg content ranging from 392.35 mg/Kg to 2951.28 mg/Kg, Mn content ranging from 16.23 mg/Kg to 178.91 mg/Kg, Ca content ranging from 1348.63 mg/Kg to 13839.86 mg/Kg, and K content ranging from 8631.09 mg/Kg to 14881.00 mg/Kg. Furthermore, the seeds of *T. tetraptera* were found to contain chlorophyll a, chlorophyll b,  $\beta$ -carotene, and xanthophyll in specific concentrations ( $\mu\text{g}/100 \text{ mL}$ ) of  $470.25 \pm 74.58$ ,  $225.50 \pm 30.13$ ,  $0.35 \pm 0.03$ , and  $180.90 \pm 16.22$ , respectively (Abugri and Pritchett, 2013).

## **2.4 BIOLOGICAL ACTIVITIES**

Herbs have a long history of use in traditional medicine for treating various ailments, and they have recently gained significant research attention. A comprehensive literature review was conducted to compile scientific investigations on these herbs. Studies have reported that the plants of interest possess antimicrobial, antioxidant, anti-inflammatory, hypolipidemic, and hypocholesterolemic properties.

### **2.4.1 ANTIOXIDANT ACTIVITY**

The ethanol extracts from stem bark and fruit exhibited a range of reductive abilities, expressed in terms of ascorbic acid equivalence (Famobuwa *et al.*, 2016). Furthermore, the stem bark and fruit extracts displayed varying degrees of efficacy in inhibiting DPPH radicals, with some concentrations of the fruit extract even showing pro-oxidant tendencies at lower levels. More studies reported the high antioxidant activity of ethanolic fruit peel extracts, evident in their ability to scavenge DPPH radicals (Erukainure *et al.*, 2017). It was observed that methanolic bark extracts outperformed aqueous extracts in terms of antioxidant properties (Mawa *et al.*,

2019). Hydroethanolic extracts of fruits showed notable antioxidant power, effectively scavenging various radicals (Saague *et al.*, 2019). Additionally, *T. tetraptera* fruit extracts demonstrated antioxidant capacity by reducing plasma malondialdehyde (MDA) levels and increasing glutathione (GSH) and superoxide dismutase (SOD) levels in a dose-dependent manner, as reported by (Kuate *et al.*, 2015) and (Onda *et al.*, 2017). Larbie *c* (2020) also highlighted the exceptional radical scavenging capabilities of hydroethanolic and methanolic leaf and stem bark extracts, surpassing the performance of gallic acid. Furthermore, Adusei *et al.* (2019) reported significant antioxidant activity in aqueous and ethanolic extracts from pulp, seeds, and whole fruit, with pulp exhibiting the highest activity.

#### **2.4.2 ANTI-INFLAMMATORY ACTIVITY**

The presence of phenolic compounds, flavonoids, and tannins in plants is associated with their possession of both antioxidant and anti-inflammatory properties, as noted by Yakubu *et al.* (2018). Ojewole and Adewunmi (2004) conducted research on the anti-inflammatory and hypoglycemic effects of *T. tetraptera* fruit aqueous extract in rats, revealing significant, dose-dependent reductions ( $P < 0.05$ – $0.001$ ) in acute inflammation induced by fresh egg albumin in the rats' hind paw edema. Additionally, Gloria *et al.* (2018) reported varying levels of anti-inflammatory activity in methanolic extracts of *T. tetraptera* leaves. These extracts were found to stabilize red blood cell membranes up to a concentration of 1000  $\mu\text{g/mL}$ , and the aqueous fraction demonstrated potent inhibition of heat-induced albumin denaturation, reaching a maximum inhibition of 63.91% at 200  $\mu\text{g/mL}$ . Kuate *et al.* (2015) demonstrated the anti-inflammatory effects of hydroethanolic extract, showing downregulation of tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), C-reactive protein (CRP), leptin, and an increase in adiponectin in a rat model of metabolic syndrome. Furthermore, Onda *et al.* (2017) revealed that *T. tetraptera* extract significantly reduced joint diameter in a carrageenan/kaolin-induced acute

monoarthritis model in rat paws, particularly at a dose of 400 mg/kg. This extract also demonstrated its maximum effect in reducing knee swelling by  $1.54 \pm 0.27$ ,  $0.67 \pm 0.14$ , and  $0.35 \pm 0.13$  (inhibition by 46.0%, 76.5%, and 87.7%, respectively) in the fifth hour, compared to the arthritic control ( $2.85 \pm 0.09$ ). The potent free radical scavenging activity (DPPH) of methanolic seed extract, ranging from 6.72% to 22.8%, using ascorbic acid as a standard (12.10% - 42.00%) (Akabuike *et al.*, 2016).



**Figure 2.1: Pods of Gum Tree (*Tetrapleura tetraptera*)**

## **2.5 SUYA**

Meat is a valuable source of essential minerals necessary for bodily functions. The consumption of meat, rich in proteins and fats, contributes significantly to the intake of various nutritional and trace elements essential for human health (Adebisi *et al.*, 2008). In Nigeria, ready-to-eat meat products are prepared in diverse forms, including the popular grilled skewered meat known as "Suya." This delicacy, commonly crafted by the Hausa community in Northern Nigeria, involves

the seasoning of boneless meat with locally sourced ingredients, followed by roasting over glowing charcoal. Suya is typically made from skewered beef, ram, or chicken, and it sometimes includes innards like kidney, liver, and tripe (Eke *et al.*, 2012).

Suya is categorized into three main forms: Tsire, Kilishi, and Balangu (Igene and Mohammed, 1981; Ahmadu and Aduwa, 2015). Among these forms, Tsire is the most commonly preferred. Although Suya originated in northern Nigeria, it has permeated all corners of the country, becoming an affordable and readily available food, often referred to as a unifying factor in Nigeria (Ekanem, 1998). Suya has earned its status as a national dish in Nigeria, with different regions proudly promoting their unique recipes and preparation techniques, though similar grilled meat recipes are prevalent across many West African countries (Egbebi and Seidu, 2011). In Benin City, located in the southern part of Nigeria, Suya has become a popular street food, primarily available in the evening, often sold by vendors from Northern Nigeria (Ahmadu and Aduwa, 2015). Suya stalls in Benin City can be found in various places, particularly near bars and busy areas, with vendors predominantly originating from the Northern region of Nigeria. While Suya is mainly sold in the evenings in Benin City, only a few vendors operate during the daytime. Concerns have been raised regarding the hygiene and safety standards associated with roadside Suya preparation (Obadina *et al.*, 2014). This review aims to contribute to the understanding of the potential public health risks associated with the consumption of street-grilled meat, commonly known as Suya, in Benin City. It also seeks to raise awareness and promote public health monitoring of street-grilled meat consumption, not only in Benin City, Edo State but across all states in Nigeria where it is available.

