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Comparative Evaluation of Aqueous Leaf Extracts of *Psidium guajava* L. and *Carica papaya* L. and Antibiotic Susceptibility Patterns of *Salmonella* spp. Isolated from Wound Infections in a Tertiary Hospital

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ABSTRACT

Antimicrobial resistance continues to complicate the management of enteric and wound infections, particularly in low-resource settings where plant-based remedies remain widely used. This study evaluated the in vitro antibacterial activity of aqueous extracts of *Psidium guajava* and *Carica papaya* leaves against clinical isolates of *Salmonella* spp. and *Shigella* spp., and further compared these findings with the antibiotic susceptibility profiles of *Salmonella* spp. isolated from wound infections among patients in the female surgical ward of the Federal Medical Centre, Owerri. Standard agar-well diffusion, MIC, and MBC assays were performed using extract concentrations ranging from 50–150 mg/mL. Wound swab samples (n = 50) were processed using conventional cultural and biochemical methods, and antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion technique. Aqueous extracts of both plants demonstrated no measurable inhibitory activity against any of the enteric isolates at all tested concentrations, whereas ciprofloxacin (positive control) produced clearly defined inhibition zones, confirming assay validity. In contrast, clinical *Salmonella* isolates from wound infections exhibited moderate to high susceptibility to conventional antibiotics, with the highest

sensitivity recorded against ciprofloxacin and levofloxacin (70% each), followed by ceftriaxone and cefixime (60%). Azithromycin and cefuroxime demonstrated comparatively lower activity (50% each). The comparative findings underscore the limited efficacy of crude aqueous plant extracts against Gram-negative pathogens, while reaffirming the continued relevance of fluoroquinolones and third-generation cephalosporins in managing *Salmonella*-associated wound infections. The study highlights the need for improved extraction methods, phytochemical concentration techniques, and more targeted exploration of solvent polarity when assessing plant-derived antimicrobials.

Keywords: *Psidium guajava*, *Carica papaya*, *Salmonella* spp., aqueous extract, wound infection, antibiotic susceptibility, agar-well diffusion, fluoroquinolones, cephalosporins, traditional medicine

1. INTRODUCTION

Antimicrobial resistance (AMR) has become a pressing global health issue, threatening the effectiveness of our current antibiotics and making it harder to manage infections that should be easily treatable. Recent estimates reveal that in 2019 alone, bacterial AMR was directly linked to around 1.27 million deaths and associated with an additional 4.95 million deaths, with the most significant impact felt in low- and middle-income countries where access to diagnostics and effective treatments is limited (Murray et al., 2022). Pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, and *Shigella* spp. are particularly worrisome due to their knack for quickly picking up multidrug-resistant traits and dodging first-line antibiotics (Tacconelli et al., 2018). At the same time, chronic and surgical wound infections are increasingly home to complex communities of resistant Gram-negative bacilli, which complicates treatment and extends hospital stays (Dowd et al., 2008; Matei et al., 2024; Schaumburg et al., 2022).

These challenges have sparked renewed interest in exploring plant-derived antimicrobial compounds as accessible, locally sourced alternatives to conventional treatments, especially in resource-limited areas where traditional medicine is still widely used. Medicinal plants produce a diverse range of secondary metabolites, such as flavonoids, alkaloids, tannins, terpenoids, and phenolic acids, many of which exhibit direct antibacterial properties, work well alongside antibiotics, and can even suppress bacterial virulence (Cushnie & Lamb, 2005; Cushnie & Lamb, 2011). Their ability to target multiple pathways is seen as a significant advantage in slowing down the emergence of resistance compared to single-target synthetic drugs.

