hhttps://doi.org/10.47430/ujmr.2492.015

Received: 19 August 2024 **Accepted:** 17 November 2024

SUSCEPTIBILITY PATTERN OF TETRACYCLINE-RESISTANT *SALMONELLA SPECIES* TO OTHER ANTIBIOTICS AND SOME PLANT EXTRACTS

Asmau Nna Sulaiman[1*], Auwalu Halliru Arzai[2], Dalha Wada Taura[2]
[1]Ahmadu Bello University -Nigeria
[2] Bayero University Kano- Nigeria
Correspondence: sulaimanasmau@yahoo.co.uk

Abstract

Antibiotic resistance is a growing concern in the treatment of gastrointestinal diseases caused by *Salmonella species*. This study investigated the antibiotic susceptibility pattern of tetracycline-resistant *Salmonella species* and their response to selected medicinal plant extracts. Faecal isolates (n=450) from hospitals in Kano were screened, leading to the identification of Tetracycline-resistant *Salmonella species* (n=4). These isolates were assessed for susceptibility to various antibiotics and ethanolic extracts of *Psidium guajava*, *Khaya senegalensis*, and *Bridelia ferruginea*. Notably, the Tetracycline-resistant *Salmonella* species exhibited susceptibility to Amoxicillin, Chloramphenicol, Cefotaxime, Ciprofloxacin, Trimethoprim, and Gentamicin. The extracts of the plant parts contained carbohydrates, flavonoids, tannins, and saponins, while anthraquinone and steroids were absent. *P. guajava* had the highest antibacterial activity against (9.5±0.29mm) *Salmonella* sp., followed by *K. senegalensis* (8.75±0.36mm) and *B. ferruginea* (8.75±0.48mm) at a concentration of 500µg/mL for all extracts. *Psidium guajava* extract with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 50mg/mL and 100mg/mL, respectively, emerged as the most active extract. Tetracycline-resistant *Salmonella species* can best be treated using *P. guajava* among the plants.

Keywords: Tetracycline, Gastrointestinal tract, *Salmonella*, Medicinal plants, *Khaya senegalensis*, *Bridelia ferruginea*, *Psidium guajava*

INTRODUCTION

Antibiotic resistance has become a global health problem (Murray *et al.*, 2022). It is alarming that in this era, the effectiveness of antibiotics is being threatened, and we are reverting to the era before the discovery of antibiotics, where there were insufficient treatment options for bacterial diseases of the gut. These resistant infections are expensive and have elevated economic impact as well as affect the mortality and morbidity of man. Nonetheless, infectious diseases of the gut continue to be the principal causative agent of death globally (Serwecińska, 2020), and the development of antibiotics has drastically declined, contributing about 0.2% to the discovery of new drugs (Ventola, 2015). The development of antibiotic resistance persists, especially in multi-resistant species, thereby making treatment difficult or sometimes ineffectual (Murray *et al.*, 2022).

The human gut is made up of about 10^{13} -10^{14} bacteria which 10 times more than human somatic cells (Thursby and Juge, 2017). There is a biological interaction that exists between the immune system of humans and their microflora. This has developed into a symbiotic relationship (Kogut *et al.*, 2020). The gut gives ideal combination of factors for antibiotic resistance genes to emanate and disperse via bacterial population. Example of this factor is high cell density (Singh *et al.*, 2019).

Regardless of the effort to halt the development of antibiotic resistance, the degree of resistance by bacteria is increasing, and the hypothesis that the gut serves as a pool for antibiotic-resistant genes is generally accepted (Singh *et al.*, 2019).During treatment or exposure to antibiotics directly or indirectly, bacteria in the body are subjected to selective pressure. As a result, the gut is highly susceptible and exposed, particularly during oral treatment. Consequently, the vital genetic pool of naturally resistant strains is responsible for transferring antibiotic-resistant genes into the gut. Furthermore, bacteria contaminants in food that possess resistant genes from farm animals and are ingested by man can also serve as donors or gene pools for antibiotic resistance genes (Prestinaci *et al.*, 2015).

The evolution of resistant genes can be traced to the transfer activity across genera due to their high percentage of similar sequences. This is present in both Gram-positive and Gram-negative bacteria (Munita and Arias, 2016).

Tetracyclines have a wide range of antibacterial activity and are commonly used to treat gastrointestinal infections. They work by binding to the 30S subunit of the ribosome, which prevents the attachment of aminoacyl-tRNA to the ribosome, thereby halting protein synthesis and bacterial growth. However, there is a limitation in its use due to its side effects (teratogenicity) and resistance. This resistance results from the acquisition of efflux genes or the synthesis of ribosomal proteins by pumping out Tetracycline from the cell and halting the binding of ribosomes with Tetracycline, respectively (Chopra and Reader, 2014).

The emergence of antimicrobial drug resistance has led to high costs, longer duration, and failure of treatment, leading to the death of patients and threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, fungi, parasites, and virus (WHO, 2014). The side effects associated with the use of antibiotics in the treatment of gastroenteritis definitely necessitates the search for suitable and friendlier alternatives (Oyetayo, 2007). It has been estimated that about 80% of the third-world population is almost entirely dependent on traditional medicine for maintaining general health and combating many diseases (Srinivasan, 2005).

Traditional medicine has a long history, and its products are the oldest healthcare product very vital, especially in rural areas and primary healthcare centres in developing countries (WHO, 2001). It is an aggregate of the skills, knowledge, and practices depending on the experiences, theories, and beliefs of different local cultures, whether interpretable or not, used in preventive medicine, health improvement, treatment, or diagnosis of diseases (WHO, 2008). The constituents of herbal medicine encompass the key components contained in the plant, its parts, combinations, or finished products, either in crude form or processed form (WHO, 2006). When properly administered, its little or no side effects have made it popular among the Western population (Jordan *et al.*, 2010). Plants form a vital part of drug development and research. Each country has a history, ethnological and medical background based on it. (WHO, 1996).

Herbal medicine has, over the years, proven its efficacy in treating gastrointestinal diseases, among others (Mukherjee and Wahile, 2006; Street and Prinsloo, 2013). They are active against viruses, bacteria, and some parasites that cause abdominal pain, diarrhoea, dysentery, gastroenteritis, constipation, and vomiting (WHO, 2008; Karki and Tiwari, 2007).

Gastrointestinal diseases are on the increase in many Nigerian cities due to the inability of the government to provide good pipeborne water, sewage farming using polluted water, and poor hygiene or sanitary practices (Ezeonwu *et al.*, 2013). High incidence of gastrointestinal diseases occurs particularly during the rainy season when manured farmlands occasionally get flooded, and rainwater carrying heavy bacterial load washes down the sewage and flows into domestic water supply (Nzeoko and Okafor, 2002). To worsen the problem, many ordinary Nigerians cannot afford modern medical treatment when they are affected by gastrointestinal disease. As a result, they resort to traditional medicine or herbal medicine. In addition, even where medical treatment is obtained and despite numerous therapeutic improvements, the treatment is sometimes ineffectual due to the phenomenon of bacterial resistance to some of the common antibiotics used to treat gastrointestinal diseases.

