

Antibacterial Activity and Gas Chromatography-Mass Spectrometry Analysis of Fractionated Ethanolic Extract of *Psidium guajava* Leaf against *Escherichia coli* and *Salmonella* Isolated from Patients with Gastrointestinal Tract Infection in Kano State

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Abstract

Gastrointestinal tract (GIT) infections remain major public health concerns, particularly in developing countries where antimicrobial resistance has reduced the effectiveness of conventional antibiotics. This study investigated the antibacterial activity and Gas Chromatography–Mass Spectrometry (GC–MS) profile of fractionated ethanolic leaf extracts of *Psidium guajava* against clinical isolates of *Escherichia coli* and *Salmonella* obtained from patients with gastrointestinal tract infections in Kano State, Nigeria. The antibacterial activity of each fraction was evaluated by the agar well diffusion method, while the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth dilution method. The most active fraction was subjected to GC–MS analysis for the identification of bioactive compounds. The ethyl acetate fraction demonstrated the highest antibacterial activity against both *E. coli* and *Salmonella*, producing significantly larger zones of inhibition than the other solvent fractions ($p < 0.05$). The antibacterial activity increased with increasing extract concentration. The MIC values of the most active fraction were 6.25 mg/mL against *E. coli* and 12.50 mg/mL against *Salmonella*, while the corresponding MBC values were 12.50 mg/mL and 25.00 mg/mL, respectively. GC–MS analysis identified several bioactive constituents, including phytol, caryophyllene oxide, *n*-hexadecanoic acid, squalene, 2,4-di-*tert*-butylphenol, 1-docosene, and 1-octadecene, compounds previously reported to possess antibacterial, antioxidant, and anti-inflammatory activities. It is concluded that the fractionated ethanolic leaf extracts of *Psidium guajava*, particularly the ethyl acetate fraction, possess significant antibacterial activity against clinical isolates of *Escherichia coli* and *Salmonella*.

Keywords: Antibacterial Activity, *Escherichia Coli*, Chromatography–Mass Spectrometry, Phytochemicals, *Psidium Guajava*, *Salmonella*.

INTRODUCTION

Gastrointestinal tract (GIT) infections remain one of the leading causes of morbidity and mortality worldwide, particularly in developing countries where poor sanitation, inadequate access to clean water, and inappropriate food handling practices contribute significantly to disease transmission. These infections are commonly caused by bacteria, viruses, fungi, and parasites, with bacterial pathogens accounting for a substantial proportion of acute diarrhoeal diseases. Among the most frequently implicated bacterial pathogens are *Escherichia coli* and *Salmonella* species, which are responsible for millions of cases of

gastroenteritis annually. These organisms cause symptoms ranging from mild diarrhoea and abdominal discomfort to severe dehydration, septicemia, and death, particularly among children, the elderly, and immunocompromised individuals (Hall *et al.*, 2023).

In Nigeria, gastrointestinal infections continue to constitute a major public health challenge due to rapid urbanization, poor environmental hygiene, contaminated food and water, and increasing antimicrobial resistance among enteric pathogens. Kano State, one of the most populous states in Nigeria, experiences a high burden of food- and water-borne diseases, making the search for alternative antimicrobial

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agents particularly important (Ali *et al.*, 2024a). The increasing prevalence of multidrug-resistant strains of *E. coli* and *Salmonella* has reduced the effectiveness of commonly prescribed antibiotics, leading to prolonged illness, increased healthcare costs, and higher mortality rates. Consequently, there is an urgent need to identify novel antimicrobial compounds from medicinal plants that can complement or replace conventional antibiotics (Gideon, 2032).

Medicinal plants have served as an important source of therapeutic agents for centuries. According to the World Health Organization, approximately 80% of the population in developing countries relies on traditional herbal medicine for primary healthcare (Silva and Fernandes-Junior, 2010). Plant-derived bioactive compounds such as flavonoids, tannins, alkaloids, terpenoids, phenolic acids, and saponins possess antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties that contribute to disease prevention and treatment (Rios and Recio, 2009). Scientific interest in medicinal plants has increased considerably because of their accessibility, affordability, relatively low toxicity, and ability to provide structurally diverse compounds with antimicrobial activity (Hemeg *et al.*, 2023).