### **2.5.1 SOCIO-ECONOMIC CHARACTERISTICS OF SUYA**

Suya production in Nigeria is primarily carried out by small-scale traditional producers, with limited information available on the economics of its production, especially in Benin City, Edo State. An economic study was conducted on Suya production, focusing on the northern region of Nigeria, specifically Borno State (Iliyasu et al., 2013). It is essential to determine whether Suya production is economically viable in the southern part of Nigeria, particularly in Benin City. Ahmadu and Aduwa (2015) conducted a study in Benin City Metropolis, which includes Oredo, Egor, and Ikpoba-Okha Local Government Areas (LGAs). The study revealed that all Suya producers in this area were male, mostly originating from Northern Nigeria, and had limited formal education. These producers relied predominantly on family labor for production, with personal savings being the primary source of finance for their businesses. Consequently, most Suya producers had relatively low initial capital investments in the Suya production business. This study found that Suya production in Benin City was profitable compared to Borno State, as indicated in Iliyasu et al.'s (2013) study. This is due to the lower production volume of Suya in Benin City, resulting in higher prices and, consequently, increased returns. Economic theory supports this phenomenon, where low production and limited supply tend to drive up prices and generate higher returns, assuming other factors remain constant (Adegeye and Dittoh, 1985). Ahmadu et al. (2008) highlighted that the costs of meat, charcoal, and labor significantly influenced gross income from Suya production. Additionally, the frequent rainfall experienced in Benin City during the wet season had an adverse impact on Suya production, particularly for those vendors with open-air stands (Ahmadu and Aduwa, 2015).

### **2.5.2 PROCESSING AND PACKAGING METHODS IMPACT ON SUYA**

The production of Suya, a complex mixture of spices and additives, lacks a standardized recipe (Akpamu *et al.*, 2011). Ingredients vary according to personal and regional preferences (Egbebi

and Seidu, 2011), including variations like clove, ginger, red pepper, black pepper, table salt, groundnut cake, and food additives such as monosodium glutamate and maggi cube (Akpamu *et al.*, 2011). Effective packaging plays a crucial role in preserving food quality and ensuring its safety during transportation from its origin to the point of consumption (Joshua *et al.*, 2016).

Many Suya processors, often located strategically, lack formal education and rely on traditional methods for handling, processing, and packaging the product. Unfortunately, these traditional methods are considered unhygienic and unsafe, leading to rapid deterioration of processed meat if not consumed promptly. Processors frequently use old newspapers obtained from various sources to wrap Suya meat, which is unsanitary and may contain dust and contaminants. In some cases, chemicals used to control insects like cockroaches and mosquitoes in homes can be inadvertently sprayed on these newspapers. If these chemicals come into contact with the meat and are subsequently consumed, they can pose serious health risks. Moreover, the use of old newspapers for packaging fails to convey a professional image and may expose consumers to harmful pigments, colorants, binders, additives, and photo initiators found in printed inks (Rokade *et al.*, 2012).

Packaging serves not only to maintain the quantity and quality of food ingredients and nutrients but also enhances sensory qualities and color stability. Food packaging is known to prolong product shelf-life, preserve the benefits of processing, and enhance food quality and safety (Marsh and Bugusu, 2007). Therefore, it is essential for food packaging materials to possess appropriate mechanical, thermal, and optical properties for food preservation. Additionally, these materials should offer anti-microbial and barrier functions against gases, vapors, and aromas (Chin *et al.*, 2015). Suya meats should be stored between 50 to 60°C to discourage microbial growth (Uzeh and Akinyemi, 2012).

The primary functions of packaging include protecting food products from external influences and damage, containing the food, and providing consumers with ingredient and nutritional information (Coles, 2003). A study by Joshua et al. (2016) indicates that processing methods and packaging materials used in the preparation of Suya can impact the quality attributes of the final products.

## **2.6 ENTERIC BACTERIA**

Enteric bacteria comprise a diverse group of microorganisms residing primarily in the gastrointestinal tracts of humans and animals. This group includes well-known species such as *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, and *Campylobacter*, among others (Kaper *et al.*, 2004). These bacteria are of paramount importance in public health and food safety due to their potential to cause various gastrointestinal infections and diseases in humans.

Enteric bacteria play vital roles in the digestive system, including the fermentation of dietary fibers, synthesis of certain vitamins, and maintenance of gut health. However, while many enteric bacteria are harmless or beneficial, some can cause a range of gastrointestinal illnesses, posing significant risks to human health (Hooper *et al.*, 2012).

*Escherichia coli* (*E. coli*) is one of the most notorious enteric bacteria. While most *E. coli* strains are harmless or even beneficial, pathogenic strains like enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) can cause severe infections, ranging from mild diarrhea to life-threatening conditions such as hemolytic-uremic syndrome (HUS) (Pavia *et al.*, 2012).

*Salmonella* is another well-known enteric bacterium responsible for salmonellosis, a common foodborne illness characterized by symptoms such as diarrhea, abdominal cramps, and fever (Majowicz *et al.*, 2010). The transmission of enteric bacteria primarily occurs through contaminated food and water supplies. These bacteria can be excreted in the feces of infected

individuals or animals, entering the food chain through various routes, including contaminated water sources, improper food handling, consumption of undercooked or raw animal products, and cross-contamination within the kitchen.

Symptoms of enteric bacterial infections can vary widely, from mild and self-limiting to severe and life-threatening, with vulnerable populations at higher risk of complications. Proper food safety measures, including adequate cooking, hygiene, and food storage practices, are crucial in preventing enteric bacterial infections.

### **2.6.1 MODES OF TRANSMISSION OF ENTERIC BACTERIA IN FOOD**

Enteric bacteria can contaminate food through various modes of transmission, highlighting their adaptability and resilience as microorganisms. These modes of transmission are critical to understanding and addressing food safety concerns comprehensively.

Contaminated water sources represent a primary route for the introduction of enteric bacteria into the food system. Agricultural practices, especially the use of untreated or polluted water for irrigation, can lead to the contamination of crops and produce. When these contaminated items are consumed without proper washing or cooking, individuals can inadvertently ingest enteric bacteria.

Livestock, such as cattle, poultry, and swine, can serve as reservoirs for enteric bacteria, which can inhabit their intestines and be shed in their feces. During the processing of meat and dairy products, cross-contamination can occur if proper hygiene practices are not followed diligently.

Improper food handling and preparation practices contribute significantly to the spread of enteric bacteria. Inadequate handwashing after restroom use, followed by food handling, can transfer bacteria from hands to food. Additionally, surfaces, cutting boards, and utensils that are not properly cleaned can harbor and transmit these pathogens.

Consuming undercooked or raw animal products is another route of transmission, as enteric bacteria are typically killed when food is cooked to recommended safe temperatures. However, consuming undercooked or raw meat, particularly ground beef, poultry, or eggs, can expose individuals to these pathogens if not cooked thoroughly.

Cross-contamination occurs when bacteria from one food item come into contact with another, facilitating the transfer of pathogens. For instance, raw meat or its juices contaminating ready-to-eat foods like salads can lead to the spread of enteric bacteria unless the food is adequately washed and cooked.

Inadequate food storage conditions can encourage the proliferation of enteric bacteria. Refrigerators that are too warm or foods left at room temperature provide an environment conducive to bacterial growth. Additionally, food containers that are not airtight can lead to cross-contamination within the refrigerator.

Person-to-person transmission, particularly in crowded or unsanitary settings, can occur through the fecal-oral route, where infected individuals shed bacteria in their stool. Contaminated surfaces, objects, or food can then cause secondary infections in others.

During food processing and packaging, enteric bacteria can be unintentionally introduced through contaminated equipment, inadequate worker hygiene, or contamination during packaging stages, potentially affecting food products before reaching consumers (Gal-Mor *et al.*, 2014).