It is against this background that this study was conceived in order to determine the extent of the relative prevalence of *Salmonella species* causing gastrointestinal diseases and to evaluate the efficacy of tetracyclines, other antibiotics and extracts of certain medicinal plants purported to be active in treatment of gastrointestinal diseases.

MATERIALS AND METHODS

Study Location

Kano State is located in Northern Nigeria. It has its capital as Kano City. It shares borders with Katsina, Jigawa, Kaduna, and Bauchi states. Established on May 27, 1967, Kano State comprises 44 local government areas, covering 20,131 Km^2 (7,772.6 sq mi). The state's coordinates are 11.7574°N, 8.6601°E. The Kano metropolis consists of six core urban LGAs (Tarauni, Nassarawa, Municipal, Gwale, Fagge, and Dala) and two peri-urban LGAs (Kumbotso and Ungogo). As of the 2006 census, the population was 9,401,288, with a 3.5% annual growth rate. This study took place in three Kano State tertiary hospitals: Aminu Kano Teaching Hospital, Muhammad Abdullahi Wase Teaching Hospital, and Murtala Muhammad Specialist Hospital (National Population Commission, NPC, 2006).

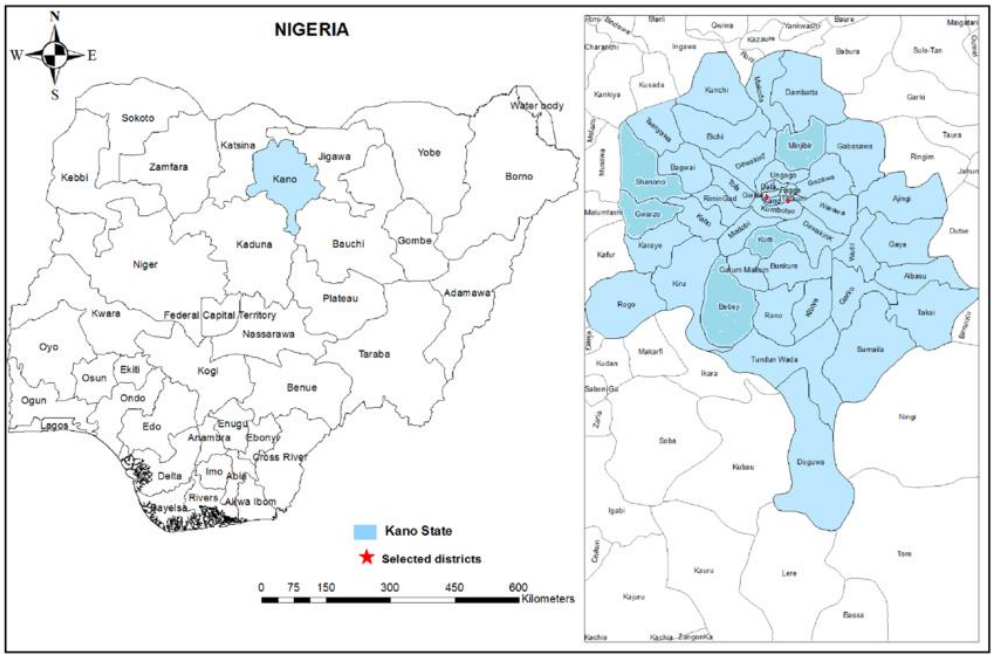

Figure 1: Some Local Government Areas within Kano State (Sulaiman *et al.*, 2022)

Study Design

The research was a cross sectional study

Sample Size

This was calculated using a prevalence rate of 34.1% obtained from a study conducted in Kaduna State- Nigeria (Sule *et al.*, 2011) and the formula below described by Aroye (2004).

$$N = \frac{z^2 pq}{l^2}$$

Where,

Z = 1.96 for 95% confidence interval
P = prevalence rate (34.1%)
q = 1 – P
l = allowable error (5%)
n = the number of samples

$$= \frac{(1.96)^2 \times 0.341 \times 0.65}{(0.05)^2}$$
$$= 345.3$$

Approximately 345. For this study, 450 samples were collected from the selected hospitals, i.e., 150 samples from each hospital.

Sample Population

Clinical isolates of the study participants were drawn from three popular hospitals in Kano: Muhammad Abdullahi Wase Teaching Hospital (MAWTH), Aminu Kano Teaching Hospital (AKTH), and Murtala Muhammad Specialist Hospital (MMSH).

Ethical Approval

Ethical approval was gotten from the ethical committee of Aminu Kano Teaching Hospital (AKTH), with ethical approval number: NHREC/21/08/2008/AKTH/EC/2726 and the Ministry of Health, with ethical approval number: MOH/Off/797/T.I/1657. This was after the submission of an introductory letter retrieved from the Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, Bayero University Kano.

Process of Collection of Bacterial Isolates

Isolates were obtained from the laboratory of the selected hospitals and transported to the laboratory on ice blocks. The isolates were screened by sub-culturing isolates colourless with or without black centres on *Salmonella Shigella* agar. These were incubated for 24 hours at a temperature of 37°C for the screening of *Salmonella species* (Cheesbrough, 2018).

Identification of Bacterial Isolates
Gram Staining Biochemical Characterization
The isolates were subjected to Gram Staining to determine their morphology and Gram reaction. Suspected Salmonella and Shigella species, characterized by colourless colonies with or without black centers on Salmonella Shigella agar, were further investigated using some biochemical test: IMViC (Indole, Methyl Red, Vogues Proskauer, Citrate Utilisation), Triple Sugar Iron (TSI), and Motility tests, as described by Sulaiman et al., (2014). The presumptive isolates were subsequently confirmed using Microbact Gram negative system kits.

Confirmation of Isolates Using Microbact Gram-Negative System
The 12A solid microplate format made up of 12 substrates was used for further biochemical characterization of isolates. From a 24hour culture, three isolated colonies were picked and emulsified in a 5.0 mL of sterile saline solution and mixed thoroughly to obtain a homogenous bacterial suspension. One hundred (100µL) of the bacterial suspension was dispensed into each well of microbact identification system and incubated at 37°C for 24hours. Reactions were evaluated as positive or negative by comparing them with the colour chart. Results were recorded in the report form provided which generated an octal coding system (four digit code). Each group of three reactions provided a single digit of the code. Using the results that was obtained, the indices of the positive reactions was circled. The sum of these indices in each group of three reactions formed the code number. The code was entered into the computer package. The microbact computer aided identification package generated percentage score against the organism's name share of the probability for that organism as a part of the total probabilities of all choices. The software permits a 75% cut off point for a probable identification.