Psidium guajava L. (guava), a member of the family Myrtaceae, is widely distributed throughout tropical and subtropical regions, including Nigeria. Beyond its nutritional value, different parts of the plant including leaves, bark, fruits, and roots have long been used in traditional medicine for the treatment of diarrhoea, dysentery, gastrointestinal disorders, wounds, diabetes, respiratory infections, and inflammatory diseases (Newman and Craigg, 2020). Guava leaves are particularly rich in phytochemicals such as quercetin, guaijaverin, catechin, gallic acid, ellagic acid, tannins, essential oils, and other phenolic compounds that have demonstrated antimicrobial and antioxidant activities against numerous pathogenic microorganisms (Gutiérrez *et al.*, 2008).

Several experimental studies have demonstrated that ethanolic extracts of *P. guajava* leaves possess inhibitory activity against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Shigella* species, and *Vibrio cholerae* (Olosunde *et al.*, 2022). Ethanol is regarded as one of the most effective extraction solvents because it dissolves a broad spectrum of bioactive phytochemicals. Fractionation of crude ethanolic extracts further separates compounds according to polarity, thereby concentrating active constituents and enhancing antibacterial activity. Consequently, fractionated extracts often exhibit greater antimicrobial efficacy than crude extracts (Metougui *et al.*, 2023).

Gas Chromatography–Mass Spectrometry (GC–MS) has become an indispensable analytical technique for identifying volatile and semi-volatile bioactive compounds in medicinal

plants. GC–MS enables accurate characterization of phytochemical constituents by separating complex mixtures and identifying compounds based on their mass spectra. The identification of bioactive molecules through GC–MS provides scientific evidence for the antimicrobial properties of medicinal plants and facilitates the discovery of potential lead compounds for pharmaceutical development (Shittu *et al.*, 2021). Recent investigations have identified numerous antibacterial constituents in *P. guajava* extracts, including fatty acids, terpenoids, phenolic derivatives, and other metabolites that contribute to antibacterial activity against enteric pathogens (Hall *et al.*, 2023).

Therefore, this study seeks to evaluate the antibacterial activity of fractionated ethanolic extracts of *Psidium guajava* leaves against clinical isolates of *Escherichia coli* and *Salmonella* recovered from patients with gastrointestinal tract infections in Kano State and to identify the bioactive compounds responsible for the observed antibacterial effects using Gas Chromatography–Mass Spectrometry (GC–MS).

MATERIALS AND METHODS

Study Area

The study was conducted in Kano State, Nigeria. Clinical isolates were obtained from selected hospitals within the state. Laboratory analyses, including extraction, fractionation, antibacterial assays, and GC–MS analysis, were carried out in Microbiology and analytical Chemistry laboratory of Bayero University Kano.

Collection and Identification of Plant Materials

Fresh, healthy leaves of *Psidium guajava* were collected from botanical garden of Bayero University, Kano State. The plant was authenticated and identified in the Herbarium, Department of Plant Biology, Bayero University Kano with voucher reference number BUKHAN 0362, and a voucher specimen was deposited in the departmental herbarium. The leaves were washed with distilled water, air-dried at room temperature for two weeks, and ground into fine powder using a sterile electric grinder (Ali *et al.*, 2025)

Collection and Identification of Clinical Isolates

Clinical isolates of *Escherichia coli* and *Salmonella* were obtained from stool specimens of patients diagnosed with gastrointestinal tract infections. Preliminary identification was carried out using colony morphology, Gram staining, and biochemical tests, while confirmation was performed using standard microbiological techniques as described by Cheesbrough, (2012).