At the same time, it's important to recognize that the antimicrobial potential of natural products should be evaluated using well-structured in vitro models that take into account factors like solvent effects, diffusion limitations, and realistic clinical concentrations (Cos et al., 2006). Among the medicinal plants known for their antimicrobial properties, *Psidium guajava* (guava) and *Carica papaya* (papaya) stand out due to their extensive ethnobotanical applications and rich phytochemical compositions. Traditionally, guava leaves have been used across Africa, Latin America, and Asia to treat diarrheal diseases, respiratory infections, and various skin or wound-related issues (Gutiérrez et al., 2008). Research into the phytochemical makeup of guava leaves reveals they are packed with phenolic compounds and flavonoids like quercetin, kaempferol, gallic acid, and ellagic acid, all of which are linked to antioxidant, anti-inflammatory, and antimicrobial effects (Jiménez-Escrig et al., 2001; Díaz-de-Cerio et al., 2017; Shah et al., 2020).

Several in vitro studies have shown that extracts from guava leaves can inhibit bacteria such as *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., and *Streptococcus* spp., although the effectiveness can vary significantly depending on the type of solvent used, extraction methods, and the susceptibility of the test strains (Biswas et al., 2013; Metwally et al., 2019; Saleh & Al-Mariri, 2020). Likewise, *Carica papaya* leaves are commonly utilized in traditional medicine for gastrointestinal issues, parasitic infections, and inflammatory conditions (Aravind et al., 2013; Vij & Prashar, 2015). Phytochemical studies have identified flavonoids, saponins, alkaloids, proteolytic enzymes (like papain and chymopapain), and various phenolic compounds, many of which are associated with antimicrobial, antioxidant, and immunomodulatory effects (Canini et al., 2007; Aravind et al., 2013).

Several lab studies have shown that papaya leaf extracts can fight off bacteria like *S. aureus*, *E. coli*, *Salmonella typhi*, and *Shigella* spp. Interestingly, the extracts made with ethanol or methanol tend to be more effective than those made with just water (Baskaran et al., 2011; Atef et al., 2019). This raises some important questions about how well water-based leaf preparations work, especially since they're commonly used in home remedies and traditional medicine. While there's growing evidence that both guava and papaya leaves have antibacterial properties under certain lab conditions, there are still significant gaps in our understanding. Many earlier studies have focused on reference strains instead of the more relevant clinical isolates, which makes their findings less applicable to real-world infections. Additionally, not many studies have looked into tough-to-treat Gram-negative bacteria like *Salmonella* and *Shigella* from actual patients, including those with wound infections. There's also been a lack of systematic comparisons between how these pathogens react to plant extracts versus standard antibiotics (Atef et al., 2019; Matei et al., 2024).

Furthermore, the generally lower effectiveness of water extracts compared to those using organic solvents especially against Gram-negative bacteria that have protective outer membranes underscores the need for research that truly reflects how these preparations are used in traditional healthcare (Al-Asmari et al., 2015; Menezes et al., 2025). With this context in mind, the current study has two main goals. First, it looks at the antibacterial activity of aqueous leaf extracts from *Psidium guajava* and *Carica papaya* against clinical isolates of *Shigella* spp. and *Salmonella* spp., which are commonly associated with gastrointestinal and fever-related illnesses.

Second, it assesses the antibiotic resistance patterns of *Salmonella* spp. isolated from wound swabs taken from patients in the female surgical ward of a tertiary hospital, providing a clinically grounded comparison between responses to conventional antibiotics and to crude aqueous plant extracts. By integrating plant-based and antibiotic susceptibility data for *Salmonella* across different clinical contexts, this work aims to generate realistic, contextually relevant evidence on the capabilities and limitations of these widely used medicinal plants, and to inform their potential complementary role within broader antimicrobial strategies.

2. MATERIALS AND METHODS

2. 1. Study Area

The study was conducted in Owerri, the capital city of Imo State in southeastern Nigeria. Imo State lies within the humid tropical rainforest zone and is predominantly inhabited by Igbo populations.

Owerri hosts several tertiary healthcare facilities, including the Federal Medical Centre (FMC), which serves as a major tertiary referral hospital in the region and hosts the Female Surgical Ward from which wound swab samples used in this study were collected. The microbiological analyses were conducted in the research laboratories of Imo State University and FMC Owerri.