Antimicrobial Susceptibility Testing
To determine the susceptibility pattern of the *Salmonella species* isolated, Kirby Bauer disc diffusion method was used in line with the standards of the Clinical Laboratory Standards Institute (CLSI, 2019). The standardized bacterial suspension (that matched 0.5 MacFarland) was inoculated onto Mueller-Hinton agar plates. The antibiotic sensitivity discs were placed on the inoculated Mueller-Hinton agar plates aseptically with adequate spacing. This was incubated at 37°C for 24 hours. The diameter of each zone of inhibiton was measured in millimetre (mm) after incubation. The antibiotic discs used were: Tetracycline (30µg), Doxycycline (5µg) and Minocycline (30µg). Isolates resistant to these antibiotics were further tested with Amoxicillin (10µg), Cefotaxime (30µg), Ciprofloxacin (5µg) Chloramphenicol (30µg), Trimethoprim (25µg), Gentamicin (10µg) (Oxoid Ltd, UK). Results were analysed as susceptible, intermediate or resistant, in line with the approved breakpoints (Clinical Laboratory Standards Institute, CLSI, 2019).

Plant Collection, Identification, and Pre-treatment
Plant species used for the study were determined after literature review, surveillance, and administration of questionnaires to traditional medical practitioners in Kano State, Nigeria. A taxonomist identified the plants at the Herbarium Unit of the Department of Biological Sciences, Bayero University Kano, Nigeria, and assigned voucher numbers BUKHAN 0049, BUKHAN 0116, and BUKHAN 0336 to *B. ferruginea, K. senegalensis*, and *P. guajava*, respectively. The plant parts were then cleaned, air-dried at room temperature, and ground into powders using a mortar and pestle. The powders were sealed in clean screw-cap containers and tagged for future use.

Extraction of Plant
The extraction procedure was done as carried out by Nurhadi *et al*. (2020). Two hundred grams (200g) of the pounded part of each plant was transferred into a thimble and poured into a Soxhlet extractor. 1 litre of 95% ethanol was used for the extraction until there was no change in the colour of ethanol, this indicated that the extraction process had been completed. The ethanolic extract was concentrated using a rotary evaporator separating extract and ethanol. The extract was placed in small volumes in porcelain dishes in an oven set at 80°C to remove remaining. This was done until the weight was constant. The extract was transferred into a clean screw cap container, properly tagged and refrigerated at a temperature of 4°C for future use.

Phytochemical Screening and Preparation of Different Concentrations of Extract
Carbohydrates, glycosides, saponins, flavonoids, tannins, alkaloids, resins, anthraquinones, triterpenes and steroids of each extract were detected as described by Onwukaeme *et al*., 2007, Sofowora, 2008 and Evans, 2002. Stock solutions of plant extracts were prepared at a concentration of 1g/mL (1000µg/mL) in DMSO, following Rahman *et al*. (2011).

Serial double-fold dilutions were then performed to obtain concentrations of 500, 250, and 125µg/mL. The solutions were prepared in four tubes, with tube 4 serving as the negative control (DMSO only) and Ciprofloxacin as the positive control. The dilutions were prepared by dissolving 10 mL of the stock solution in 10 mL of DMSO in tube 1 to obtain 500µg/mL, then mixing 10 mL of this solution with 10 mL of DMSO in tube 2 to obtain 250µg/mL, and similarly preparing tube 3 to obtain 125µg/mL.

Screening of Plant Extract for Antibacterial Activity

This was performed using paper disc diffusion assay, a procedure used by Elayaraja *et al.*, (2008). Paper discs of uniform size (8mm in diameter) were prepared using Whatmann No. 1 filter paper. Paper discs were sterilized at 160ºC in hot air oven for one hour. The discs were impregnated with 0.1 mL of 500, 250 and 125µg/mL concentrations of the plant extract. Dimethyl sulfoxide (DMSO) was used as negative control. Isolates were standardised and compared to 0.5 McFarland standard. An aliquot of 0.1mL broth suspension was used to streak the petri dish of sterile Mueller-Hinton agar. This was left to dry for about 5 minutes. Subsequently, the extract paper discs were placed on the agar plates using sterile forceps. Plates was incubated within 30 minutes at 37ºC for 24 hours. The diameter of the zone of inhibition was measured using a transparent metre rule. The test was done in duplicates and the mean values were recorded.

Minimum Inhibition Concentration (MIC)

Double dilution of each plant extract was carried out in nutrient broth using five concentrations of the extract i.e 100, 50, 25, 12.5 and 62.5mg/mL. A small amount (1 mL) of nutrient broth was placed in a test tube, followed by an equal amount (1 mL) of the extract. Serial dilution was performed, discarding the last 1 mL. Each microorganism was then incubated in 5 mL of nutrient broth overnight. The next day, 0.1 mL of the microorganism suspension was added to each test tube, and the mixture was incubated at 37°C for 18 hours. The Minimum Inhibitory Concentration (MIC) was determined as the lowest extract concentration that prevented visible growth of the microorganisms after overnight incubation. (Cheruiyot *et al.*, 2009).

Minimum Bactericidal Concentration (MBC)

The MBC of the extracts were determined using a method previously described by Adegboye *et al.* (2008). Samples from tubes that showed no growth from the MIC assay were cultured on fresh nutrient agar plates and incubated at 37°C for 48 hours. The MBC was determined as the lowest extract concentration that completely inhibited bacterial growth on the agar plates.

Data Analysis

Chi-square was employed to establish association between the organisms isolated and the hospitals. Subsequently, other results were analysed using Statistical Package for Social Sciences (SPSS) 19 version by using Analysis of Variance (ANOVA) for the comparison of means. P-values of <0.05 were considered statistically significant.

RESULTS

Qualitative Phytochemical Composition of the Plant Extract

The qualitative phytochemical screening of ethanolic extract of the *B. ferruginea* bark, bark of *K. senegalensis* and leaves of *P. guajava* were carried out as presented in Table 1. Among the bioactive substances tested, the plant extracts were all found to contain carbohydrates, saponins, flavonoids and tannins while anthraquinones and steroids were absent. *P. guajava* and *B. ferruginea* were also found to contain glycosides and resins. *B. ferruginea* and *K.senegalensis* were found to contain alkaloids.

The Distribution of Bacterial Isolates from the Selected Hospitals

Out of 450 isolates collected, 140 (31.1%) isolates were identified. They were characterised using conventional biochemical tests and Microbact Gram negative system. The Distribution of bacterial isolates collected from MMSH, MAWTH and AKTH were presented in Table 2. Among these were: *Proteus mirabilis* 17(3.8 %), *Proteus rettgeri* 1 (0.2 %), *Escherichia coli* non-O157 107(23. 8%), *Enterobacter agglomerans* 1(0.2 %), *Salmonella choleraesuis*/ *Salmonella enterica* 5 (1.1%), other *Salmonella* species 4 (0.9%), *Tatumella ptyseos* 3 (0.7%), *Citrobacter freundii* 1(0.2%), *Yersinia pseudotuberculosis* 1(0.2%). However, *Salmonella* species had an overall prevalence of 9 (2.0%).