Preparation of Ethanolic Extract

Approximately 500 g of powdered guava leaves were soaked in 1000 mL of 90% ethanol for 72 hours with intermittent shaking. The mixture was filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated

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under reduced pressure using a rotary evaporator at temperatures below 45°C, after which the concentrated extract was further dried in a water bath to obtain the crude ethanolic extract (Muazu *et al.*, 2024).

Fractionation of the Crude Extract

The crude ethanolic extract was suspended in distilled water and successively partitioned using solvents of increasing polarity, including n-hexane, chloroform, ethyl acetate, and n-butanol. Each solvent fraction was concentrated separately using a rotary evaporator and stored in sterile amber bottles at 4°C until use.

Preparation of Test Organisms

Pure colonies of *E. coli* and *Salmonella* were inoculated into nutrient broth and incubated at 37°C for 24 hours. The bacterial suspension was standardized to 0.5 McFarland turbidity (approximately 1.5×10^8 CFU/mL) before antibacterial testing (Ali *et al.*, 2024b).

Antibacterial Activity Assay

The agar well diffusion method was employed as described by Ali *et al.* (2019). Mueller Hinton agar plates were inoculated uniformly with the standardized bacterial suspension. Wells of 6 mm diameter were bored aseptically into the agar and filled with different concentrations namely 25, 50, 75, and 100 mg/mL of each solvent fraction. Ciprofloxacin (10 µg) served as the positive control, while 5% dimethyl sulfoxide (DMSO) served as the negative control. The plates were incubated at 37°C for 24 hours, after which the diameters of inhibition zones were measured in millimetres. All experiments were performed in triplicate, and mean inhibition zones were calculated.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the most active fraction was determined using the broth microdilution method. Serial two-fold dilutions of the extract were prepared in Mueller Hinton broth, inoculated with standardized bacterial suspensions, and incubated at 37°C for 24 hours. The MIC was recorded as the lowest concentration that showed no visible bacterial growth (Nas *et al.*, 2018).

Determination of Minimum Bactericidal Concentration (MBC)

Aliquots from tubes showing no visible growth during the MIC assay were sub-cultured onto fresh Mueller Hinton agar plates and incubated at 37°C for 24 hours. The lowest concentration producing no bacterial growth on agar was recorded as the Minimum Bactericidal Concentration (Nas *et al.*, 2018).

Gas Chromatography–Mass Spectrometry Analysis

The fraction exhibiting the highest antibacterial activity was

subjected to GC–MS analysis. The sample was filtered and injected into a Gas Chromatography–Mass Spectrometry system equipped with a suitable capillary column. Helium was used as the carrier gas, and electron ionization was performed at 70 eV. The chromatographic peaks were identified by comparing their mass spectra with those contained in the National Institute of Standards and Technology (NIST) spectral library. Identified compounds were recorded together with their retention times and percentage peak areas (Mazu *et al.*, 2024).

Data Analysis

Experimental data were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using SPSS version 25. One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test was used to compare antibacterial activities among extract fractions and concentrations. Differences were considered statistically significant at $p < 0.05$.

Ethical Considerations

Ethical approval was obtained from Health Service Management Board Research Ethics Committee with the following reference number NHREC/17/03/203 before commencement of the study. Permission was also obtained from participating hospitals. Patient confidentiality was maintained by using coded laboratory isolates without personal identifiers.

RESULTS

Isolation and Identification of Isolates

A total of 60 bacterial isolates were recovered from stool samples collected from patients with gastrointestinal tract infections. Of these, 35 (58.3%) were identified as *Escherichia coli* and 25 (41.7%) as *Salmonella* species based on colony morphology, Gram staining, and biochemical characteristics as shown in Table 1.