2. 2. Materials and Reagents

Laboratory materials included sterile Petri dishes, test tubes, glass slides, inoculating loops and needles, conical flasks, beakers, Bijou bottles, Durham tubes, micropipettes, cotton swabs, aluminium foil, and Bunsen burners. Equipment comprised an autoclave, hot-air oven, analytical balance, incubator, and laminar-flow cabinet.

Reagents used for microbiological and biochemical analyses included: crystal violet, Lugol's iodine, acetone–alcohol (decolourizer), safranin, 3% hydrogen peroxide, Kovac's reagent, methyl red indicator, α -naphthol, potassium hydroxide, oxidase reagent, peptone water, Simmons citrate agar, triple sugar iron agar, and sterile normal saline. All media and reagents were sourced from Oxoid (UK) and were prepared according to manufacturer specifications.

2. 3. Source of Isolates

Isolates of *Salmonella* spp. were obtained from two sources:

1. Wound swab samples collected from patients admitted to the Female Surgical Ward, Federal Medical Centre (FMC) Owerri.
2. Previously confirmed laboratory isolates from the Microbiology Laboratory of Imo State University Owerri.

2. 4. Culture Media and Preparation

Selective media used for confirmation included Salmonella–Shigella (SS) agar. Media were prepared by dissolving dehydrated powder in distilled water according to manufacturer instructions, followed by sterilisation at 121 °C for 15 min. Media were cooled to 45-50 °C, dispensed into sterile Petri dishes, and allowed to solidify under aseptic conditions.

2. 5. Sterilization Procedures

All glassware was washed, rinsed with distilled water, and oven-sterilised at 160 °C for 2 h. Culture media and solutions were autoclaved at 121 °C, 15 psi for 15 min. Inoculating loops were flamed to red heat before each use, and work surfaces were disinfected with 70% ethanol before and after laboratory procedures. A Bunsen flame was maintained throughout procedures to create an aseptic working environment (Ohazuruike et al., 2017).

2. 6. Isolation and Purification of Bacteria

Wound swab samples from FMC were streaked onto SS agar and incubated aerobically at 37 °C for 24 hours. Following incubation, colonies with distinct *Salmonella*-like morphology were picked and subcultured onto nutrient agar plates for purification. Pure isolates were preserved on nutrient agar slants at 4 °C for subsequent biochemical identification and susceptibility testing (Ohazuruike et al., 2017).

2. 7. Identification of Bacterial Isolates

Bacterial identification followed standard morphological, microscopic, and biochemical procedures (Cheesbrough, 2005). Procedures included:

2. 7. 1. Gram Staining

Smears were heat-fixed and stained sequentially with crystal violet, iodine, alcohol, and safranin. Gram-positive organisms appeared purple, while Gram-negative organisms appeared pink (Ohazuruike et al., 2017)..

2. 7. 2. Biochemical Characterisation

The following standard tests were performed:

Catalase test: using 3% H₂O₂ to detect bubble formation.

Coagulase test: slide method using human plasma for differentiation of *Staphylococcus aureus*.

Oxidase test: using oxidase reagent-soaked filter paper for rapid colour change.

Indole test: using tryptone broth and Kovac's reagent after 48 h incubation.

Simmons citrate utilisation test: monitored for colour change from green to blue.

MR–VP test: methyl red indicator for mixed-acid fermentation and α -naphthol/KOH for acetoin detection.

Sugar fermentation (TSI): evaluated for glucose/lactose/sucrose fermentation, H₂S production, and gas formation.

Morphological features such as colony size, margin, elevation, pigmentation, and surface texture were recorded.

2. 8. Preparation of Plant Materials

Fresh leaves of *Psidium guajava* and *Carica papaya* were collected from healthy plants and taxonomically authenticated by a botanist. Leaves were washed, surface-sterilised with 70% ethanol, air-dried, and homogenised aseptically using a sterile mortar and pestle (Davis & Kuttan, 2000).