Antibiotics Resistance Pattern of *Salmonella* species to Tetracyclines

The tetracycline resistance pattern of *Salmonella* species were presented in Table 3. Out of 9 isolates, 2 (22.2 %) showed resistance to Tetracycline, 4 (44.4 %) showed resistance to minocycline and 2 (2.2 %) showed resistance to Doxycycline.

Table 1: Phytochemical Constituents of the Plant Extracts

PHYTOCHEMICAL CONSTITUENT	TEST	PLANT EXTRACT (ETHANOL)		
		PSIDIUM GUAJAVA	***KHAYA SENEGALENSIS***	***BRIDELIA FERRUGINEA***
Carbohydrate				
	Molisch's Test	+	+	+
	Fehling's Test (Reducing sugar)	+	+	+
Glycosides				
	Fehling's Test	+	_	+
	Ferric chloride Test	+	_	+
	Cardiac glycosides (Kella Killiani's Test)	-	_	+
	Kadde' s Test	-	_	+
Saponins				
	Frothing Test	+	+	+
Flavonoids				
	Sodium Hydroxide Test	+	+	+
Tannins				
	Ferric chloride Test	+	+	+
Alkaloids				
	Mayer's Test	-	+	+
	Dragendoff's Test	-	+	+
	Wagner's Test	-	+	+
Resins				
	Acetic Anhydride Test	+	_	+
Anthraquinone derivatives				
	Free anthraquinones (Borntrager's Test)	-	-	-
	Combined anthracene (Modified Borntrager's Test)	-	-	-
Steroids				
	Salkowsk's Test	-	-	-
Triterpenoids				
	Lieberman-Burchard's Test	-	-	+

KEY: + Presence - Absence

Table 2: The Distribution of the Bacterial Isolates from the Selected Hospitals

BACTERIA	HOSPITAL			TOTAL FREQUENCY (%) N=450	x^2	p-value
	AKTH N=150	MAWTH N=150	MMSH N=150			
Proteus mirabilis	3 (2.0)	11 (7.3)	3 (2.0)	17(3.8)	59.597	0.000
Proteus rettgeri	1 (0.7)	0 (0)	0 (0)	1(0.2)		00061
Escherichia coli non O157	37(24.7)	11(7.3)	59(39.3)	107 (23.8)		
Enterobacter agglomerans	0 (0)	1 (0.67)	0 (0)	1(0.2)		
Salmonella choleraesuis/Salmonell enterica	2 (1.3)	3 (2.0)	0 (0.0)	5 (1.1)		
Salmonella species	0 (0)	4 (2.7)	0 (0)	4 (0.9)		
Tatumella ptyseos	2 (1.3)	1 (0.7)	0 (0.0)	3 (0.7)		
Citrobacter freundii	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.2)		
Yersinia pseudotuberculosis	0 (0.0)	1 (0.7)	0 (0)	1 (0.2)		
TOTAL	45(30.0)	33 (22.0)	62(41.3)	140 (31.1)		

KEY:
x^2= Chi square N= Total number of isolates AKTH= Aminu Kano Teaching Hospital
MMSH= Murtala Muhammad Specialist Hospital MAWTH= Muhammad Abdullahi Wase Teaching Hospital

Table 3: Antibiotics Resistance Pattern of *Salmonella* species (n=9) to Tetracyclines

ANTIBIOTICS	RESISTANCE		
	SUSCEPTIBLE (%)	**INTERMEDIATE (%)**	**RESISTANT (%)**
Tetracycline (30µG)	7 (77.8)	0 (0.0)	2 (22.2)
Minocycline (30µG)	0 (0)	5 (55.6)	4 (44.4)
Doxycycline (5µG)	7 (77.8)	0 (0)	2 (22.2)

*Zone sizes are measured and interpreted using CLSI, 2019.
KEY: N= Total number of isolates

Antibiotic Susceptibility Pattern of Tetracycline Resistant *Salmonella* species to other Selected Antibiotics

The susceptibility pattern of Tetracycline resistant *Salmonella* species to other selected antibiotics were presented in Table 4. All the *Salmonella* isolates showed susceptibility to Amoxicillin, Cefotaxime, Ciprofloxacin, Chloramphenicol, Gentamicin and Trimethoprim.

Antibacterial Susceptibility Pattern of Ethanolic Extract of Bark of *Khaya senegalensis*, Bark of *Bridelia ferruginea* and Leaves of *Psidium guajava* Against Tetracycline Resistant *Salmonella* species

The susceptibility of the isolates to the ethanolic extract of the bark of *K. senegalensis, bark of B.ferruginea and leaves of P.guajava* were carried out using the disc method. The disc measured 8mm in diameter. The various concentrations used were 500 µg/mL, 250 µg/mL and 125 µg/mL with Ciprofloxacin (5µg) and dimethyl sulfoxide serving as positive and negative controls respectively. The isolates were found to be susceptible at the concentration of 500 µg/mL with highest zone of inhibition of 9.5±0.29mm for *Psidium guajava*, 8.75±0.48 mm for *B. ferruginea*, and 8.75±0.36 mm for *K. senegalensis* as presented in Table 5.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic extract of *Khaya senegalensis*, *Bridelia ferruginea* and *Psidium guajava*The Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration of the three plant extracts were found to be 50mg/mL and 100mg/mL for each of the extracts respectively. This is indicated in Table 6

.

Table 4: Antibiotics Susceptibility Pattern of Tetracycline Resistant *Salmonella* species (n=4) to Other Antibiotics

ANTIBIOTICS	SUSCEPTIBILITY		
	SUSCEPTIBLE (%)	**INTERMEDIATE (%)**	**RESISTANT (%)**
AMOXICILLIN(10µg)	4 (100)	0 (0)	0 (0)
CEFOTAXIME(30µg)	4 (100)	0 (0)	0 (0)
CIPROFLOXACIN(5µg)	1 (25)	3 (75)	0 (0)
CHLORAMPHENICOL(30µg)	4 (100)	0 (0)	0 (0)
GENTAMICIN(10µg)	4 (100)	0 (0)	0 (0)
TRIMETHOPRIM(5µg)	4(100)	0 (0)	0 (0)

*Zone sizes are measured and interpreted using CLSI, 2019.

