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Phytochemical Profile, Antibacterial Activity and Effect of Infusions from Polyherbal Tea on Gut Microbiota of Albino Rats

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ABSTRACT
Herbal teas unique taste, aroma and health benefits have led to its increased interest worldwide. Some of the health benefits are attributed to the interaction between the tea's bioactive compounds and the gut microbiota. This study investigated the phytochemical composition, antibacterial activities, and gut microbiota-modulating effects of a polyherbal tea infusion using an albino rat model. Thirty rats were divided into ten groups receiving different formulations (F1–F6), sweetened variants (with sugar or honey), a commercial tea and water as control over 21 days. The gastrointestinal contents were analyzed using the spread plate method; reverse-phase high-performance liquid chromatography (RP-HPLC) method was used for phytochemical analysis, while antibacterial activities were assessed against *Escherichia coli* and *Staphylococcus aureus* using the disk diffusion method. Quercetin was the most abundant polyphenol (3.82 mg/L), followed by kaempferol (1.53 mg/L). Formulation 5 consistently showed higher concentrations of gallic acid, chlorogenic acid, Quercetin, kaempferol and apigenin. All polyherbal formulations showed antibacterial activity and *E. coli* were more susceptible than *S. aureus*. The tea significantly increased mesophilic aerobic bacterial counts in the duodenum compared to the water control group, suggesting prebiotic-like stimulation of beneficial microbiota. *Escherichia coli* was detected only in the water control group of the caecum, while *Salmonella* was absent across all groups. These findings indicate that polyherbal tea can modulate gut microbiota composition, potentially suppress pathogenic organisms and promote beneficial microbial growth, which supports its role as a functional beverage for improving gastrointestinal health.

Keywords: Tea, Rats, Quercetin, Microbiota, Antibacterial, Duodenum

INTRODUCTION

Herbal medicine has been at the forefront of primary healthcare and prophylaxis in developing countries (Hassali *et al*., 2016; Gayani *et al*., 2023). Globally, herbal tea is consumed as functional beverage. They may consist of mono- or polyherbal plant materials brewed as decoction or infusion and drank for their therapeutic and prophylactic benefits (Poswal *et al*., 2019; Liu *et al*., 2023). The perceived health benefits derived from consuming herbal tea, as well as its unique flavour, taste and aroma, have led to increased interest in herbal tea (Abdullah *et al*., 2023).

Tea, an infusion derived from *Camellia sinensis,* is consumed worldwide as a functional beverage; and it is cultivated in over 30 countries, with China and India leading in the production (Namita *et al*., 2012). Many studies have reported that tea is rich in polyphenols, amino acids, alkaloids, polysaccharides and other substances that contribute to its antioxidant, anti-tumour, anti-inflammatory, antimicrobial, immune-regulating, anticancer, cardiovascular health promotion, diabetes prevention, obesity prevention, and liver protection (Foster *et al*., 2016; Guo *et al*., 2016; Tang *et al*., 2019; Zhou *et al*., 2020). Numerous studies attributed some of these health benefits to interplay between tea bioactive compounds and the gut microbiota (Bond and Derbyshire, 2019). The impact of tea on the gut microbiota could be a route by which tea exerts its health benefits, as the gut microbiome plays a role in health (Liu *et al*., 2018; Zhao *et al*., 2024). The microbiota of the gastrointestinal tract (GIT) of mammals is a home to an enormous number of symbiotic microorganisms estimated to be about 10 trillion, which is 10 times higher than the number of human cells, that coexist and provide several ecosystem services, and functions that their host's genome is unable to encode (Singh, 2021; Almeida *et al*., 2021). The GIT of animals functions in a symbiotic ecosystem with the human body (Zhou *et al*., 2021). They play a role in host health, and in chronic diseases such as obesity, diabetes, inflammatory bowel disease, and neurological disorders (Strati *et al*., 2017; Rowland *et al*., 2018). The gut microbiota plays a vital role in the digestion of food as well as protection against infection resulting from the presence of pathogens (Peck *et al*., 2019; Wlazlo *et al*., 2021). They function as a barrier against harmful exogenous substances, while ensuring normal metabolic, immune and neurological functions in the host (Wlazlo *et al*., 2021).

Tea's polyphenols have been reported to have low bioavailability; and over 90 % of them are not absorbed in the small intestine; most tea that reaches the large intestine is metabolized by gut microbes (Liu *et al*., 2018). Numerous studies have shown that intestinal microbiota can transform tea polyphenols into short-chain fatty acids, c-pentalactone, phenolic acids, and other metabolites, which can effectively improve insulin sensitivity, adiponectin levels, total

cholesterol, and triglyceride levels, and prevent the accumulation of fat in the liver (Barroso *et al*., 2019), thereby improving parameters related to obesity regulation (Zhou *et al*., 2021). Chen *et al*. (2019) reported that tea polyphenols and other bioactive substances in green tea can reduce fasting blood glucose level, mesenteric fat and increase serum insulin levels, thereby lowering blood lipid and preventing beta cell damage. Tea polyphenols were included among different substances that could act as prebiotics because catechins can favour the growth of potentially beneficial microbes, additionally, hinder some potentially harmful ones (Gibson *et al*., 2017). Phenolic compounds are secondary metabolites in plants that protect them against certain aggressions or infections, either by bacteria or insects (Liu *et al*., 2018). Tea phenolic compounds (especially catechins) have shown antibacterial activity against bacteria such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli*, *Helicobacter pylori*, *Legionella pneumophila*, and *Mycobacterium* sp (Liu *et al*., 2018).