2. 9. Extraction of Plant Materials

Approximately 150 g of each leaf type was homogenised and filtered through sterile muslin cloth. The filtrate obtained was considered the 100% crude extract. Extracts were stored at –20 °C until use. sterility of extracts was confirmed by inoculating aliquots onto nutrient agar. Working concentrations (10%, 25%, 50%, and 100%) were prepared by diluting the crude extract with sterile distilled water following the method of Davis & Kuttan, (2000).

2. 10. Preparation of Standard Inoculum

Isolates were grown in nutrient broth for 18 h and adjusted to the turbidity of a 0.5 McFarland standard (approximately 1×10^7 CFU/mL) by comparison against a visual turbidity scale (Davis & Kuttan, 2000).

2. 11. Antimicrobial Susceptibility Testing

Antimicrobial activity of the plant extracts was evaluated using the agar-well diffusion assay in accordance with standard methods (Marasini et al., 2015). Mueller–Hinton agar (MHA) plates were inoculated uniformly with the standardised inoculum using sterile swabs. Wells (5 mm diameter) were aseptically bored into the agar and filled with 50 μ L of each extract concentration. Sterile distilled water served as the negative control. Plates were allowed to pre-diffuse for 1 h at room temperature and incubated at 37 °C for 18–24 h. Zones of inhibition were measured in millimetres. All assays were performed in triplicate, and mean values were recorded.

2. 12. Antibiotic Susceptibility Testing (Added – Missing Section Completed)

Antibiotic susceptibility of *Salmonella* spp. isolated from wound samples was determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar following CLSI guidelines (CLSI, 2023).

Procedure

- MHA plates were inoculated uniformly with the standardised 0.5 McFarland suspension of *Salmonella* isolates.
- Commercial antibiotic discs (Oxoid, UK) were placed aseptically on the inoculated plates, including:
 - Azithromycin (30 μ g)
 - Cefuroxime (30 μ g)
 - Cefixime (30 μ g)
 - Levofloxacin (5 μ g)
 - Ceftriaxone (30 μ g)
 - Ciprofloxacin (5 μ g)
- Plates were incubated at 37 °C for 18–24 h.
- Zones of inhibition were measured in millimetres using a calibrated ruler.
- Sensitivity or resistance was interpreted based on CLSI breakpoints.

3. RESULTS

3. 1. Demographic Characteristics of Study Population

Table 1. Demographic Characteristics of Study Participants.

Variable	Number of Patients (n = 50)	Percentage (%)
Age Group (Years)		
20–29	8	16
30–39	12	24
40–49	14	28

50–59	10	20
≥60	6	12
Gender		
Male	30	60
Female	20	40
Underlying Conditions		
Diabetes Mellitus	15	30
Hypertension	12	24
No Comorbidities	23	46

The demographic characteristics of the 50 study participants involved in the antimicrobial susceptibility profiling of Gram-negative organism (*Salmonella* spp) in leg ulcerations. The age group 40–49 years had the highest representation (28%), followed by 30–39 years (24%), while the lowest representation was among participants aged ≥60 years (12%). Males constituted the majority of the participants (60%), while females accounted for 40%. Regarding underlying conditions, 30% of the participants had diabetes mellitus, and 24% had hypertension. Notably, 46% of the participants reported no comorbidities. Shown in Table 1, Fig. 1 and Fig. 2.