Table 1. Distribution of Clinical Isolates

Organism	Number of isolates	Percentage (%)
<i>Escherichia coli</i>	35	58.3
<i>Salmonella</i> spp.	25	41.7
Total	60	100.0

Percentage Yield of Ethanolic Extract and Solvent Fractions

The percentage yield of ethanolic extract and solvent fractions is presented in Table 2. the result showed that the crude ethanolic extraction yielded 68.4 g (13.68%) from the powdered leaves of *Psidium guajava*. Following solvent fractionation, the ethyl acetate fraction recorded the highest yield (18.7 g; 27.3%), followed by the chloroform fraction (15.2 g; 22.2%), n-hexane fraction (12.5 g; 18.3%), and n-butanol fraction (10.9 g; 15.9%).

Table 2. Percentage Yield of Ethanolic Extract and Solvent Fractions

Fraction	Weight (g)	Percentage Yield (%)
Crude ethanolic extract	68.4	13.68
n-Hexane fraction	12.5	18.3
Chloroform fraction	15.2	22.2
Ethyl acetate fraction	18.7	27.3
n-Butanol fraction	10.9	15.9

Antibacterial Activity of Fractionated Ethanolic Extract Against Escherichia coli

Table 3 shows the antibacterial activity of the different solvent fractions against *Escherichia coli* at concentrations of 25, 50, 75, and 100 mg/mL. All fractions exhibited concentration-dependent antibacterial activity, with inhibition zones increasing as extract concentration increased. Among the fractions, the ethyl acetate fraction demonstrated the greatest antibacterial activity, producing inhibition zones ranging from 12.5 ± 0.6 mm at 25 mg/mL to 25.3 ± 0.5 mm at 100 mg/mL. This was followed by the chloroform fraction (10.1–19.4 mm), n-butanol fraction (9.3–18.2 mm), and n-hexane fraction (8.2–14.0 mm). Ciprofloxacin, used as the positive control, produced the highest inhibition zone of 30.4 ± 0.3 mm, while DMSO, the negative control, produced no inhibition.

Table 3. Mean Zone of Inhibition (mm) against *E.Coli*

Extracts	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	p-value
n-Hexane	8.2 ± 0.4	10.3 ± 0.5	12.4 ± 0.6	14.0 ± 0.5	0.01357
Chloroform	10.1 ± 0.5	13.6 ± 0.7	16.9 ± 0.4	19.4 ± 0.6	
Ethyl acetate	12.5 ± 0.6	16.8 ± 0.5	21.2 ± 0.7	25.3 ± 0.5	
n-Butanol	9.3 ± 0.4	12.0 ± 0.5	15.1 ± 0.5	18.2 ± 0.6	
Ciprofloxacin	30.4 ± 0.3	30.4 ± 0.3	30.4 ± 0.3	30.4 ± 0.3	
DMSO	0.0	0.0	0.0	0.0	

Key: * = The result is statistically significant, Control = Ciprofloxacin 10 µg

Antibacterial Activity Against Salmonella Species

Table 4 presents the antibacterial activity of the solvent fractions against *Salmonella* species. Similar to the findings for *E. coli*, antibacterial activity increased with increasing extract concentration. The ethyl acetate fraction exhibited the highest inhibitory effect, with inhibition zones increasing from 11.8 ± 0.6 mm at 25 mg/mL to 24.1 ± 0.6 mm at 100 mg/mL. The chloroform fraction showed moderate activity (9.5–18.6 mm), followed by n-butanol (8.7–17.4 mm) and n-hexane (7.6–13.5 mm). Ciprofloxacin maintained the highest inhibition zone (31.2 ± 0.2 mm) across all concentrations, whereas DMSO produced no inhibition.

Table 4. Mean Zone of Inhibition (mm) against *Salmonella*

Extracts	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	p-value
n-Hexane	7.6 ± 0.5	9.8 ± 0.4	11.7 ± 0.6	13.5 ± 0.4	0.01042*
Chloroform	9.5 ± 0.5	12.8 ± 0.6	15.8 ± 0.5	18.6 ± 0.5	
Ethyl acetate	11.8 ± 0.6	15.7 ± 0.5	20.3 ± 0.4	24.1 ± 0.6	
n-Butanol	8.7 ± 0.4	11.5 ± 0.4	14.6 ± 0.5	17.4 ± 0.4	
Control	31.2 ± 0.2	31.2 ± 0.2	31.2 ± 0.2	31.2 ± 0.2	
DMSO	0.0	0.0	0.0	0.0	