KEY:

N= Total number of isolates

Table 5: Zones of Inhibition (Mean ± Standard Error) of Tetracycline Resistant *Salmonella* species (n=4) in Ethanolic Extract of Bark of *Khaya senegalensis*, Bark of *Bridelia ferruginea* and Leaves of *Psidium guajava*

CONCENTRATION (µg/mL)	**ZONE OF INHIBITION(mm)**		
	Khaya senegalensis	***Bridelia ferruginea***	***Psidium guajava***
500	8.75±0.36[b]	8.75±0.48[b]	9.50±0.29[b]
250	8.00±0.00[b]	8.00±0.00[b]	9.00±0.00[b]
125	8.00±0.00[b]	8.00±0.00[b]	8.00±0.00[b]
Dimethyl sulfoxide	8.00±0.0[b]	8.00±0.00[b]	8.00±0.00[b]
Ciprofloxacin(5µg)	27.25±0.80[a]	33.50±1.71[a]	35.25±1.60[a]
p-value	0.00	0.00	0.00

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanolic Extract of *Khaya senegalensis*, *Bridelia ferruginea* and *Psidium guajava* Against *Salmonella species* (N=4)

Plant Extract	**Minimum Inhibitory Concentration (mg/mL)**					**Minimum Bactericidal Concentration (mg/mL)**				
	6.25	12.5	25	50	100	6.25	12.5	25	50	100
***Khaya senegalensis* (%)**	0 (0)	0 (0)	0 (0)	1(25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(25)
***Psidium guajava* (%)**	0 (0)	0 (0)	0 (0)	4(100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4(100)
***Bridelia ferruginea* (%)**	0 (0)	0 (0)	0 (0)	1(25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(25)

DISCUSSION

Several studies have shown that different bacteria are found in the gastrointestinal tract, and a few can cause gastrointestinal diseases. In this study, *Proteus mirabilis*, *Proteus rettgeri*, *Escherichia coli* non O157, *Enterobacter agglomerans*, *Salmonella enterica*, other *Salmonella species*, *Tatumella ptyseos*, *Citrobacter freundii* and *Yersinia pseudotuberculosis* were found in faeces but *Salmonella species* had a high frequency among the pathogenic bacteria isolated. This is similar to the findings of Mbuthia *et al.*, 2018 who identified *Salmonella species* as the most frequent causative agent of bacterial gastroenteritis.

The varying differences in the prevalence rate of bacteria with *Escherichia coli* non O157 from MMSH dominating, can be attributed to the bacteria being a normal flora of the gut and its higher frequency is due to differences in patient demographics or underlying health conditions, variations in gut microbiota composition among patient populations, distinct dietary habits or nutritional influences. On the other hand, the higher prevalence of *Salmonella* species in AKTH and MAWTH can be attributed to the consumption of contaminated food or water among its patients, inadequate hand hygiene and illicit antibiotic usage patterns and environmental contamination. Additionally, the presence of more virulent or transmissible *Salmonella* strains, asymptomatic carriers among the patients and their local community during outbreaks or food/water contamination may contribute more to its prevalence .*Bridelia ferruginea* (bark), *Khaya senegalensis* (bark) and *Psidium guajava* (leaves) are popular medicinal plants used in treatment of gastrointestinal diseases (Sulaiman *et al.*, 2022).They were found to contain flavonoids, saponins, carbohydrates and tannins while anthraquinones and steroids were absent. Carbohydrates, being primary photosynthetic products in plants, serve as energy storage molecules and structural components of cell walls. Saponins, derived from the mevalonate or 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways, are amphipathic compounds that interact with cell membranes, exhibiting antimicrobial, anti-inflammatory, and antioxidant properties. Tannins, synthesized via the shikimate pathway, are polyphenolic compounds that complex with proteins and polysaccharides, contributing to their astringent, antimicrobial, and antioxidant activities. Flavonoids, produced through the phenylpropanoid pathway, are a diverse group of benzo-γ-pyrone derivatives that play key roles in plant defense, UV protection, and antioxidant mechanisms. They likely contribute to the plant's adaptability, modulating enzyme activities, scavenging free radicals, and interacting with other phytochemicals. The co-occurrence of these phytochemicals is likely due to their shared biosynthetic precursors and the plant's defense strategies, where carbohydrates provide structural support, saponins modulate membrane interactions, tannins confer chemical defense, and flavonoids fine-tune antioxidant and UV-protective responses. This is in line with Kiruba, 2014 and Abdulhamid *et al.*, 2013 who stated that the ethanolic leaves extract of *Psidium guajava* obtained from and Tamil Nadu, India and Kebbi State, Nigeria respectively showed that flavonoid, tannins, alkaloids, saponins and steroid glycosides were present and had antibacterial activity. Similarly, Alfa *et al.*, 2019 showed that ethanolic extract of bark of *Bridelia ferruginea* obtained from Kogi State also contained saponins, tannins, carbohydrate, alkaloid, flavonoids, and cardiac glycosides which is in line with this study and that of Adebayo and Ishola, 2009 from Oyo State who reported *Bridelia ferruginea* as having antimicrobial activities and contained very important phytochemicals (Akuodor *et al.*, 2011; Owoseni *et al.*, 2010).In addition, according to Agouru *et al.*, 2017 and Makut *et al.*, 2008 *Khaya senegalensis* obtained from Benue and Nassarawa State respectively also contained same phytochemicals as seen in this study while those obtained by Kubmarawa *et al.*, 2008 from Adamawa State only contained saponins and tannis as seen in this study but disagrees by showing that alkaloids, glycosides and flavonoids were absent.

Anthraquinone was absent in all the ethanolic extract of *Khaya senegalensis*, *Bridelia ferruginea* and *Psidium guajava* this is because anthraquinones are derived from shikimate pathway specific to plants with certain enzymatic capabilities such as those that belong to Rhamnaceae and Polygonaceae family which these specific plants of interest in this study lacked. This result is in agreement with the works of Elisha *et al.*, 2012 in Jos, Adebayo and Ishola, 2009 in Ogun State, and Abdulhamid *et al.*, 2013 in Kebbi State respectively and in disagreement with the study of Ugoh *et al.*, 2014 in Abuja, Owoseni *et al.*, 2010 in Osun State and Abdallah *et al.*, 2019 in Kano State respectively. Steroids were absent as a result of the absence of the mutli-enzyme pathway responsible for its production in all the ethanolic extract of *Khaya senegalensis*, *Bridelia ferruginea* and *Psidium guajava*. This was not in tandem with the works of Ugoh *et al.*, 2014 in Abuja, Adebayo, and Ishola, 2009 in Oyo State, and Abdulhamid *et al.*, 2013 in Kebbi State, respectively who had steroids present in their extracts. This variation in the phytochemical compounds in the plant extracts are as a result of the differences in the geographical location which has been previously said to affect the chemical constituents of plants that belong to same genus but found in other environments. This may explain the absence of anthroquinone and steroids in *Khaya senegalensis*, *Bridelia ferruginea* and *Psidium guajava* obtained in Kano State, Nigeria in their study.