Age Distribution of Study Participants

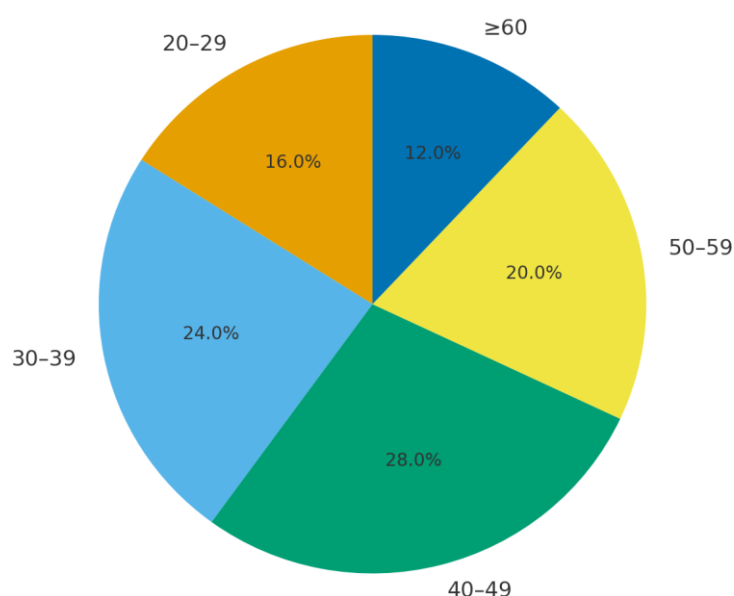


Fig. 1. Age Representation of study Participants.

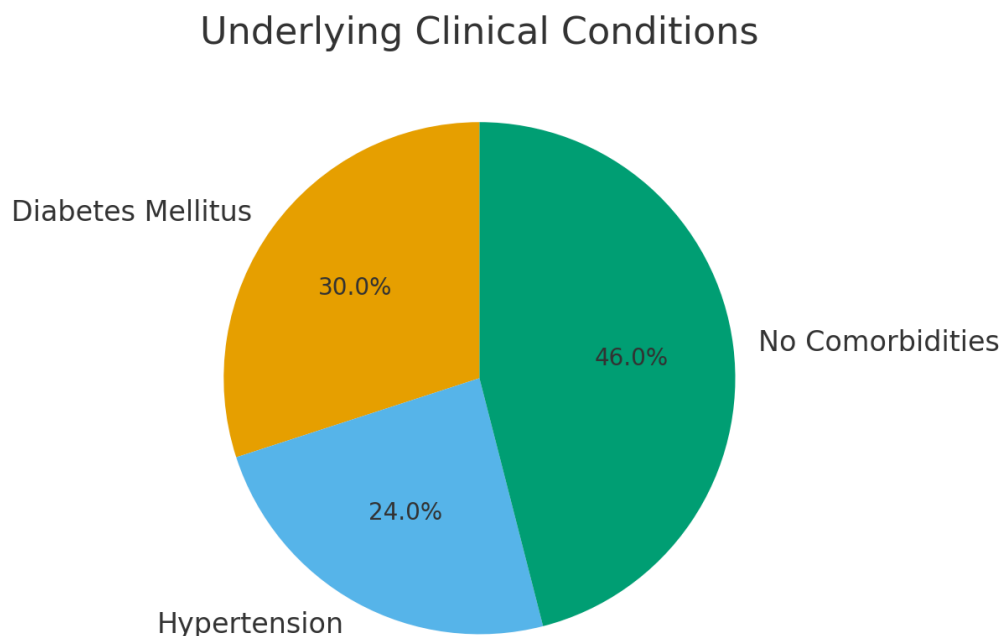


Fig. 2. Underlying Clinical Conditions.

3. 2. Identification of Salmonella Isolate

Morphological and Biochemical characterisation confirmed the identity and viability of the laboratory isolates used for testing plant extracts and Conventional Antibiotics. *Salmonella* spp. exhibited motility, citrate positivity, methyl red positivity, and typical Gram-negative rod morphology.

Table 2. Biochemical properties of Clinical Isolates.

			BIOCHEMICAL IDENTIFICATION TEST										
Gram reaction test	Cel. Arrangement	Mot. Test	Catalase test	Indole test	Oxidase test	Coagulase test	Citrate test	V.P test	MR test	Glucose Test	Lactose Test	Sucrose Test	Probable organism
-	Rod shape	+	+	-	-	-	-	-	+	+	-	-	<i>Salmonella</i> spp.