Key: * = The result is statistically significant, Control = Ciprofloxacin 10 µg

Minimum Inhibitory Concentration (MIC) and MBC of the Most Active Fraction

The minimum inhibitory concentration (MIC) and MBC of the most active fraction in Table 5 showed that the MIC against *Escherichia coli* was 6.25 mg/mL, while the corresponding MBC was 12.50 mg/mL. For *Salmonella* species, the MIC and MBC were 12.50 mg/mL and 25.00 mg/mL, respectively. These findings indicate that lower concentrations of the ethyl acetate fraction were required to inhibit and kill *E. coli* than *Salmonella*, demonstrating that *E. coli* was more susceptible to the extract.

Table 5. MIC of the Most Active Fraction

Organism	MIC (mg/mL)	MBC (mg/mL)
<i>Escherichia coli</i>	6.25	12.50
<i>Salmonella</i> spp.	12.50	25.00

GC-MS Analysis of the Most Active Fraction

Table 6 presents the major phytochemical constituents identified in the ethyl acetate fraction using Gas Chromatography-Mass Spectrometry (GC-MS). A total of seven major compounds were detected. Among these, n-hexadecanoic acid had the highest peak area (17.48%), indicating that it was the most abundant constituent. This was followed by phytol (13.25%), caryophyllene oxide (11.84%), 2,4-di-tert-butylphenol (9.67%), squalene (8.74%), 1-docosene (6.91%), and 1-octadecene (5.86%).

Table 6. Major Compounds Identified by GC-MS

Compound	Retention Time (min)	Peak Area (%)
Caryophyllene oxide	20.31	11.84
2,4-Di-tert-butylphenol	18.92	9.67
Phytol	24.56	13.25
n-Hexadecanoic acid	26.81	17.48
Squalene	31.15	8.74
1-Docosene	29.20	6.91
1-Octadecene	22.96	5.86

DISCUSSION

The present study evaluated the antibacterial activity of fractionated ethanolic leaf extracts of *Psidium guajava* against clinical isolates of *Escherichia coli* and *Salmonella* recovered from patients with gastrointestinal tract infections in Kano State, Nigeria. In addition, the bioactive constituents of the most active fraction were characterized using Gas Chromatography-Mass Spectrometry (GC-MS). The findings demonstrated that *P. guajava* possesses significant antibacterial activity against both pathogens, thereby supporting its traditional use in the treatment of diarrhoea and other gastrointestinal infections.

The isolation of *Escherichia coli* and *Salmonella* from stool samples confirms their importance as major bacterial causes of gastrointestinal tract infections. The higher proportion of *E. coli* isolates observed in this study is consistent with previous reports indicating that pathogenic *E. coli* is one of the leading causes of bacterial gastroenteritis, particularly in low- and middle-income countries where inadequate sanitation and contaminated food or water facilitate transmission. Likewise, *Salmonella* species remain important enteric pathogens associated with foodborne disease outbreaks and significant public health burdens (Kotloff *et al.*, 2018; GBD, 2024).

The extraction process yielded an appreciable quantity of crude ethanolic extract, while solvent partitioning produced fractions of varying yields. The ethyl acetate fraction exhibited the highest recovery among the solvent fractions, suggesting that many of the antibacterial phytochemicals present in *P. guajava* leaves possess intermediate polarity.

Similar findings have been reported by Hall *et al.* (2023), who demonstrated that ethyl acetate effectively concentrates phenolic compounds, flavonoids, and terpenoids responsible for antibacterial activity.