The overall status of the intestinal microbiota of animals can be improved by promoting the growth of a beneficial microbiota (like *Lactobacillus*, *Bifidobacterium* and *Akkermansia*), reducing harmful bacteria such as those from the Enterobacteriaceae family, activating certain host genes, regulating metabolism, increasing intestinal butyrate and other short-chain fatty acids (SCFA) (Canfora *et al*., 2019; Zhou *et al*., 2021).

Several studies have reported antibacterial activity of tea infusions due to its bioactive compounds such as catechin-3-gallates and theaflavins (Tiwari *et al*., 2005; Liu *et al*. 2022). Catechins and their derivatives present in herbal teas plays a key role in their biological activities (Sharangi, 2009; Zhang *et al*., 2019). According to Friedman (2007) and Liu *et al*. (2022), (-) – epigallocatechin (EGCG), the main catechin compound in total catechins from green tea extracts, had strong antibacterial effects on *Bacillus anthracis*, *E. coli* and *S. aureus*.

The tea plant (*Camellia sinensis*, L. Kuntze), belonging to the family Theaceae, is native to Southeast Asia but is currently cultivated in more than 30 countries (Namita *et al*., 2012). Tea is widely consumed in Africa because of its natural origin and pharmacological effects, such as hypoglycemic, anti-obesity, anti-carcinogenic, antioxidant, anti-arterisclerotic and antibacterial activity associated with the high contents of catechins, flavanols (Zaveri *et al*., 2006; Cabrera *et al*., 2006; Zhu *et al*., 2016; Ng *et al*., 2018;Yan *et al*., 2020; Liu *et al*., 2022). Results of molecular docking analysis carried out by Badeji (2026) revealed Petunidin, Cyanidin, and Epigallocatechin as potent compounds with high affinity for relieve of neurodegenerative diseases (Pakinson's disease). In Nigeria, they are sold in supermarkets or shops. The tea plant has been reported to have good inhibitory effects against pathogenic bacteria such as *E. coli, Salmonella typhimurium, Enterobacter faecalis* and *Staphylococcus aureus* (Cushnie & Lamb, 2005; Liu *et al*., 2022).

Ginger (*Zingiber officinale* var rubrum) belongs to the family Zingiberaceae. It originated from the tropical rainforests of the Indian subcontinent to Southern Asia (McGee, 2004). Ginger oil contains sesquiterpenes (which include bisabolene, zingiberol and zingiberene), vanilloids, flavonoids, diterpenes and monoterpenes believed to be responsible for its medicinal properties (Zhang *et al*., 2022). Medicinal properties of ginger include: anti-cancer, anticholinergic, antioxidant, anti-inflammatory and rheumatologic properties, blood circulation and anti-cramp effects, cholesterol regulation and hypotensive properties, analgesic and antimicrobial effects (Zhang *et al*., 2022). Ginger inhibits the growth of *Escherichia coli*, *Proteus* sp, *Staphylococci*, *Streptococci* and *Salmonella* (White, 2007; Philip *et al*., 2009).

Syzygium aromaticum, also known as clove, is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku Islands in Indonesia but has recently been farmed in different places worldwide (Cortes-Rojas *et al*., 2014; Batiha *et al*., 2019). *S. aromaticum* is rich in many phytochemicals, such as sesquiterpenes, monoterpenes, hydrocarbons, and phenolic compounds (Batiha *et al*., 2020). Several reports documented the analgesic, antioxidant, anticancer, antiseptic, anti-depressant, antispasmodic, anti-inflammatory, antiviral, antifungal, and antibacterial activity of eugenol against several pathogenic bacteria, including methicillin-resistant *Staphylococcus epidermidis* and *S. aureus* (Batiha *et al*., 2020). Joshi *et al*. (2011) found that *S. aromaticum* was the most effective against *Salmonella typhi*. Jirovetz *et al*. (2006) showed that the flower bud extract of *S. aromaticum* has antibacterial efficacy toward *Bacillus* and *Serratia marcescens*. In addition, Oulkheir *et al*. (2017) found that the clove essential oil produced an inhibition zone of 16 mm against *E. coli* and a higher inhibitory zone of 20 mm against *Salmonella* species, while it had no antibacterial effect on *Klebsiella pneumoniae* (Han and Parker, 2017).