## **2.6.2 SYMPTOMS AND RISK FACTORS**

The presentations of enteric bacterial infections can vary significantly, encompassing mild, self-limiting cases to severe, life-threatening conditions. Common indications comprise diarrhea, abdominal discomfort, vomiting, and elevated body temperature. Individuals at greater risk of

experiencing severe complications due to enteric bacterial infections include young children, the elderly, and those with weakened immune systems.

### **2.6.3 PREVENTION**

Effective measures to safeguard against enteric bacterial infections include proper adherence to food safety practices, encompassing thorough cooking, meticulous personal hygiene, and appropriate food storage techniques. It is imperative to ensure the safety of both food and water sources while implementing sound food handling procedures. These measures are pivotal in reducing the likelihood of contamination and the resultant illnesses linked to enteric bacteria in food.

In conclusion, enteric bacteria, though essential for gut health, can pose significant risks to human health when present in contaminated food and water sources. Understanding their characteristics, modes of transmission, and potential health risks is crucial for effective prevention and mitigation of foodborne illnesses associated with these bacteria. Proper food safety measures, including adequate cooking, hygiene, and food storage practices, are crucial in preventing enteric bacterial infections (Todd *et al.*, 2010).

Vulnerable populations, such as the very young, the elderly, and individuals with compromised immune systems, are at higher risk of developing severe complications from enteric bacterial infections. In these groups, the bacteria can lead to more profound and persistent symptoms, with potentially fatal consequences.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 SAMPLE COLLECTION

Gum tree (*Tetrapleura tetraptera*) pods were bought from New Benin market in Benin city, Edo State, Nigeria. The pods were cut broken into pieces and the seeds were removed and the pods retrieved. The entire collected samples were subjected to surface cleaning by rinsing with sterile water, in order to remove dirt which may be present on the plant materials. The debris free pods were allowed to air dry at room temperature (35°C) for about 3 weeks. The air dried gum tree pods were ground in a blender to reduce the size.

24 meat samples, 12 smoked and 12 unsmoked, were obtained from the following locations: Ugbowo and Isihor axis (Uwasota, Uselu, Ekosodin, Evidence, Agen, and Aico gas). Ring road and New Benin axis (Mobil, Ibiwe, Akpakpava, New Benin1, New Benin2, and New Benin3) all in Benin city, Edo state.

#### 3.2 SAMPLE PREPARATION

##### 3.2.1 Pod Sample Preparation

The *Tetrapleura tetraptera* pods were dried at room temperature. Then, it was macerated using a sterile pestle and mortar. The samples were further blended to obtain a fine powder. It was then extracted. The extraction was done by slightly modifying the soaking technique that Kigigha *et al.*, and Izah *et al.* previously described. 10 ml each of ethanol, aqueous, and acetone were used to extract 5g of the blended pod sample. For three days, the sample was soaked. After that, it was filtered via muslin cloth and Whiteman filter paper. Before concentration, the ethanol and acetone were allowed to evaporate.

### **3.2.2 Suya sample preparation**

90ml of water was used to homogenize 10g of prepared, ready-to-eat Suya. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, and *Proteus* species were isolated from the ready-to-eat suya, and their identities were established using Gram staining, differentials, and biochemical testing. These enteric bacteria were used in this study.

## **3.3 PREPARATION OF CULTURE MEDIA**

All media used were prepared according to the manufacturer's instructions and were sterilized in the autoclave at 121°C for 15 minutes.

### **3.3.1 Nutrient Agar**

*Contents:* Peptone, Lab-Lemco powder, yeast extract, sodium chloride, agar. A conical flask dissolved 2.8g of Nutrient agar in 1000 ml of distilled water. The medium was placed in an autoclave for 15 minutes at 121°C to sterilize the media. After sterilization, the flask was removed from the autoclave and left to cool.

### **3.3.2 Muller Hinton Agar**

*Contents:* Beef extract, Casein Hydrolysate, agar, distilled water, starch.

38 g of agar is dissolved in 1000 ml of distilled water and placed in a conical flask. the conical flask was placed in an autoclave for 15 minutes at 15 psi at 121°C. It was then taken out of the autoclave and placed to cool.

### **3.3.3 Violet Red Bile Agar**

*Contents:* Peptone, lactose, crystal violet, Bile Salts (usually sodium cholate), agar, neutral red, distilled water.

41.53 g of VRBA is dispensed into 1000 ml of distilled water and heated to dissolve the solution completely. It is then left to cool at 45°C.

### **3.3.4 Serial dilution**

Six test tubes containing 0.9 ml of sterile diluents are used to collect the sample. A clean pipette containing 0.1 ml of properly integrated sample is dispensed. Sample is added to the first tube to make the first 10<sup>-1</sup> ml dilution. Repeatedly emptying and filing the pipette lets the dilution be blended thoroughly. After the old pipette tip is discarded, a fresh tip is used to remove 0.1 ml of the 10<sup>-1</sup> ml diluent and place it into the 10<sup>-2</sup> second tube. This procedure is repeated with 1 ml from the initial dilution being added to subsequent 0.9 ml diluents. The final dilution of the bacteria cells will be 10<sup>-6</sup> (1 in 1,000,000) because 6 tubes were used.

### **3.3.5 Microbial Plate Count**

After incubating the plates, the colonies formed on the media were counted using the digital colony counter. The total population of the colonies was expressed as colony forming unit per gram (CFU/g) (Iche, I., Anyanwu, G. O., 2022). This was calculated using the formula below:

$$\frac{cfu}{ml} = \frac{\text{number of colonies} \times \text{dilution fold/series}}{\text{volume of inoculum}}$$

(Willey *et al.*, 2008)

## **3.6 MATERIALS AND MEDIA USED**

Materials such as Petri dishes, pipettes, glass containers (conical flask, round bottom flask), and bottles were drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved using a Bunsen burner flame and the disinfection of work surfaces with alcohol.

All media used were obtained from Oxoid and were prepared according to manufacturers' instructions. The media used in this study include Plate count agar, *Bacillus cereus* agar (BCA), Eosin methylene blue agar (EMB), Mannitol salt agar (MSA), *Pseudomonas cetrinide* agar (PCA), Triple sugar iron agar (TSI), Simmons citrate agar (SCA) and Mueller Hinton agar (MHA) (Willey *et al.*, 2008).

### **Phenotypic Identification of Bacteria from Samples**

Pure cultures of the bacterial isolates were obtained from the subculture of a single colony from the successful pour plate technique and were characterized using cultural, morphological and biochemical methods. Several tests, such as Gram reaction, catalase, urease, indole, oxidase, citrate utilization and respective reactions of bacteria on triple sugar iron agar, were carried out to identify bacterial isolates presumptively (Holt *et al.*, 1994).

#### **3.6.1 GRAM STAINING TEST**

A Gram staining test was carried out to determine the presence of Gram-positive and Gram-negative isolates. Neat, grease-free and sterile-dried microscope slides with labels were smeared using a sterilized loop, and the organism was air-dried and heat-fixed over a flaming Bunsen burner. The fixed smear was saturated with drops of crystal violet, left for one minute to react and washed off with distilled water. Lugol's iodine, which serves as a mordant, was added, left for one minute, and washed off with distilled water. The smear was decolourized with 95% ethyl alcohol, went for 30 seconds and washed off with distilled water. Then, the streak underwent counter staining using safranin solution for one minute and was rinsed with distilled water. Lastly, the smear was allowed to air dry and immersion oil was added for a microscopic view on an immersion objective lens light microscope. Colours, shapes and arrangements were used in

identifying the organisms. Gram-positive organisms maintained the crystal's purple colour, while Gram-negative retained the pink of safranin.