KEYS: - = Negative; + = Positive; Cel. = Cellular; Mot. = Motility; V.P = VogesProskuer; spp. = Species

Ethnobotanical Activity of *Psidium guajava* (guava) and *Carica papaya* (papaya) leaves

The antibacterial activity of the aqueous extracts of *Psidium guajava* (guava) and *Carica papaya* (papaya) leaves was evaluated against clinical isolates of *Shigella* spp. and *Salmonella* spp. using agar well diffusion, MIC, and MBC assays. Across all tested concentrations, no measurable zones of inhibition were recorded for either plant extract. Ciprofloxacin, the positive control, produced well-defined inhibition zones, confirming the validity of the assays, while distilled water produced no inhibition as expected. Biochemical characterisation confirmed the identity and viability of the organisms used in the assays.

Table 3. Antimicrobial Activity of for *Guava* leaf Extract.

Concentration (mg/mL)	Zone of Inhibition Against <i>Salmonella</i> spp. (mm)
50	0
100	0
150	0
Positive Control (Ciprofloxacin)	24
Negative Control (Distilled Water)	0

Table 4. Antimicrobial Activity of *Paw paw* leaf Extract.

Concentration (mg/mL)	Zone of Inhibition Against <i>Salmonella</i> spp. (mm)
50	0
100	0
150	0
Positive Control (Ciprofloxacin)	24
Negative Control (Distilled Water)	0

Antibacterial Susceptibility of *Salmonella* spp.

The antibiotic susceptibility pattern of *Salmonella* spp. isolated from wound samples revealed a moderate to high level of responsiveness to commonly used antimicrobial agents. As shown in Table 5, and Fig. 3 fluoroquinolones Levofloxacin and Ciprofloxacin demonstrated

the highest activity, each achieving a sensitivity rate of 70%. Third-generation cephalosporins, including Cefixime and Ceftriaxone, also showed substantial effectiveness, with 60% sensitivity. In contrast, Azithromycin and Cefuroxime exhibited lower activity, each with a sensitivity rate of 50%. These findings indicate that while *Salmonella* displays variable susceptibility across antibiotic classes, fluoroquinolones remain the most reliable therapeutic option in this clinical context, followed by select cephalosporins. The pattern aligns with global trends reporting sustained fluoroquinolone efficacy against invasive *Salmonella* strains despite rising antimicrobial resistance in other Gram-negative pathogens.

Table 5. Antibiotic Susceptibility of *Salmonella* spp. from Wound Samples (n = 10).

Antibiotic	% Sensitive
Azithromycin	50%
Cefuroxime	50%
Cefixime	60%
Levofloxacin	70%
Ceftriaxone	60%
Ciprofloxacin	70%

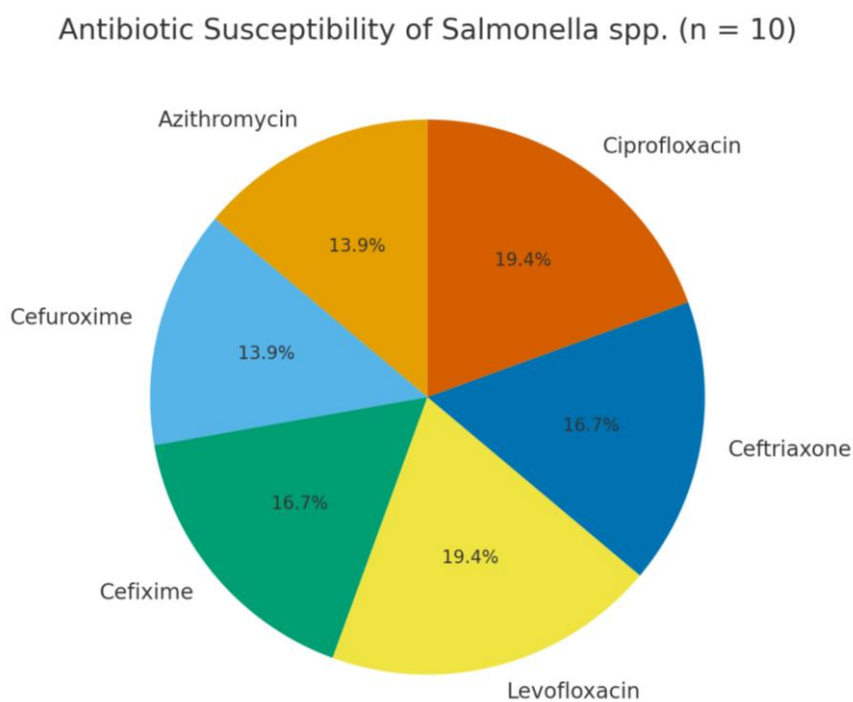


Fig. 3. Antibiotics Susceptibility of *Salmonella* Isolates.