In northern Nigeria, a traditional beverage combining green and black tea with ginger and clove is widely consumed for general wellness, though prepared arbitrarily without scientific validation. Given the recognized pharmacological properties of these plants, this study introduces standardized polyherbal formulations to evaluate its phytochemical composition, antibacterial potential, and modulatory effect on the microbiota of selected sections of gastrointestinal tract (i.e., the caecum, small intestine, large intestine and duodenum) of albino rats.

MATERIALS AND METHODS

Plant Materials

Dried leaves of black and green tea (*Camellia sinensis*), dried rhizomes of ginger (*Zingiber officinale)* and dried clove (*Syzygium aromaticum*) buds were purchased from the traders in Kano State, Nigeria and shipped to Akanu Ibiam Federal Polytechnic, Unwana, Afikpo Ebonyi State, Nigeria. They were screened for impurities and then ground separately into powder using a mechanical blender and stored in different airtight glass bottles for further studies.

Formulation and Bagging of Plant Materials

One hundred grams (100 grams) each of the different formulations containing adequately mixed dried and ground plant materials were processed into 40 tea bags and heat sealed such that each tea bag contains 2.5g of green tea, black tea, ginger rhizome and clove buds in the following percentage composition (Table 1).

Table 1: Percentage Composition of Plants Materials

Formulation (F)	Percentage Composition of Plant Materials			
	Green tea	**Black tea**	**Ginger**	**Clove**
F1	40	40	15	5
F2	55	25	15	5
F3	25	55	15	5
F4	80	0	15	5
F5	0	80	15	5
F6	50	50	0	0

Experimental Animals

Thirty (30) healthy male (8 to 10 weeks old) Albino Wistar rats were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and used as the animal model for this study. The animals were housed in a controlled environment with standard conditions of temperature, humidity and light-dark cycles. The animals were allowed free access to feed and water *ad libitum.* They were allowed to acclimatize to the environment for 7 days before the commencement of treatment.

Extraction of Infusion

A tea bag was randomly selected from individual formulations and placed in 100 ml of boiling drinking water at 100 °C, and allowed to extract with occasional stirring for about 15 minutes. The resulting infusion was allowed to cool to room temperature before being used for analysis.

Administration of Tea Infusion

Exactly 0.3ml of water (for Group A) and 0.3ml of the cooled infusion obtained from one tea bag were orally administered to each rat using a steel cannula for 21 days.

Experimental Design

This study was conducted by randomizing 30 male albino rats into 10 groups of 3 animals each. The rats were randomly assigned to different treatment groups according to the experimental design below:

Group A: 3 albino rats orally administered with water for 21 days

Group B: 3 albino rats orally administered with an infusion of Formulation 1 for 21 days

Group C: 3 albino rats orally administered with an infusion of formulation 2 for 21 days

Group D: 3 albino rats orally administered with an infusion of Formulation 3 for 21 days

Group E: 3 albino rats orally administered with an infusion of Formulation 4 for 21 days

Group F: 3 albino rats orally administered with an infusion of Formulation 5 for 21 days

Group G: 3 albino rats orally administered with an infusion of Formulation 6 for 21 days

Group H: 3 albino rats orally administered with an infusion of Formulation 1+Sucrose for 21 days

Group I: 3 albino rats orally administered with an infusion of Formulation 1+ Honey for 21 days

Group J: 3 albino rats orally administered with an infusion of commercially available tea for 21 days.

HPLC Analysis of Infusions

The quantification of phytochemical constituents of the polyherbal tea infusions was performed using a reverse-phase high-performance liquid chromatography (RP-HPLC) method. Infusions were prepared by steeping standardized tea bags in boiling water (100 °C) for 10 minutes. Phytochemical constituents were extracted using an acetonitrile–methanol (1:1, v/v) binary solvent system and separated on a µBondapak C18 column (100 × 4.6 mm, 10 µm) under gradient elution conditions using acetonitrile and ultra-pure water as the mobile phase at a flow rate of 1.0 mL/min. Detection was performed using a diode array detector (DAD) set at 254 nm. Compounds quantification was determined by external standard calibration, comparing sample peak areas against certified reference standard chromatograms (Mathivha *et al*., 2020).

Microbiological Analysis

Determination of Antibacterial Activity

Antibacterial activity of the polyherbal tea infusions were assessed against *Escherichia coli* and *Staphylococcus aureus* using the disk diffusion method (Clinical and Laboratory Standard Institute (CLSI), 2020). A standardized inoculum equivalent to 0.5 McFarland standards (1.5×10^8 cfu/ml) was prepared from the bacterial culture by choosing well-isolated colonies of the test bacteria. Each sterile Petri dish was prepared with about 25 ml of Mueller-Hinton agar. After solidifying, a sterile swab stick dipped into the inoculum of the test bacteria was used to rub the entire surface of the agar. A sterile filter paper disc (6 mm) immersed in test infusion (25 mg/ml) was placed on the plates and incubated at 37 °C for 18-24 hours. Ciprofloxacin (5 µg) was used as a positive control. After incubation, the diameter of the inhibition zone (DIZ) was observed and measured in millimetres (mm). The experiment was performed in triplicate.