### **3.6.2 POTASSIUM HYDROXIDE (KOH) TEST**

The potassium hydroxide (KOH) test was used to determine or confirm Gram-negative bacteria, to quickly differentiate between Gram-negative and Gram-positive bacteria as a complement to Gram staining. KOH breaks down the thin peptidoglycan bacterial cell walls of Gram-negative bacteria but does not affect the thick layer of Gram-positive cell walls. Disruption of bacterial cell walls lyses the cell and releases its contents, including the genetic material. A drop of 3% KOH solution was applied on a labelled clean microscope slide and smeared with pure isolated culture using a loop. It was stirred carefully and observed that the solution turned to be a viscous or dense suspension, which formed a slimy or mucoid string within 60 seconds, and the appearance of that indicated a positive result as the presence of Gram-negative isolates. While non-slimy viscous suspensions remained negative results.

### **3.6.3 BIOCHEMICAL TEST**

To better characterize these isolates, biochemical tests were conducted, which included:

#### **3.6.3.1 Indole Test**

An indole test was carried out to demonstrate the ability of certain bacteria that can decompose amino acid **tryptophane** to **indole**. The indole production test is essential in identifying the *Enterobacteriaceae* family that breaks down the amino acid tryptophan by releasing indole in the presence of intracellular enzymes called "tryptophanase." Several drops of Kovac's indole reagent were placed on a filter paper. A portion of a pure isolated colony picked from the TSA pure culture with an inoculating loop was smeared onto the reagent-saturated area of the filter paper. It was allowed and examined to observe for colour development within 2 - 3 minutes. **In**

**this spot test**, indole combined with the reagents in the filter paper matrix to produce a **blue-to-blue-green colour change** on the bacterial smear **and adverse** reactions remained colourless or light pink.

### 3.6.3.2 Oxidase Test

The oxidase test was carried out to detect the presence of a cytochrome oxidase or indophenol oxidase that will catalyze electrons between electron donors in the bacteria and a redox dye known as tetramethyl-*p*-phenylene-diamine. The dye would be reduced to deep purple colour if yielded to positive reactions.

Several reagents can be used for this study, but Kovacs oxidase reagent: 1% tetra-methyl-*p*-phenylenediamine dihydrochloride in water, was used. The filter paper was saturated with a Kovacs oxidase reagent solution, and a speck of the pure culture was smeared on it with a platinum loop. It was allowed and observed for colour development within 10 - 60 seconds. The appearance of a deep purple-blue/blue colour indicated oxidase production and the negative result was when no colour changed.

### 3.6.3.3 Catalase Test

This test was used to distinguish between bacteria that produce the catalase enzyme, such as *Staphylococci*, and bacteria that do not, such as *Streptococci*. Catalase catalyzes the breakdown of hydrogen peroxide ( $H_2O_2$ ) to oxygen ( $O_2$ ) and water ( $H_2O$ ). In this test, 2mL of hydrogen peroxide solution was poured into a test tube, and some colonies of the test organism were picked and immersed into the  $H_2O_2$  solution using a sterile glass rod. The bacteria that generated catalase (positive result) produced gas bubbles (oxygen), but those that did not possess catalase enzyme had none (negative result).

#### **3.6.3.4 Citrate Utilization Tests**

**The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source.** A citrate test was performed to differentiate members of *Enterobacteriaceae* capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test was demonstrated with no growth, no colour change, or the colour of the medium remains green.

#### **3.6.3.5 Urease test**

The urease test is used to identify bacteria capable of producing the urease enzyme. The organisms that secrete urease can hydrolyze urea to ammonia and carbon dioxide. This test was used to distinguish urease-positive bacteria from other *Enterobacteriaceae*. The isolated pure bacteria were inoculated into well-prepared and autoclaved Christensen-modified urea broth and incubated for 24 hours at 37°C. Urease-positive cultures produced a pink colour due to a change in the indicator's colour in the presence of ammonia, while the negative result remains no colour change or yellow-orange colour.

#### **3.6.4 Mannitol Test**

The mannitol test is selective (the presence of high salt concentration; sodium chloride inhibits most Gram-negative and Gram-positive bacteria) and differential test (the ability of the organism

to ferment or not the mannitol). The ability to ferment mannitol induces acidification, changing the medium's coloration from red to yellow. A well-prepared mannitol salt agar was autoclaved at 121°C for 15 minutes, cooled and plated. Inoculated with pure isolates and incubated for 24 hours at 37°C. Growths were observed with yellow zones and yellow colonies as positive results. The negative result remained red-pink colour with growths.

### **3.6.5 Triple Sugar Iron (TSI) test**

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H<sub>2</sub>S) or gas (O<sub>2</sub>), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns and from other Gram-negative rods. An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H<sub>2</sub>S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H<sub>2</sub>S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO<sub>2</sub> and H<sub>2</sub>).

### **3.7 GROWTH ON DIFFERENTIAL MEDIA**

#### **3.7.1 Bacillus Cereus Agar Base**

*Bacillus cereus* agar was used to identify and isolate *Bacillus* species and pathogenic *Staphylococci* species. *Bacillus cereus* agar helps to restrict the growth of Gram-negative bacteria, and this differentiating media allows the differentiation of Gram-positive *Bacillus* species. Autoclaved dissolved *Bacillus cereus* agar at 121°C for 15 minutes; then was allowed to cool and poured into Petri dishes. Isolated pure cultures were inoculated by streaking on the medium and incubated the plates at 37°C for 24 hours. Plates were examined and observed for typical growths by colony forms, colours and spore morphology.

#### **3.7.2 Mannitol Salt Agar (MSA)**

MSA is used in differentiating and selecting mostly *Staphylococcus* species, which was prepared and autoclaved at 121°C for 15 minutes, then was allowed to cool and poured into Petri dishes. The isolated pure cultures were inoculated by streaking on the medium, and the plates were incubated at 37°C for 24 hours. Plates were examined and observed for typical growth.

#### **3.7.3 Pseudomonas Agar**

*Pseudomonas* species produce a variety of pigments, and fluorescein is commonly produced. *Pseudomonas* agar was used to determine pigment production by *Pseudomonas* species. *Pseudomonas* agar is a selective and differential medium that inhibits Gram-positive and Gram-negative bacteria other than isolating *Pseudomonas* species. *Pseudomonas* agar Petri dishes were prepared for inoculation after the medium was autoclaved at 121°C for 15 minutes, cooled, and poured into plates. Plates were inoculated with the isolated pure inoculums and incubated at 37°C for 24 hours. It was then examined and observed for growths, where the positive result was cream to greenish-yellow colouration in the agar, which can fluoresce under UV lighting.

### **3.7.4 Eosin Methylene Blue (EMB) agar**

**Eosin Methylene Blue (EMB) agar** is a differential medium that inhibits the growth of Gram-positive bacteria and is used to indicate Gram-negative pathogenic enteric bacteria by distinguishing between organisms that ferment lactose and those that cannot cope with a colour indication. A sterile petri plate was prepared with EMB, which was autoclaved at 121°C for 15 minutes, allowed to cool and inoculated with pure inoculums by streaking. Inoculated plates were incubated at 37°C for 24 hours and examined plates for colonial morphological changes. Lactose fermenting bacteria produced dark colonies with green metallic sheen or pink mucoid colonies (positive result), and lactose non-fermenters were colourless (negative result).