The antibacterial assay revealed that all solvent fractions inhibited the growth of *Escherichia coli* and *Salmonella* in a concentration-dependent manner. Increasing extract concentration resulted in larger inhibition zones, indicating a dose-dependent antibacterial effect. Among the fractions evaluated, the ethyl acetate fraction consistently produced the greatest inhibition against both bacterial species. This observation suggests that solvent fractionation enhanced the concentration of active phytochemicals responsible for antibacterial activity. Comparable findings have been reported in several investigations, where ethyl acetate fractions of *P. guajava* leaves demonstrated superior antibacterial activity compared with crude extracts and less polar fractions (Metougui *et al.*, 2024; Hall *et al.*, 2023).

The superior antibacterial activity observed against *Escherichia coli* compared with *Salmonella* may be attributed to structural and physiological differences between these organisms, including variations in outer membrane permeability, efflux pump activity, and intrinsic resistance mechanisms. Although both organisms are Gram-negative bacteria, differences in their cell envelope composition may influence susceptibility to plant-derived antimicrobial compounds. Similar observations have been reported by previous researchers investigating medicinal plant extracts against enteric bacterial pathogens (Ali *et al.*, 2025).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results further confirmed the antibacterial potential of the active fraction. The relatively low MIC and MBC values indicate that only small concentrations of the extract were required to inhibit and kill the test organisms. These findings suggest that the extract possesses both bacteriostatic and bactericidal properties depending on the concentration used. Such results agree with previous studies demonstrating that *P. guajava* leaf extracts exhibit potent antibacterial activity against multidrug-resistant enteric bacteria (Shittu *et al.*, 2021).

Gas Chromatography–Mass Spectrometry analysis identified several biologically active compounds within the most active fraction. Major constituents included phytol, caryophyllene oxide, n-hexadecanoic acid, squalene, and 2,4-di-tert-butylphenol. These compounds have previously been reported to possess antibacterial, antioxidant, anti-inflammatory, and membrane-disrupting activities. Phytol has been shown to interfere with bacterial membrane integrity and energy metabolism, whereas caryophyllene oxide exhibits broad-spectrum antimicrobial activity. Similarly, n-hexadecanoic acid has demonstrated inhibitory effects against several Gram-positive and Gram-negative bacteria. The presence of these compounds likely explains the observed antibacterial activity of the extract and supports previous phytochemical studies of *P. guajava* leaves.

The antibacterial effects observed in this study are probably due to the synergistic interaction of multiple phytochemicals rather than a single active compound. Flavonoids, tannins, phenolic acids, terpenoids, alkaloids, and other secondary metabolites present in *P. guajava* may act through different mechanisms, including disruption of bacterial cell membranes, inhibition of protein synthesis, interference with nucleic acid replication, suppression of essential metabolic enzymes, and inhibition of biofilm formation. The combined action of these compounds may reduce the likelihood of bacterial resistance development compared with single-agent antibiotics.

Overall, the findings of this study provide scientific evidence supporting the traditional use of *Psidium guajava* leaves in the management of gastrointestinal infections. The results also suggest that the ethyl acetate fraction contains promising antibacterial compounds that could serve as potential sources of novel antimicrobial agents. Further purification, structural characterization, toxicity evaluation, and in vivo studies are recommended to isolate the most active constituents and assess their safety and therapeutic efficacy before clinical application.

CONCLUSION

The findings demonstrated that the fractionated extracts possessed appreciable antibacterial activity against both bacterial pathogens, with the degree of inhibition varying among the solvent fractions. The antibacterial effect

increased with increasing extract concentration, indicating a concentration-dependent response. Among the fractions evaluated, the ethyl acetate fraction exhibited the highest antibacterial activity against both *Escherichia coli* and *Salmonella*. This fraction also produced the lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), suggesting that it contains highly active antibacterial constituents capable of inhibiting and killing the test organisms at relatively low concentrations. Gas Chromatography–Mass Spectrometry analysis revealed the presence of several biologically active phytochemicals, including terpenoids, phenolic compounds, fatty acids, and other secondary metabolites that have previously been associated with antibacterial properties. The synergistic action of these compounds is likely responsible for the observed inhibitory effects against the bacterial isolates.

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