These compounds have been reported in previous studies and shown to have adequate inhibitory action against various microorganisms. For example, saponins, flavonoids and tannins are well known to have antimicrobial activities (Akiyama *et al.*, 2001). Flavonoids are synthesized by plants and act by inhibiting the nucleic acid, energy metabolism, damaging cytoplasmic membrane of bacteria making it antimicrobial in nature (Kujumgiev *et al.*, 1999).The presence of flavonoids in the *Khaya senegalensis* (bark), *Bridelia ferruginea* (bark) and *Psidium guajava* (leaves) could be responsible for their anti-diarrhoeal activity (Bylka *et al.*,2004). It is shown to inhibit contraction induced by spasmogens and inhibit intestinal transit (Abdullahi *et al.*, 2001) which accounts for the use of the plant as natural anti-diarrhoeal agents and as cure for colic. Nuhu *et al.*, 2000; Ejimadu and Ogbeide, 2001; Okerulu and Chinwe, 2001 reported that these natural constituents were reported to have inhibitory activity on the growth of many pathogenic bacteria. Tannins have also been reported to possess anti-diarrhoeal activities (Wangensteen *et al.*, 2013) due to their capacity to form complexes with soluble and extracellular and proteins such as enzymes involved in active transport across the cell membrane (Tsuchiya *et al.*, 1996). *Salmonella species* from this study exhibited higher resistance to minocycline among the tetracyclines tested .This could be due to increased lipophilicity and distinct molecular structure of minocycline that affects its penetration and ribosomal binding. It may possess efflux pumps that preferentially export minocycline, produce ribosomal protein protections that interfere with its ribosomal binding, or harbour resistance genes on plasmids. The selective pressure from overuse or misuse of minocycline, varying prescription practices, and genetic factors like plasmid-mediated resistance genes and chromosomal mutations could further exacerbate its resistance. All *Salmonella* species that were resistant to Tetracycline were susceptible to other antibiotics such as Amoxicillin, Cefotaxime, Ciprofloxacin, Gentamicin, Chloramphenicol and Trimethoprim indicating that these antibiotics were probably not abused as compared to Tetracycline. This finding is in tandem with Gargano *et al.*, 2021 who also revealed that these antibiotics were active against *Salmonella* species. Thus, they could be used as therapeutic agents in treating gastrointestinal disease caused by *Salmonella* species. However, due to susceptibility of *Salmonella* to the three plant extracts using disc diffusion method, further analysis using MIC and MBC revealed that Minimum Inhibitory Concentration of 50mg/mL and Minimum Bactericidal Concentration of 100mg/mL indicating that these extracts had both bacteriostatic and bactericidal effects. Although, *Psidium guajava* had a higher frequency of MIC and MBC among the strains. This finding is similar to the studies by Adebayo and Ishola (2009), Mohammed *et al.*, 2016, and Ugoh *et al.*, 2014 respectively who reported that, *Psidium guajava* exhibited a higher antibacterial activity against the isolates than the extracts of *Khaya senegalensis* and *Bridelia ferruginea*. Furthermore, the ethanolic extract of *Psidium guajava* exhibited a higher antimicrobial activity than *Khaya senegalensis* and *Bridelia ferruginea*. The variation in level of activity among the plant extracts could be due to the difference in solubility of active ingredient in ethanolic solvent on one hand and to the constitutional or structural variability of the tested organisms on the other hand (Adewunmi *et al.*, 2001).

CONCLUSION

Based on the findings of this study, it could be deduced that bark of *Khaya senegalensis,* bark of *Bridelia ferruginea* and leaves of *Psidium guajava)* contain bioactive substances with antibacterial properties. Also, there are various enteric bacteria that cause gastrointestinal diseases. *Salmonella species* have been found to be resistant to some antibiotics belonging to the group of tetracyclines which are used in the treatment of gastrointestinal diseases. The tetracycline-resistant *Salmonella species* are susceptible to some commonly used antibiotics such as Amoxicillin, Cefotaxime, Ciprofloxacin, Chloramphenicol, Gentamicin and Trimethoprim as well as ethanolic extracts of *Khaya senegalensis* stem bark, *Bridelia ferruginea* stem bark and *Psidium guajava* leaves. Thus, Salmonellosis caused by tetracycline-resistant *Salmonella species* can be treated with some the antibiotics tested in the study. Similarly, these three medicinal plants screened in the study have potential to develop medications for the treatment of several gastrointestinal bacterial infections, particularly tetracycline-resistant Salmonellosis.

REFERENCES

Abdulhamid, A., Fakai, I.M., Sani, I., Argungu, A.U., and Bello, F. (2013). Phytochemical screening and antibacterial activity of ethanolic and aqueous extracts of *Psidium guajava*.

World Research Journal of Medical and Aromatic Plants,2(1). 44-46. **[Crossref]**

Abdullahi, A.L., Agbo, M.O.; Garmaniel, K.S. and Wambebe, C. (2001). Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennioides* Roots, *Phytotherapy Research*, 19: 431-434. **[Crossref]**

Adebayo, E.A., and Ishola, O.R. (2009). Phytochemical and antimicrobial screening of the crude extract of the root, stem bark and leaves of *Bridelia feruginea*. *African Journal of Biotechnology*, 8(4). 650-653.

Adegboye, M.F, Akinpelu, D.A, Okoli, A.I (2008). The bioactive and phytochemical properties of G. kola (Heckel) seed extract on some pathogens. *African Journal Biotechnology*. 7(21): 3938-3938.

Adewunmi, C.O., Agbedahunsi J.M., Adebajo A.C., Aladesanmi A. J., Murphy N. and Wando J. (2001). Ethano-veterinary medicine; screening of Nigerian medicinal plants for trypanocidal properties. *Journal of Ethnopharmacology* 77.19-22. **[Crossref]**

Aguoru, U.C., Bashayi, G.C., and Ogbonna, O.I. (2017). Phytochemical profile of stem bark extracts of *Khaya senegalensis* by Gas Chromatography- Mass Spectrometry (GC-MS) analysis. *Journal of Pharmacognosy and Phytotherapy*.9(3).35-43. **[Crossref]**

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., and Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 48(4), 487-491. **[Crossref]**

Akuodor, G.C., Mbah, C.C., Anyawelechi, N.A., Idris-Usman, M., Iwuanyanwu, T.C.,and Osunkwo, U.A.(2011). Pharmacological profile of aqueous extract of *Bridelia ferruginea* stem bark in the relief of pain and fever. *Journal of Medical Plant Research*, 5(22).5366-5369.

Alfa, A.A., Ayodeji, A.O., Teru, D.A.G., Tijani, B.K. (2019). Studies on the Phytochemical Compounds in the Ethanolic Leaf Extract (ELE), Ethanolic Bark Extract (EBE) and Ethanolic Root Extract (ERE) of *Bridelia ferruginea* Benth (Euphorbiaceae) .*Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2(4).1-8. **[Crossref]**

Aronsson, L., Huang, Y., Parini, P., Korach-André, M., and Håkansson, J. (2010). Decreased fat storage by Lactobacillus paracasei is associated with increased levels of angiopoietin-like 4 protein (ANGPTL4). *Public Library of Science One*, 5. **[Crossref]**

Aroye, M.O. (2004): *Research Methodology with Statistics for Health and Social Sciences*. Nathadex Publishers, Ilorin. 118.