Collection and Analysis of Biological Material

After 21 days of treatment, the rats were slaughtered, and the GIT sections of interest were harvested. The animal slaughter was carried out in accordance with Council Regulation (EC) No: 1099/2009 of September 2009 on the protection of animals at the time of killing (Council Regulation (EC) No: 1099/2009, 2009). Exactly 10 g of the GIT sections (i.e., caecum, small intestine, large intestine and duodenum) were weighed out and placed in beakers containing 90 ml Ringer's solution. They were homogenized with the use of a mortar and pestle. The homogenized samples were returned to the sterile beakers and shaken for 5 minutes, and left to settle for about 15 minutes. Then, a ten-fold serial dilution was prepared. One millilitre of each of the intestinal contents in the beaker was deposited into 9 ml of the Ringer's solution and shaken vigorously. Exactly, 0.1 ml from the 6^{th} dilution was inoculated on the prepared Petri dishes containing the microbiological medium using the spread plate method. The following were determined:

i. The total number of mesophilic aerobic bacteria on Nutrient agar medium for 18-24 hours at 37°C.
ii. The total number of fungi on Sabouraud dextrose agar, for 3-5 days at 28±2°C.
iii. The total number of *E. coli* on MacConkey agar for 18-24 hours at 37 °C.
iv. The presence of *Salmonella* on Salmonella-Shigella agar for 18-24 hours at 37°C.

Each sample was plated in triplicate. After incubation, the colonies were counted using a digital colony counter. The number of each morphological type was determined and expressed as log colony-forming units per gram of intestinal contents [log CFU/g contents] (Wlazlo *et al*., 2021).
Ethical clearance was obtained from our Institutions Ethical Committee for all tests on animals.

Statistical Analysis
The diameter of the inhibition zone was presented as Mean ± Standard error of the mean (Mean ± SE) of three replicates. The total aerobic microbial count and total mould and yeast counts were presented as Mean ± Standard deviation (Mean ± SD) of three replicates. The results were analyzed using a one-way Analysis of Variance (ANOVA) and a Post-Hoc Tukey HSD. Values were considered significant at $p< 0.05$.

RESULTS AND DISCUSSION

Phytochemical Analysis
High-performance liquid chromatography analysis carried out to identify and quantify the phytochemical compounds present in the polyherbal infusions revealed the presence of varying compounds (Table 2). Quercetin was the most abundant polyphenolic compound in the ratio F5>F3>F6>F2>F4>F1 with the values 3.82 mg/L > 3.28 mg/L>2.90 mg/L > 2.59 mg/L> 2.34 mg/L>2.31 mg/L respectively; followed by kaempferol in the ratio F5>F3>F6>F2>F1>F4 with values 1.53 mg/L> 1.29 mg/L>0.88 mg/L>0.76 mg/L>0.56 mg/L>0.03 mg/L respectively. Gallic acid was found to be more abundant in F5 (1.00 mg/L), followed by F6 (0.90 mg/L) and F3 (0.87 mg/L). Chlorogenic acid and apigenin (a natural flavonoid) was more abundant in F5 with 0.44 mg/L and 1.24 mg/L respectively. Catechin concentration in F3 (0.14 mg/L) was found to be higher compared to other formulations. Alkaloids such as theophylline, and theogallin was detected in formulations F1, F4, F5 and F6 while, caffeine was present in all formulations.

Table 2: Phytochemical Constituents of Infusions of Polyherbal Tea Formulated from Green and Black Tea, Ginger Rhizome and Clove Bud

Identified compounds	Concentration (mg/L)					
	F1	F2	F3	F4	F5	F6
Chlorogenic acid	0.20	0.16	0.38	0.14	0.44	0.40
Gallic acid	0.40	0.35	0.87	0.21	1.00	0.90
Caffeine	0.15	0.23	0.28	0.10	0.22	0.22
Catechin	0.03	0.10	0.14	0.05	0.11	0.07
Epicatechin	0.05	0.06	0.09	0.06	0.07	0.03
Epicatechin gallate	0.03	0.02	0.03	0.03	0.02	0.03
Epigallocatechin	0.05	0.04	0.06	0.05	0.03	0.04
Epigallocatechin gallate	0.04	0.02	0.04	0.04	0.04	0.03
Coumarrylquinic acid	0.03	0.03	0.04	-	0.04	-
Quercetin	2.31	2.59	3.28	2.34	3.82	2.90
Kaempferol	0.56	0.76	1.29	0.03	1.53	0.88
Apigenin	0.15	0.30	1.11	0.12	1.24	0.31
Myricetin	0.12	0.15	0.04	0.03	0.03	0.07
Luteolin	0.02	0.11	0.10	0.03	0.18	0.61
Chrysin	0.02	0.17	-	0.03	0.02	0.04
Tricin	0.16	-	-	0.07	0.04	0.04
Theophylline	0.03	-	-	0.08	0.03	0.02
Theogallin	0.02	-	-	0.02	0.03	0.02