## **3.8 PHYTOCHEMICAL SCREENING**

### **3.8.1 Detection of Flavonoids**

Sulfuric acid test: A few drops of Sulfuric acid are added to the extracts and the formation of orange colour indicates the presence of flavonoids.

### **3.8.2 Detection of Terpenoids**

Salkowski's test: extract of 5mg of the selected plant part is mixed with 2ml Chloroform and 3ml concentrated Sulfuric acid added carefully to form a layer. A reddish-brown colour indicates the presence of terpenoids.

### **3.8.3 Detection of Alkaloids**

Add a few drops of Dragendorff's reagent to the extract and the formation of a colored precipitate indicates the presence of alkaloids.

### **3.8.4 Detection of Phenols**

Ferric chloride test: A few drops of Ferric chloride are added to 10ml extract. A bluish-black colour indicates the presence of phenol.

### **3.8.5 Detection of Saponins**

0.5mg of the extract is mixed vigorously with 5ml of Distilled water. The formation of frothing indicates the presence of saponins.

### **3.8.6 Detection of Tannins**

A few millilitres of the extract are mixed with a few millilitres of water and heated in a water bath and the mixture is filtered. Ferric chloride is added to the filtrate. The dark green colour indicates the presence of tannins.

### **3.8.7 Detection of Glycosides**

- Keller-Kiliani test: A few drops of extract are mixed with concentrated Acetic acid and a few drops of Ferric chloride solution are added. The formation of a green or bluish-green colour indicates the presence of glycosides.
- Fehling's test: The extract is dissolved in water, and equal volumes of Fehling's A and B solutions are added to the mixture and heated over a flame. The formation of a brick-red precipitate indicates the presence of glycoside

### **3.8.8 Detection of Steroids**

A few drops of concentrated Acetic acid are added to a small amount of test extract solution. The appearance of pink, violet or purple colour indicates the presence of steroids.

## **3.9 ANTIBIOTIC SUSCEPTIBILITY TESTING USING PLANT EXTRACTS**

Paper disc diffusion experiment was used to confirm the efficacy of the extracts on the aforementioned bacterial isolates obtained from suya samples. A sterile filter paper disc of 6mm size was obtained from the whole filter paper which was cut using a file punching machine. The cut paper size was placed in a container and sterilized by autoclaving at 121<sup>0</sup>C for 15 minutes.

Different concentrations of the extracts were prepared in stoichiometric ratios by dissolving 1 g of the extract in 1 ml of sterile distilled water (SDW) from whence other concentrations were obtained. The following concentrations, 100% to 25% of the extracts were prepared in separate sample bottles and sterile paper discs were placed in the bottles and allowed to stand for hours before the antibacterial activity was carried out. The antibacterial activity was done using standardized bacterial cells, which were streaked on Mueller Hinton agar plates before the discs with different concentrations of the extracts were impregnated on the agar. The plates were well labelled and afterwards, they were incubated for 24 hours to monitor and measure any zone of inhibition that may occur.

The formula below was useful for obtaining the different concentrations of the extracts from the 1000 mg/ml stock prepared by dissolving 1 g in 1 ml of SDW:

$$C_1V_1 = C_2V_2$$

Where  $C_1$  = original concentration (1000mg/ml)

$V_1$  = original volume of 1 ml

$C_2$  = desired concentration

$V_2$  = final volume of desired concentration

$V_2 = V_1 + V_d$  (and  $V_d$  is volume of diluent)

### **3.9.1 Antibiotic Susceptibility Testing**

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc

diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA. The inoculum corresponding to  $1.5 \times 10^8$  cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps. The susceptibility results were recorded after incubation for 24 hours at 37 °C. Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S). The antibiotic discs used in the study with their corresponding codes and concentrations include: colistin, clindamycin, ciprofloxacin, amoxicillin + clavulanic, metronidazole, gentamicin, erythromycin, cefuroxime, and tetracycline.

### **3.9.2 Determination of minimum inhibitory concentrations (MIC)**

After measuring about 10 ml of nutrient broth into sterile tubes that were previously empty, the minimum inhibitory concentration of the extract was determined. Based on the calculations of the different concentrations of the extracts, 2 ml was added to 0.1 ml of the organisms. This was then incubated for 24hr at 37°C. The tubes were then observed for any apparent turbidity. Tubes that had turbidity in them were tubes that had microbial growth in them. The minimum inhibitory concentration, or MIC, was reported as the lowest concentration that inhibited observable growth (Cheesbrough, 2000).



**Plate 1: A plate showing the growth of microorganisms on nutrient agar**

# CHAPTER FOUR

## RESULTS

### 4.1 Results

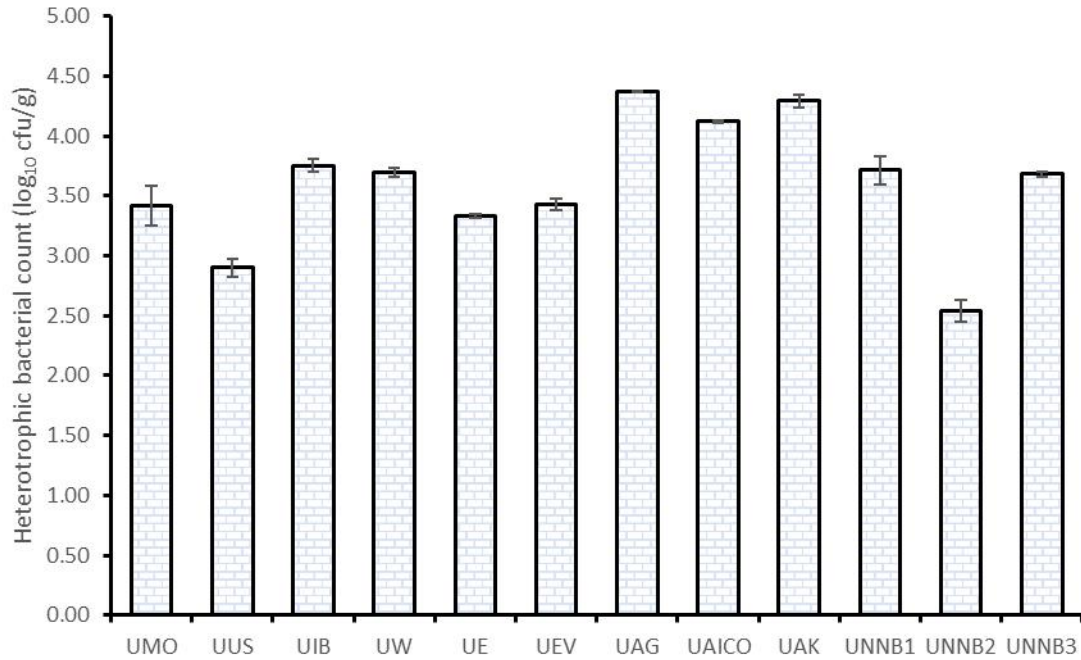
This study was carried out to determine the antimicrobial efficacy of *Tetrapleura tetraptera* pods on enteric bacteria found in ready-to-eat suya.