Bettleheim, K.A. and Beutin, L. (2003).Rapid laboratory identification and characterization of verocytotoxigenic (shiga-toxin producing) *Escherichia coli* (VTEC/STEC).*Journal of Applied Microbiology*, **95**:205-217. **[Crossref]**

Bylka, W.E., Matlawska, I., Pilewski, N.A. (2004). Natural flavonoids as Antimicrobial Agents. *Journal of American Nutraceutical Association*. 7(2): 21–28.

Cannel, R.J.P (2000). *Methods in Biotechnology*. Natural Products Isolation. Human press. New Jessy.14-36.

Cheesbrough, M. (2018): *District Laboratory Practice for Tropical Countries*. Macmillan Publishing Company, U.S.A. 67-68, 80, 137,141,180.

Cheruiyot, K.R., Olila, D., Kateregga, J., (2009). In vitro antibacterial activity of selected medicinal plants from Longisa region of Bonset district, *Kenya African Health Science*. 01(04). 100-105.

Chopra S., and Reader J. tRNAs as Antibiotic Targets. *International Journal of Molecular Sciences*. 2015; 16(1):321-349. **[Crossref]**

Clinical Laboratory and Standards Institute manual. (2019). Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement. CLSI document M100-S29.

De Angelis, M., Piccolo, M., Vannini, L., Siragusa, S., and De Giacomo, A. (2013). Faecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *Public Library of Science One*, 8: e76993. **[Crossref]**

Ejimadu, I.M. and Ogbeide O.W. (2001).Antimicrobial activities of petroleum ether and ethanolic extracts of leaf-stem and root bark of *Impomoea involucrate* Beaur. *Journal of Chemical Society of Nigeria*, 26(1):56-59.

Elayaraja, A., Ramaha, S.A., Samantalu, G. and Garikapati, D.R. (2008). Antibacterial Activity of Various Crude Extracts of

Justicia tranquebariensis. *Journal of Pure and Applied Microbiology* 2(1)232.

Elisha, L.I., Makoshi, S.M., Makama, S., Dawurung, J.C., Offiah, V.N., Gotep, G.J., Oladipo, O.O., and Shamaki, D. (2012). Antidiarrheal Evaluation of Aqueous and Ethanolic Stem Bark Extracts of *Khaya senegalensis* A. Juss (Meliaceae) in Albino Rats. *Pakistan Veterinary Journal, 33(1) .32-36.*

Erickson, A.R., Cantarel, B.L., Lamendella, R., Darzi, Y., and Mongodin, E.F. (2012). Integrated metagenomics/metaproteomics reveals human host-microbiota signatures of Crohn's disease. *Public Library of Science One*, 7: e49138. **[Crossref]**

Evans, W. C. (2002). Trease and Evans Pharmacognosy. 15th Ed. Saunders Publishers, London. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393, 542-543.

Evans, W.C. (2009) Trease and Evans Pharmacognosy. 16th edition. Saunders Elsevier Publishers, UK, 513.

Ezeonwu, B.U., Ibeneme., C.A., Aneke, F., and Oguonu, T. (2013). Clinical features of acute gastrenteristis in children at University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. *Annals of Medical and Health Sciences Research*. 3. (3). 361-364. **[Crossref]**

Gargano, V.; Sciortino, S.; Gambino, D.; Costa, A.; Agozzino, V.; Reale, S.; Alduina, R.; Vicari, D. (2021). Antibiotic Susceptibility Profile and Tetracycline Resistance Genes Detection in *Salmonella* spp. Strains Isolated from Animals and Food. *Antibiotics*, 10, 809. **[Crossref]**

Gosalbes, M.J., Durbán, A., Pignatelli, M., Abellan, J.J., and Jiménez-Hernández, N. (2011). Metatranscriptomic approach to analyze the functional human gut microbiota. *Public Library of Science One*, 6. **[Crossref]**

Ibrahim, G., Mahmaoud, N., Yaro, A.H. and Ahmed, A. (2006). Phytochemical and Toxicity evaluation on the Stem bark of *Ficus sycomorous* Linn. (Moraceae). *Biological and Environmental Sciences Journal for the Tropics*, 3(3)38.

Jordan, S.A., Cunningham, D.G., and Marles, R.J., (2010). Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and Applied Pharmacology*, 243:198-216. **[Crossref]**

Karki, A., and Tiwari, B.R. (2007). Prevalence of acute diarrhoea in Kathmandu valley. *Journal of Nepal Medical Association*, 46,175–179. **[Crossref]**

Kiruba, G.V.D. (2014). Preliminary Phytochemical analysis of leaf extracts of *Psidium guajava* L. *International Journal of Pharmacognosy and Phytochemical Research*. 6(2).332-334.

Kogut, M. H., Lee, A., & Santin, E. (2020). Microbiome and pathogen interaction with the immune system. *Poultry Science*, *99*(4), 1906–1913. **[Crossref]**

Kubmarawa, D., Khan, M.E., Punah, M.A and Hassan, M.(2008).Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria. *African Journal of Biotechnology*, 7 (24).4563-4566.

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., Popov, S. (1999).Antibacterial, antifungal, and antiviral activity of propolis of different geographic origin. *Journal of Ethnopharmacology*. 64: 235-240. **[Crossref]**

Makut, M.D., Gyar, S.D., Pennap, G.R.I., and Anthony, P.(2008). Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of the leaf and bark of *Khaya senegalensis*. *African Journal of Biotechnology*. 7(9). 1216-1219.

Maslowski, K.M., Vieira, A.T., Ng, A., Kranich, J., and Sierro, F. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, 461: 1282-1286. **[Crossref]**

Mazodier, P., and Davies, J. (1991). Gene transfer between distantly related bacteria. *Annual Review of Genetics*, 25. 147–171. **[Crossref]**

Mbuthia, O.W., Mathenge, S.G., Oyaro, M.O., Ngáyo, M.O. (2018). Etiology and Pathogenicity of bacterial isolates: a cross sectional study among diarrheal children below five years in central regions of Kenya.*Pan African Medical Journal*, 31, 88. **[Crossref]**

Mohammed, A.H., Na'inna, S.Z., Yushau, M., Salisu, B., Adamu, U., and Garba, S.A. (2016). Invitro antibacterial activity of *Psidium guajava* leaves extract against isolates of *Salmonella* species. *UMYU Journal of Microbiology Research*. 2(1). 1-5. **[Crossref]**

Mukherjee, P.K., and Wahile, A., (2006). Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *Journal of Ethnopharmacology*, 103: 25–35. [Crossref]

Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiology Spectrum*, *4*(2),10.1128/microbiolspec. VMBF-0016-2015. [Crossref]

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., & Tasak, N. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet*, *399* (10325), 629-655. [Crossref]

National Population Commission (NPC) (2006) .Nigeria National Census: Population Distribution by Sex, State, LGAs and Senatorial District: 2006 Census Priority Tables.3. http://www.population.gov.ng/index.php/publication/140-popn-distri-by-sex-state-jgas-and-senatorial-distr-2006

Nuhu, A. M., Mshelis, M. S. and Yakubu, Y. (2000). Antimicrobial screening of the bark extract of *Pterocarpus erinaceus* Tree. *Journal of Chemical Society of Nigeria*, 25:85-86.