Key: F1 to F6 are Different Formulations of the Polyherbal Tea

Antibacterial Activity
In this study, we evaluated the antibacterial activity of six polyherbal infusions designated F1-F6 (i.e. Formulations F1-F6) based on the composition and concentration of green tea, black tea, ginger and clove as stated in Table 1. The analyzed polyherbal infusions demonstrated a stronger antibacterial efficacy against Gram-negative bacteria (*E. coli*) more than Gram-positive bacteria (*S. aureus*) used in the study (Table 3). The highest antibacterial activity was recorded against *E. coli* by F5 (19.33 ± 0.88 mm), followed by F4 (15.67 ± 1.20 mm), F6 (12.00 ± 3.06 mm), F2 (11.00 ± 2.08 mm), and F3 (11.00 ± 4.51 mm), while the least was F1 (10.67 ± 2.33 mm). The antibacterial activity of F5 against *E. coli* was significantly higher ($p<0.05$) than F1, F2 and F3. Against *S. aureus*, F2 had the highest antibacterial activity of 9.67 ± 0.33 mm, followed by F6 and F5 with 8.67 ± 0.67 mm and 8.33 ± 0.88 mm, respectively. Formulation 2 (F2) exhibited a statistically significant ($p<0.05$) antibacterial activity against *S. aureus* compared to F1 and F4.

Table 3: Antibacterial Activity of Polyherbal Tea Formulated from Green and Black Tea, Ginger Rhizome and Clove Bud

Tested polyherbal tea formulations	Concentration (mg/ml)	Diameter of inhibition zone expressed as Mean± Standard error of mean (mm)	
		Escherichia coli	*Staphylococcus aureus*
F1	25	10.67 ± 2.33^{b}	6.67 ± 0.67^{b}
F2	25	11.00 ± 2.08^{b}	9.67 ± 0.33^{a}
F3	25	11.00 ± 4.51^{b}	7.67 ± 1.20^{ab}
F4	25	15.67 ± 1.20^{ab}	7.00 ± 0.58^{b}
F5	25	19.33 ± 0.88^{a}	8.33 ± 0.88^{ab}
F6	25	12.00 ± 3.06^{ab}	8.67 ± 0.67^{ab}

Columns with same letter do not differ significantly (Tukey HSD, α= 0.05). Key: mm= Millimetres

Microbial Count of Sections of the Gastrointestinal Tract

In the caecum, a statistically significant increase in the number of mesophilic aerobic bacteria was observed only in the contents of group B (F1) relative to the control group (group A) that received only water for 21 days. Groups G (F6), H (F1 + Sugar), I (F1+Honey) and J (F1+Lipton tea) recorded counts too numerous to count (TNTC), indicating a higher microbial load that could not be quantified (Table 4). In the small intestine, the number of mesophilic aerobic bacteria in group D (F3) was significantly lower ($p<0.05$) than that of the control group (group J), treated with commercially available tea. The control group treated with water (group A) did not vary significantly from groups B to G, treated with infusions from F1 to F6, respectively. Similar to our result in the caecum, a higher microbial load, too numerous to count, was recorded in groups I and J of the large intestine. In the duodenum, a highly significant ($p<0.05$) increase in bacteria count was observed in groups C (F2), D (F3), E (F4), F (F5), G (F6), group H (F1 + Sugar) and I (F1+Honey) relative to the control group (group A) treated only with water. Similarly, the control group J (F1+Lipton tea) was found to be significantly higher than control group A. *Escherichia coli* were detected only in the water control group (group A) of the caecum (Table 5). *Salmonella* sp were absent in the experimental and control groups analyzed.

Table 4: Total Aerobic Microbial Count (TAMC) in Each Section of the GIT of Albino Rat (Log cfu/g)