**Figure 4.1:** shows the heterotrophic bacterial count in unsmoked meat ( $\log_{10}$  cfu/g) from a vendor in a location (UAG) having the highest mean of 4.37 and a vendor from another location (UNNB2) having the lowest mean of 2.54. The keys are represented accordingly.

**Figure 4.2:** shows the coliform count in unsmoked meat ( $\log_{10}$  cfu/g) from a vendor in a location (UAG) having the highest mean of 3.92 and a vendor from another location (UE) having the lowest mean of 2.06. The keys represented accordingly.

**Figure 4.3:** shows the heterotrophic bacterial count of smoked meat ( $\log_{10}$  cfu/g) from two vendors at two different locations (SIB and SAK) having the highest mean of 2.83 and three vendors from different locations (SW, SE and SAG) having the lowest mean of 1.00. The keys are represented accordingly.

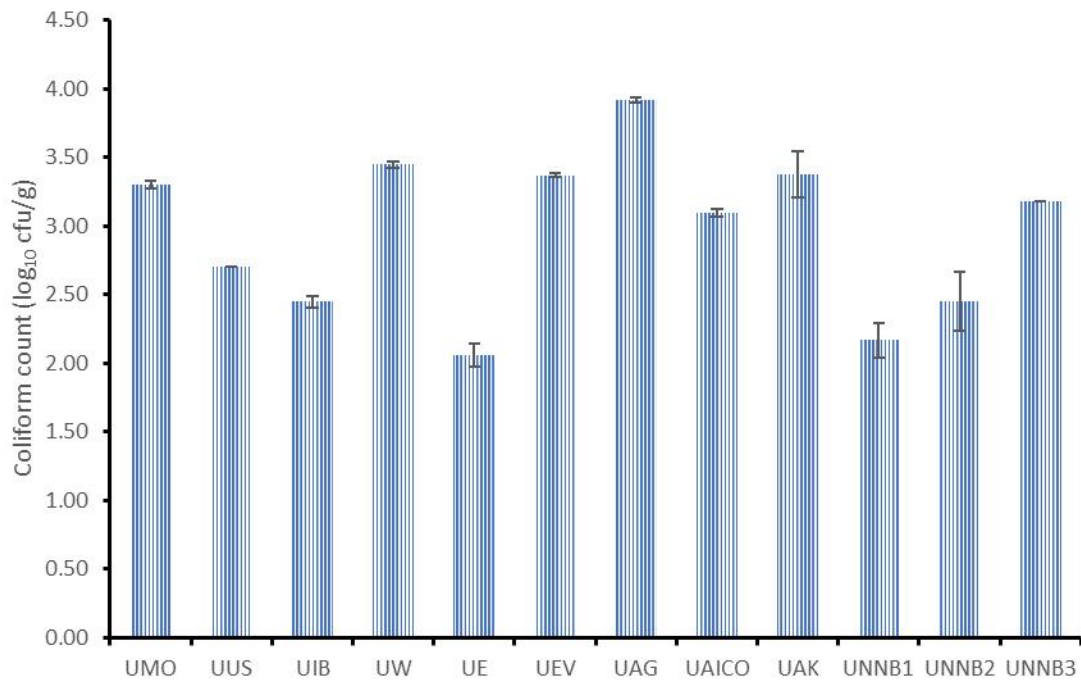
**Figure 4.4:** shows the coliform count of smoked meat ( $\log_{10}$  cfu/g) from a vendor at one location (SW) having the highest mean of 2.91 and six vendors from the four different locations (SIB, SE, SAG, SUS, SNNBI, SAICO, and SAG) having the lowest mean of 1.00. The keys represented accordingly.



**Figure 4.1: Heterotrophic bacterial count for unsmoked meat**

Keys:

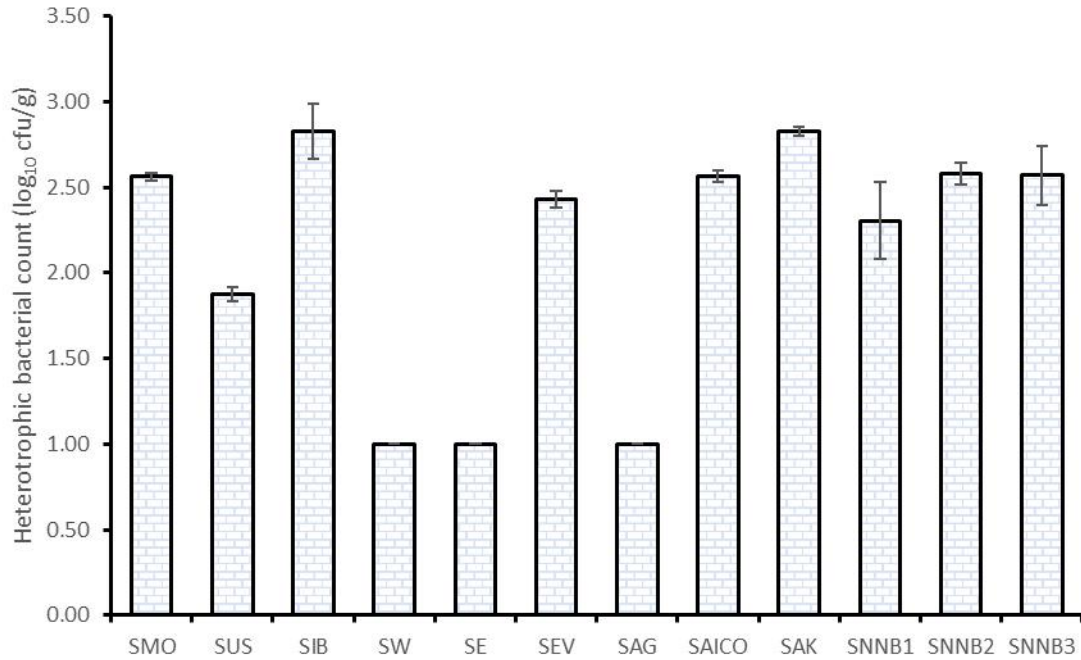
- UMO: unsmoked mobil
- UUS: unsmoked uselu
- UIB: unsmoked Ibiwe
- UW: unsmoked Uwasota
- UE: unsmoked ekosodin
- UEV: unsmoked evidence
- UAG: unsmoked agen
- UAICO: unsmoked aico gas
- UAK: unsmoked akpapava
- UNNB1: unsmoked new benin 1
- UNNB2: unsmoked new benin 2
- UNNB3: unsmoked new benin 3



**Figure 4.2: Coliform count for unsmoked meat**

Keys:

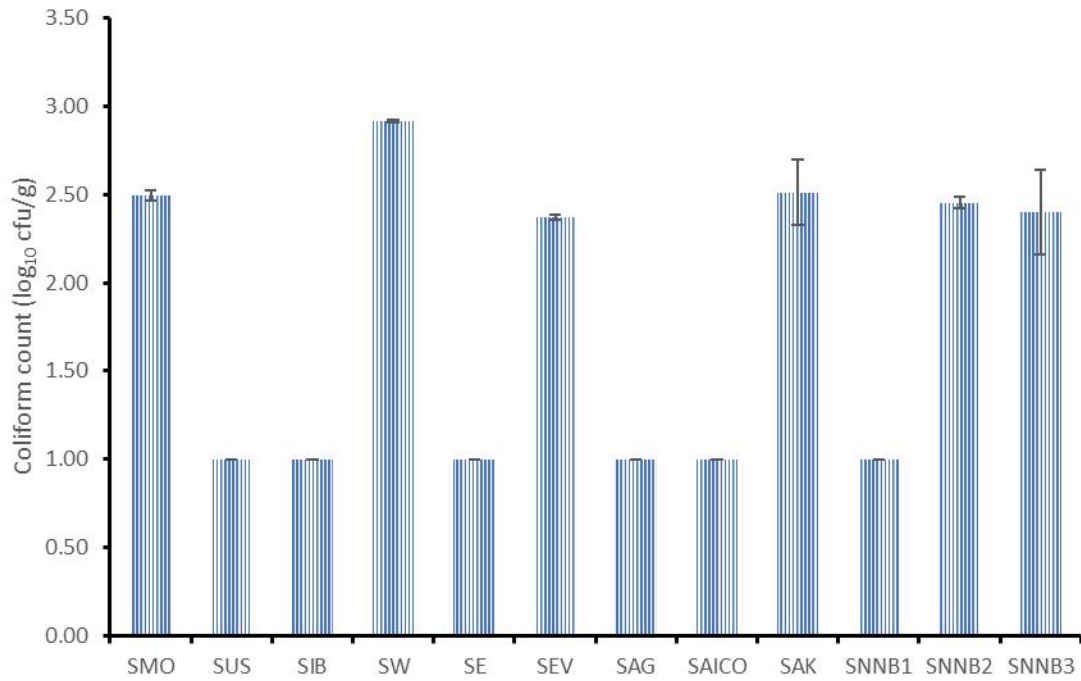
- UMO: unsmoked mobil
- UUS: unsmoked uselu
- UIB: unsmoked Ibiwe
- UW: unsmoked Uwasota
- UE: unsmoked ekosodin
- UEV: unsmoked evidence
- UAG: unsmoked agen
- UAICO: unsmoked aico gas
- UAK: unsmoked akpapava
- UNNB1: unsmoked new benin 1
- UNNB2: unsmoked new benin 2
- UNNB3: unsmoked new benin 3



**Figure 4.3: Heterotrophic bacterial count for smoked meat**

Keys:

- SMO: Smoked mobil
- SUS: Smoked uselu
- SIB: Smoked Ibiwe
- SW: Smoked Uwasota
- SE: Smoked ekosodin
- SEV: Smoked evidence
- SAG: Smoked agen
- SAICO: Smoked aico gas
- SAK: Smoked akpapava
- SNNB1: Smoked new benin 1
- SNNB2: Smoked new benin 2
- SNNB3: Smoked new benin 3



**Figure 4.4: Coliform count for smoked meat**

Keys:

- SMO: Smoked mobil
- SUS: Smoked uselu
- SIB: Smoked Ibiwe
- SW: Smoked Uwasota
- SE: Smoked ekosodin
- SEV: Smoked evidence
- SAG: Smoked agen
- SAICO: Smoked aico gas
- SAK: Smoked akpapava
- SNNB1: Smoked new benin 1
- SNNB2: Smoked new benin 2
- SNNB3: Smoked new benin 3

**Table 4.1:** The morphological and biochemical characteristics of isolates used for the study was carried out to confirm the isolates obtained from the laboratory. It was observed that this study was carried out with five Gram negative bacteria (*Morgenella spp*, *Enterobacter aerogenes*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*) and a Gram positive bacteria (*Bacillus subtilis*).

**Table 4.2:** Table 4.2. Shows the quantitative phytochemical characteristics of the ethanolic extracts of the pod of the gum plant. The parameters analyzed included saponins, flavonoids, terpenoids, steroids, phenols, glycoside, alkanoids and tannins.

**Figure 4.5:** Shows the Zones of diameter/zones of inhibition (mm).

**Figure 4.6:** Shows the MIC of the pod extract of gum tree.

## Morphological

Elevation	Flat	Flat	Raised	Flat	Flat	Flat
Margin	Curled	Undulate	Entire	Undulate	Entire	Undulate
Color	Cream	Cream	Lemon	Cream	Cream	Cream
Shape	Irregular	Irregular	Circular	Irregular	Circular	Irregular
Size	Medium	Large	Medium	Large	Small	large
Gr. diff. agar	EMB	EMB	PCA	EMB	EMB	BCA
Colour	purple	Pink	Green	green	Pink	Straw

## Staining

Gram stain	-	-	-	-	-	+
cell type	rod	Rod	Rod	Rod	Rod	Rod
Arrangement	disperse	disperse	Disperse	disperse	disperse	disperse
Color	pink	Pink	Pink	pink	Pink	purple
Spore staining	-	-	-	-	-	+

## Biochemical

KOH String Test	+	+	+	+	+	-
Catalase	+	+	+	+	+	+
Indole	+	-	-	+	-	-
Citrate	+	+	+	-	+	+
Oxidase	-	-	+	-	-	-
Motility	+	+	+	+	-	+
Urease	+	-	+	-	+	-
Glucose	+	+	-	+	+	+
Sucrose	-	+	-	-	+	+
Lactose	-	+	-	+	+	+
Mannitol	-	-	-	-	-	+
Gas formation	-	-	-	+	+	-
H <sub>2</sub> S formation	-	-	-	-	-	-
TSI (Slant/Butt) reaction	A/K	A/AG*	K/K	A/AG	A/AG	A/A
Esculin Hydrolysis	+	+	-	-	+	-
Possible identity	<i>Morgenella</i> spp	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Klebsiella oxytoca</i>	<i>Bacillus subtilis</i>