Nurhadi, B., Saputra, R.A., Setiawati, T.A.,Husein, S.N., Faressi, F.R., Utari, C.D., Sukri, N., Kayaputri, I.L and Setiasih, I.S. (2020). *IOP Conference Series: Earth Environmental Science,* 443: 012074. [Crossref]

Nzeoko, B., and Okafor, N. (2002). Bacterial enteropathogens and factors associated with seasonal episodes of gastroenteritis in Nsukka, Nigeria. *British Journal of Biomedical Science*. 59(2), 76-79. [Crossref]

Okerulu, I.O. and Chinwe, J.A. (2001). The phytochemical analysis and antimicrobial screening of extracts of *Tetracarpidium conophorum*. *Journal of Chemical Society of Nigeria*, 20(1): 53-55.

Onwukaeme, D.N., Ikuegbvweha, T.B. and Asonye, C.C. (2007). Evaluation of Phytochemical Constituents, Antibacterial Activities and Effects of Exudates of *Pycanthus angolensis* Weld Warb (Myristiceae) on corneal ulcers in Rabbits. *Tropical Journal of Pharmaceutical Research,* 6(2):726. [Crossref]

Owoseni, A.A., Ayanbamiji, T., Ajayi, Y.O., Ewegbenro, I.B. (2010). Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea*. *African Journal of Biotechnology,* 9. 1031-1036. [Crossref]

Oyetayo, O.V. (2007). Control of Gastroenteritis: the probiotic therapy alternative in Nigeria. *International Journal of Biomedical and Pharmaceutical Sciences*. 1(1): 60-64.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and global health*, *109*(7), 309–318. [Crossref]

Rahman, S., Imran, M., Muhammed, N., Hassan, N., Chisthi, A.K., Khan, A.F., Sadozai, K. S. and Khan, S.M. (2011). Antibacterial Screening of Leaves and Stem of *Carica papaya*. *Journal of Medicinal Plants Research* 5 (20) 5167-5171.

Rajili, Ä., StojanoviÄ, M., Smidt, H., and De Vos, W.M. (2007). Diversity of the human gastrointestinal tract microbiota revisited. *Environmental Microbiology,* 9: 2125-2136. [Crossref]

Rocha, C.S., De Azevedo,M., Pontes,D.S., Agresti, M.P., Pereira,B.V., De Castro,P.C., Da Cunha,P.V., De Oliveira Junior, A.F., De Sousa,S.C., Saraiva, L.D.T., Preisser,M.T., Souza,M.B., Turk, Z.M., Azevedo,V., Van de Guchte,M., Miyoshi,A., and De Oliveira Carvalho, D.R. (2015). Human GI microbiota in Health and disease: a focus on inflammatory bowel diseases. Innovative immunology. *Autin Publishing Group*. 4.

Serwecińska L. (2020). Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. *Water*. 12(12):3313. [Crossref]

Singh, S., Verma, N., & Taneja, N. (2019). The human gut resistome: Current concepts & future prospects. *The Indian journal of medical research*, *150*(4), 345–358. [Crossref]

Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., and Gallini, C.A. (2013). The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science,* 341: 569 573. [Crossref]

Sofowora, A. (2008). *Medicinal Plants and Traditional Medicine in Africa,* Spectrum Books Limited, Ibadan, Nigeria. 3rd edition. 70-74; 175-180; 201-202.

Sonnenburg, J.L., Chen, C.T., and Gordon, J.I. (2006). Genomic and metabolic studies of the impact of probiotics on a model

gut symbiont and host. *Public Library of Science Biology,* 4. **[Crossref]**

Srinivasan, K. (2005). Plant foods in the management of Diabetes mellitus: *species* as beneficial antidiabetic food adjuncts. *International Journal of Food Sciences and Nutrition*. 56: 399-414. **[Crossref]**

Street, R. A., and Prinsloo, G. (2013). Commercially important medicinal plants of South Africa: a review. *Journal of Chemistry,* 1–16. **[Crossref]**

Sulaiman, A.N., Arzai, A.H., Taura, D.W. (2022). Ethnobotanical survey: A Comprehensive Review of Medicinal Plants Used in Treatment of Gastrointestinal Diseases in Kano Nigeria. *Phytomedicine Plus,* 2, (1), 100180. **[Crossref]**

Sulaiman, A.N., Ameh, J.B., Inabo, H.I. (2014). Viability of Clinical *Staphylococcus aureus* and *Staphylococcus aureus*ATCC 25923 Under Different Preservation Methods. *Journal of Medical Science and Clinical Research,* 2 (7). 1824-1837.

Sule, E.I., Aliyu, A.M and Abdulaziz, B.M. (2011). Isolation of diarrhoeagenic bacteria in children attending some selected hospitals within Kaduna metropolis. *Continental Journal of Applied Sciences,* **6**(1):1-6.

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *The Biochemical Journal*, *474*(11), 1823-1836. **[Crossref]**

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T., Linuma, M. (1996). Comparative studies of the antibacterial activity of phytochemical flavones against Methicillin Resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology,* 50: 27- 34. **[Crossref]**

Ugoh, C.S., Agarry, O.O., and Garba, A.S. (2014). Studies on the antibacterial activity of *Khaya senegalensis* [(Desr.) A. Juss)] stem bark extract on *Salmonella enterica* subsp. *enterica* serovar Typhi [(ex Kauffmann and Edwards) Le Minor and Popoff]. *Asian Pacific Journal of Tropical Biomedicine*. 4(1). S270-S279. **[Crossref]**

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T : a peer-reviewed journal for formulary management*, *40*(4), 277–283.

Wangensteen, H., Klarpås, L., Alamgir, M., Samuelsen, A. B., and Malterud, K. E. (2013). Can scientific evidence support using Bangladeshi traditional medicinal plants in the treatment of diarrhoea? A review on seven plants. *Nutrients*, *5*(5), 1757-1800. **[Crossref]**

World Health Organization. (1996). Traditional Medicine from http://www.chiro.org/acupuncture/FULL/TRADITIONAL MEDICINE.shtml retrieved 18/02/2011

World Health Organization. (2001). *Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A World Wide Review*. Geneva. Retrieved from http://apps.who.int/medicinedocs/en/d/Jh2943e/.

World Health Organization. (2006): Contribution of traditional medicine to public health: coca leaf. Report by the Sectretariat, World Health Organization, EB120/36

World Health Organization. (2008). Traditional Medicine obtained from *http://www.who.int/mediacentre/factsheets/fs134/en/* retrieved 18/02/201

World Health Organization. (2014). Global report on surveillance of antimicrobial resistance, with data provided by 114 countries. No 194. Updated April, 2014.