Formulation	Group	Number of mesophilic aerobic bacteria (Mean ± Standard deviation)			
		Caecum	Small Intestine	Large Intestine	Duodenum
F1	B	7.67 ± 0.13^{a}	7.49 ± 0.91^{ab}	6.54 ± 0.34^{ab}	6.69 ± 0.13^{bc}
F2	C	6.83 ± 0.75^{ab}	7.40 ± 0.19^{ab}	7.13 ± 0.18^{ab}	7.61 ± 0.25^{a}
F3	D	7.38 ± 0.48^{ab}	6.65 ± 0.07^{b}	7.65 ± 0.21^{a}	7.15 ± 0.02^{ab}
F4	E	7.55 ± 0.35^{ab}	7.78 ± 0.78^{ab}	7.25 ± 0.89^{ab}	7.73 ± 0.28^{a}
F5	F	6.73 ± 0.18^{ab}	6.96 ± 0.68^{ab}	6.50 ± 0.28^{b}	7.38 ± 0.32^{a}
F6	G	TNTC	7.22 ± 0.52^{ab}	7.52 ± 0.06^{ab}	7.55 ± 0.13^{a}
	H	TNTC	7.39 ± 0.30^{ab}	7.05 ± 0.14^{ab}	7.12 ± 0.05^{ab}
	I	TNTC	7.23 ± 0.54^{ab}	TNTC	7.12 ± 0.31^{ab}
	A(Ctrl)	6.45 ± 0.64^{b}	6.63 ± 0.21^{b}	7.12 ± 0.48^{ab}	6.39 ± 0.13^{c}
	J(Ctrl)	TNTC	8.16 ± 0.31^{a}	TNTC	7.63 ± 0.40^{a}
F-value		3.106	2.955	3.071	3.457
P-value		0.014	0.026	0.028	0.0001

Groups with same letter are not significantly different (Tukey HSD, α =0.05).

Key:

Group A: Albino rats orally administered with 0.3mls of water for 21 days

Group B: Albino rats orally administered with 0.3mls of the first test infusion for 21 days

Group C: Albino rats orally administered with 0.3mls of the second test brew for 21 days

Group D: Albino rats orally administered with 0.3mls of Formulation 3 infusion for 21 days

Group E: Albino rats orally administered with 0.3mls of Formulation 4 infusion for 21 days

Group F: Albino rats orally administered with 0.3mls of Formulation 5 infusion for 21 days

Group G: Albino rats orally administered with 0.3mls of Formulation 6 infusion for 21 days

Group H: Albino rats orally administered with 0.3mls of Formulation 1 + Sugar for 21 days

Group I: Albino rats orally administered with 0.3mls of Formulation 1 + Honey for 21 days

Group J: Albino rats orally administered with 0.3mls of Lipton tea infusion for 21 days

Ctrl = Control

GIT = Gastrointestinal tract.

TNTC = Too numerous to count.

Table 5: Total *Escherichia coli* Count (Log cfu/g)

Formulation	Group	Number of *Escherichia coli* count (Mean ± Standard deviation)			
		Caecum	Small Intestine	Large Intestine	Duodenum
F1	B	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F2	C	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F3	D	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F4	E	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F5	F	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F6	G	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	H	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	I	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	A(Ctrl)	6.05±0.13	0.00±0.00	0.00±0.00	0.00±0.00
	J(Ctrl)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

There was no significant difference observed in the fungal count of the caecum and large intestine in all the groups (Table 6). The small intestine recorded a significantly higher fungal count in group E (F4) relative to groups C (F2), G (F6) and the control group J. In the duodenum, group B (F1) fungal count was significantly higher ($p<0.05$) than that of groups C (F2), D (F3), F (F5) and the control group J.

Table 6: Total Yeast and Mould Count (TYMC) in Each Section of the GIT of Albino Rat (Log cfu/g)

Formulation	Group	Number of mesophilic aerobic fungi (Mean ± Standard deviation)			
		Caecum	Small Intestine	Large Intestine	Duodenum
F1	B	7.10±0.78	$7.06±0.28^{ab}$	6.74±0.37	$7.36±0.14^{a}$
F2	C	6.45±0.64	$6.60±0.04^{b}$	6.24±0.34	$6.65±0.07^{b}$
F3	D	6.87±0.45	$6.78±0.05^{ab}$	6.41±0.53	$6.65±0.07^{b}$
F4	E	6.39±0.13	$7.41±0.53^{a}$	6.76±0.40	$6.84±0.08^{ab}$
F5	F	7.34±0.20	$6.69±0.13^{ab}$	7.18±0.56	$6.50±0.28^{b}$
F6	G	6.72±0.33	$6.60±0.03^{b}$	6.50±0.28	$6.80±0.28^{ab}$
	H	6.74±0.06	$6.88±0.45^{ab}$	6.84±0.85	$6.97±0.16^{ab}$
	I	6.98±0.54	$7.41±0.18^{a}$	7.13±0.25	$6.87±0.12^{ab}$
	A(Crtl)	7.12±0.31	$7.12±0.05^{ab}$	7.06±0.78	$6.88±0.26^{ab}$
	J(Ctrl)	6.95±0.07	$6.60±0.42^{b}$	6.63±0.21	$6.54±0.34^{b}$
F-value		1.684	3.860	1.171	4.471
P-value		0.170	0.006	0.360	0.003