**Table 4.1: Morphological and Biochemical Characteristics of cultured isolates**

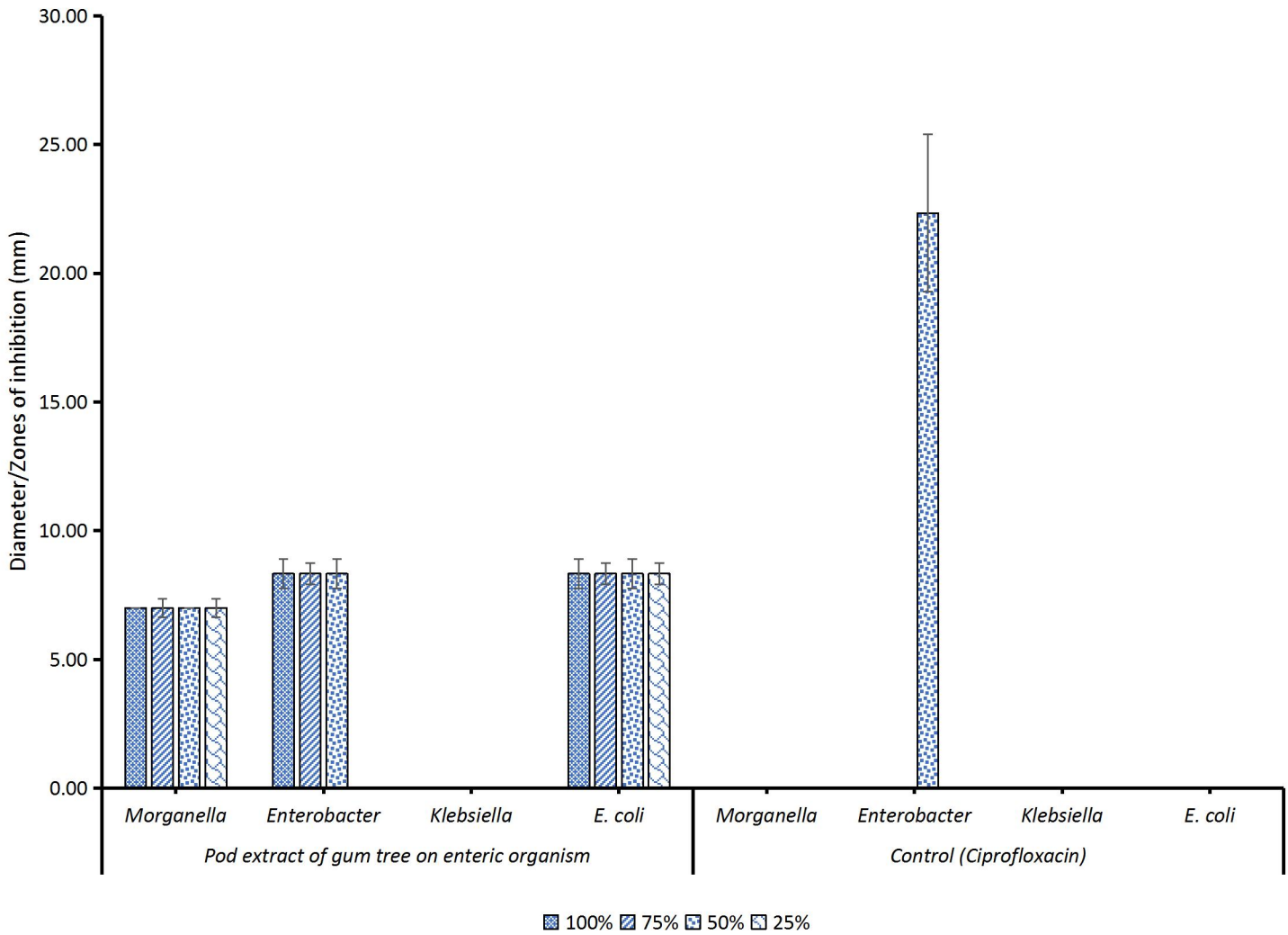
Key:

+ve = Positive

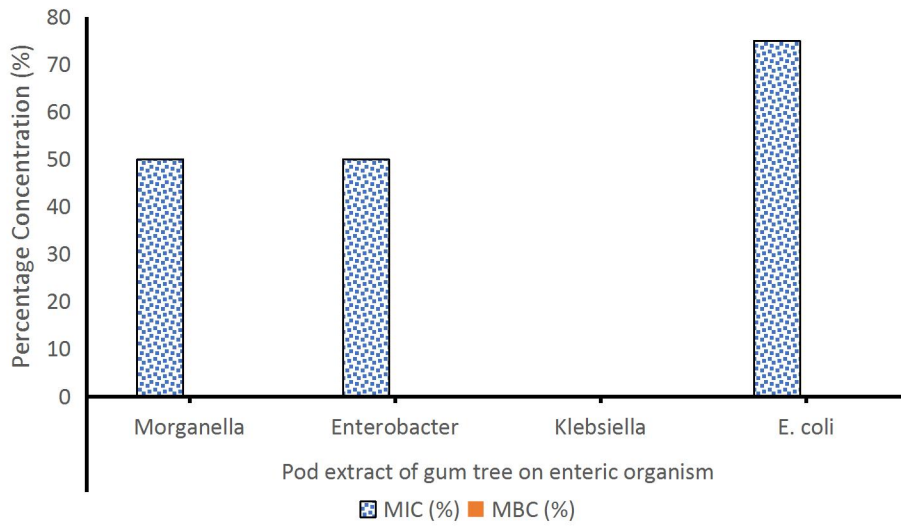
-ve = Negative

<b>Parameter</b>	<b>Bioactive constituents</b>	<b>Presence/Absence</b>
	Saponin	+
	Flavonoids	-
	Terpenoids	+
	Steroids	-
	Phenols	+
	Alkaloids	+
	Tannins	-
	Glycoside	+
	(+ = Present)	
	(- = Absent)	

**Table 4.2: Qualitative Phytochemical Screening of Plant Extract**



**Figure 4.5: Zones of diameter/zones of inhibition (mm)**



**Figure 4.6: MIC of the pod extract of gum tree**

## CHAPTER FIVE

### DISCUSSION AND CONCLUSION

#### 5.1 Discussion

This study clarifies the prevalence of enteric bacteria in ready-to-eat suya and highlights the demand for improved food safety procedures in handling and preparing it. The prevalence of enteric bacteria in ready-to-eat *Suya* and the antibacterial activity of the *Tetrapleura tetraptera* pod extract was carried out in this experiment. Suya, a typical meat dish made from beef that has been flavored with peanut cake, salt, vegetable oil, and other flavors before being roasted over a roaring charcoal fire, is a popular ready-to-eat meal in West Africa. However, roadside meat consumers are unaware of the high health risks they face. Street foods are exposed to various forms of contamination at every stage of handling. Etok (1998) identified insufficient roasting/heating duration, uneven temperature distribution, and exposure to unhygienic environments as crucial factors of infection and contamination. The Suya samples were collected and examined microbiologically. This experiment aimed to determine the degree of microbial contamination in Suya meat. From the microbiological examination of Suya meat, the following isolates were discovered: *E. coli*, *Klebisella oxytoca*, *Enterobacter sp* , *Morganella sp* , *Shigella sp* , *Bacillus subtillis* and *Pseudomonas aruginosa*.

The Total coliform bacteria for smoked and unsmoked was done and represented in figures 1 and 2 with values ranging from 3.9 to 2.0 (Log10cfu/g), 2.9 to 1.0, respectively. Represented in Table 1 are the morphological and biochemical characteristics of the bacteria used. The zone of inhibition with ciprofloxacin for *E. coli*, *Klebisella oxytoca* , *Enterobacter sp*, *Morganella sp*, *Shigella sp*, *Bacillus subtillis*, and *Pseudomonas aruginosa* was 0mm, 0mm, 22.33mm, 0mm, 0mm, 0mm, and 0mm respectively. Figure 3 shows that *Enterobacter sp* showed susceptibility to

*Tetrapleura tetraptera* extract combination with 8.33 mm at 100% concentration, 8.00 mm at 75% concentration, and 7.00 mm at 50% concentration. *Klebisella oxytoca*, *Morganella sp*, *Shigella sp*, *Bacillus subtilis*, and *Pseudomonas aruginosa* showed no inhibition to extract from *Tetrapleura tetraptera*. MIC of the plant extract against the isolates is shown in Figure 4.

Although it differs from those previously reported by authors, the zone of inhibition displayed by the test organisms employed in this investigation. Variations in extract concentration, extract solvents, physical state, such as the age of the plant material, and strain of the microbial isolates, among other things, could bring on the fluctuation. (Izah, 2018).

Various authors have claimed that the presence of bioactive components is what gives plants, particularly *Tetrapleura tetraptera*, their therapeutic properties. (Epidi *et al.*, 2016a).

## **5.2 Conclusion:**

In conclusion, this effort makes a substantial addition to the field of microbial control and food safety, especially in the context of street meals like *suya*. The results highlight the necessity of ongoing attention to ensure food safety standards among street sellers. The discovery of *Tetrapleura tetraptera* extracts' antibacterial activity also opens up new options for scientific study and technological innovation in food safety and preservation in West Africa and beyond.

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