Groups with Same Letter are not Significantly Different (Tukey HSD, α =0.05)
Key:
Group A: Albino rats orally administered with 0.3mls of water for 21 days
Group B: Albino rats orally administered with 0.3mls of the first test infusion for 21 days
Group C: Albino rats orally administered with 0.3mls of the second test brew for 21 days
Group D: Albino rats orally administered with 0.3mls of Formulation 3 infusion for 21 days
Group E: Albino rats orally administered with 0.3mls of Formulation 4 infusion for 21 days
Group F: Albino rats orally administered with 0.3mls of Formulation 5 infusion for 21 days
Group G: Albino rats orally administered with 0.3mls of Formulation 6 infusion for 21 days
Group H: Albino rats orally administered with 0.3mls of Formulation 1 + Sugar for 21 days
Group I: Albino rats orally administered with 0.3mls of Formulation 1 + Honey for 21 days
Group J: Albino rats orally administered with 0.3mls of Lipton tea infusion for 21 days
Ctrl = Control
GIT = Gastrointestinal tract.

Discussion

Herbal teas consumed as functional beverages are gaining more importance due to the problem of antibiotic resistance and their rich source of natural bioactive compounds such as flavonoids, alkaloids, carotenoids, coumarins, polyacetylenes and terpenoids (Chandrasekara and Shahidi, 2018). Consumption of herbal infusions is beneficial to health due to their antioxidant, anti-inflammatory, and antibacterial properties (Liu *et al*., 2022; Nowak *et al*., 2025).

High performance liquid chromatography detection and quantitative analysis of infusions from polyherbal tea (F1 – F6) shows the presence and concentration of various phenolic acids, flavonoids, catechins, and alkaloids in the infusions. Phenolic acids such as chlorogenic acid and gallic acid were detected in all formulations, with relatively higher concentrations observed in F3, F5, and F6. Gallic acid was found to be more abundant, reaching the highest level in F5 (1.00 mg/L), suggesting a contribution from *Camellia sinensis* and *Syzygium aromaticum*, both known sources of this compound (Zhang *et al*., 2019; Batiha *et al*., 2020; Liu *et al*., 2022). These phenolic acids are recognized for their antioxidant and antimicrobial properties and may have contributed significantly to the antibacterial activity observed in this study (Liu *et al*., 2022. Catechins and their derivatives, including catechin, epicatechin, epigallocatechin, and epigallocatechin gallate, were present in varying amounts across all formulations. These compounds are characteristic bioactive constituents of *Camellia sinensis* and are known to exert antimicrobial effects (Friedman, 2007; Sharangi, 2009; Zhang *et al*., 2019; Yan *et al*., 2020; Liu *et al*., 2022). Formulation F5 showed higher levels of catechins, which may partly explain their stronger antibacterial activity against *E. coli*. Quercetin was the most abundant flavonoid identified in the infusions with the highest concentration recorded in F5 (3.82 mg/L), followed by F3 (3.28 mg/L). Researchers found Quercetin and Kaempferol as the two major flavonoids

abundantly present in plants (Rahmatullah *et al*., 2022). This assertion was confirmed in this study. The high levels of quercetin and other flavonoids are significant, as these compounds are known to possess strong antioxidant, anti-inflammatory, antibacterial activities among other health benefits (Foster *et al*., 2016; Guo *et al*., 2016; Chen *et al*. (2019; Tang *et al*., 2019; Zhou *et al*., 2020; Yan *et al*., 2020; Liu *et al*., 2022; Nowak *et al*., 2025). The higher flavonoid content observed in F5 further supports its enhanced antibacterial activity against *E. coli*.

The results of the antibacterial activity revealed that all the polyherbal infusions exhibited measurable antibacterial activity against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*), indicating that the polyherbal infusions possess broad-spectrum antibacterial activity. These results are consistent with earlier reports on similar studies carried out by Akanksha *et al*. (2022), Liu *et al*. (2022), Sarkar *et al*. (2023), Dincer & Baglam (2023), Harfoush *et al*. (2024) and Norwak *et al*. (2025). This broad-spectrum activity may be attributed to the effects of bioactive compounds such as the flavonoids, quercetin and kaempferol found in the polyherbal tea (Rahmatullah *et al*., 2022; Dincer & Baglam, 2023). Yang & Zhang (2019) reported that flavonoids (especially EGCG) exhibit its effectiveness by damaging the liposome membrane of *E. coli* and *S. aureus.*

Formulation F5 showed a statistically significant ($p<0.05$) higher antibacterial activity against *E. coli* relative to F1, F2 and F3, with a mean inhibition zone diameter of 19.33 ± 0.88 mm, followed by F4 (15.67 ± 1.20 mm). The higher susceptibility of *E. coli* to specific formulations suggests that the formulation possesses the highest bioactive secondary metabolites, which it uses to disrupt the integrity of the lipopolysaccharide outer membrane characteristic of Gram-negative species. Notably, F5 that demonstrated the highest antibacterial activity had the highest polyphenols content, which suggests that the polyphenols content contributed more to antibacterial activity of the polyherbal infusions. In contrast, *S. aureus* exhibited a comparatively lower inhibition zone diameter across all formulations, with values ranging from 6.67 ± 0.67 mm (F1) to 9.67 ± 0.33 mm (F2). However, a statistically significant ($p<0.05$) antibacterial activity was recorded with F2 relative to F1 and F4. The relatively lower sensitivity of *S. aureus* may be attributed to its thick peptidoglycan cell wall, and are less susceptible to the effects of polyphenols present in the polyherbal infusions. In contrast to the present findings, previous studies have demonstrated that plant extracts exhibit greater antibacterial activity against Gram positive bacteria than Gram negative bacteria (Hemeg *et al*., 2020; Liu *et al*., 2022; Sarker *et al*., 2023; Nowark *et al*., 2025). We hypothesized that the variation is attributable to differences in the microbial strain as well as the proportion or synergistic interaction of the bioactive compounds present in the tea formulations. In addition, it is noteworthy that most of the observations were based on mono- than polyherbal formulations assessed in this study.

The study on microbiota of gastrointestinal tract of the albino rats showed that the polyherbal infusion could modulate the gut microbiota of rats. The result from this study agrees with Bakari *et al*. (2017) that tea infusion can have an effect on gut microbiota. A statistically significant increase in the number of mesophilic aerobic bacteria was found in the caecum of group B treated with infusion from F1 relative to the control (group A) that was given water for 21 days. This suggests that the infusion may have bacteria growth promotion potential in the rats. The TNTC values recorded with Groups G (F6), H (F1 + Sugar), I (F1+Honey) and J (F1+commercial Lipton tea) under the caecum, groups I and J under the large intestine and the overall highest microbial density in group J under the small intestine suggest that the sweeteners can promote microbial proliferation rather than suppress them. However, the caecum and large intestine are anatomically known to harbour the highest microbial densities up to 10^{12} cfu/g of faeces in mammalian gut (Wlazlo *et al*., 2021). The TNTC microbial densities observed in groups H and I may be a result of the prebiotic properties of sugar and honey supplementation. The duodenum recorded a highly significant increase in bacteria count in groups C (F2), D (F3), E (F4), F (F5), G (F6), group H (F1 + Sugar) and I (F1+Honey) relative to the control group (group A) treated only with water. According to the reports of Wlazlo *et al*. (2021), the type and number of microorganisms in the GIT depend on the section of the GIT. The stomach and superior part of the duodenum, which is the first section, is dominated by *Lactobacillus* sp and *Enterococci* sp due to acidic pH, presence of bile, shorter transit time and limited mucus production in the sections. *Lactobacillus* sp and *Enterococci* sp are probiotic bacteria important in maintaining gut health, inhibiting pathogenic bacteria and improving immunity (Wlazlo *et al*., 2021; Zhao *et al*., 2024). This suggests that the polyherbal infusion has the potential to enhance the growth of probiotic bacteria in the gut of albino rats. Similar to the present study, Perez-Burillo *et al*. (2021) reported an increase in gut bacteria growth in rats treated with green tea. Also, Emmanuel *et al*. (2023) reported a statistically significant increase in bacterial plate count in treatment groups after 21 days of treatment with green tea.

The study found no significant difference in fungal counts of the caecum and large intestine in all the groups. However, in the duodenum, group B (F1) fungal count was significantly higher than that of groups C (F2), D (F3), F (F5) and the control group J supplemented with commercial Lipton tea. The small intestine recorded a significantly higher fungal count in group E (F4) relative to groups C (F2), G (F6) and the control group J.

Escherichia coli were detected only in the water control group (group A) of the caecum, while *Salmonella* sp were absent in the experimental and control groups analyzed.

CONCLUSION

HPLC detection and quantitative analysis of infusions from polyherbal tea showed the presence and concentration of various phenolic acids, flavonoids, catechins, and alkaloids in the infusions. Quercetin was the most abundant polyphenol, followed by kaempferol. This study reported that all the polyherbal infusions exhibited measurable antibacterial activity against Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus,* indicating that the polyherbal infusions possesses broad-spectrum antibacterial activity. The gut bacteria count of the rats treated with infusions from the polyherbal tea increased significantly in the duodenum, known to harbour microbes such as *Lactobacillus* sp and *Enterococci* sp. *Escherichia coli* were detected only in the water control group (group A) of the caecum while *Salmonella* sp were absent in the experimental and control groups analyzed. Findings show that consumption of polyherbal tea has the potential to regulate and stimulate beneficial microbiota of the GIT of rats, which can inhibit the growth of pathogenic microbes and improve gut health.

ETHICAL APPROVAL

Ethical approval was sought and approved by the Akanu Ibiam Federal Polytechnic, Unwana, Nigeria Research Ethics Committee. The animal slaughter was carried out in accordance with Council Regulation (EC) No: 1099/2009 of

September 2009 on the protection of animals at the time of killing (Council Regulation (EC) No: 1099/2009, 2009).

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