4. DISCUSSION

Antimicrobial resistance (AMR) has become a pressing global health issue, threatening the effectiveness of our current antibiotics and making it harder to manage infections that should be easily treatable. Recent estimates reveal that in 2019 alone, bacterial AMR was directly linked to around 1.27 million deaths and associated with an additional 4.95 million deaths, with the most significant impact felt in low- and middle-income countries where access to diagnostics and effective treatments is limited (Murray et al., 2022). Pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, and *Shigella spp.* are particularly worrisome due to their knack for quickly picking up multidrug-resistant traits and dodging first-line antibiotics (Tacconelli et al., 2018). At the same time, chronic and surgical wound infections are increasingly home to complex communities of resistant Gram-negative bacilli, which complicates treatment and extends hospital stays (Dowd et al., 2008; Matei et al., 2024; Schaumburg et al., 2022). These challenges have sparked renewed interest in exploring plant-derived antimicrobial compounds as accessible, locally sourced alternatives to conventional treatments, especially in resource-limited areas where traditional medicine is still widely used. Medicinal plants produce a diverse range of secondary metabolites, such as flavonoids, alkaloids, tannins, terpenoids, and phenolic acids, many of which exhibit direct antibacterial properties, work well alongside antibiotics, and can even suppress bacterial virulence (Cushnie & Lamb, 2005; Cushnie & Lamb, 2011). Their ability to target multiple pathways is seen as a significant advantage in slowing down the emergence of resistance compared to single-target synthetic drugs.

At the same time, it is important to recognize that the antimicrobial potential of natural products should be evaluated using well-structured in vitro models that take into account factors like solvent effects, diffusion limitations, and realistic clinical concentrations (Cos et al., 2006). Among medicinal plants known for their antimicrobial activities, *Psidium guajava* (guava) and *Carica papaya* (papaya) stand out due to their extensive ethnobotanical applications and rich phytochemical profiles. Traditionally, guava leaves have been used across Africa, Latin America, and Asia for treating diarrheal diseases, respiratory infections, and a wide range of skin or wound-related conditions (Gutiérrez et al., 2008). Their chemical composition includes phenolic compounds and flavonoids such as quercetin, kaempferol, gallic acid, and ellagic acid—all known for antimicrobial, anti-inflammatory, and antioxidant properties (Jiménez-Escrig et al., 2001; Díaz-de-Cerio et al., 2017; Shah et al., 2020). Several in vitro investigations have confirmed the antibacterial activity of guava extracts against organisms including *Staphylococcus aureus*, *E. coli*, *Salmonella spp.*, and *Streptococcus spp.*, although results vary widely depending on the solvent system and extraction method employed (Biswas et al., 2013; Metwally et al., 2019; Saleh & Al-Mariri, 2020).

Similarly, papaya leaves contain an array of flavonoids, alkaloids, saponins, proteolytic enzymes, and phenolic compounds that have been linked to antimicrobial and antioxidant activity (Canini et al., 2007; Aravind et al., 2013). Ethanol and methanol extracts of papaya leaves have repeatedly demonstrated inhibitory effects against bacteria such as *S. aureus*, *E. coli*, and *Salmonella typhi*, while aqueous preparations are generally less potent (Baskaran et al., 2011; Atef et al., 2019). Despite promising literature, the findings from the present study did not replicate this pattern. Across all test concentrations, aqueous extracts of *P. guajava* and *C. papaya* failed to produce measurable zones of inhibition against clinical isolates of *Salmonella spp.* This negative outcome is consistent with earlier reports showing that water is

a weak solvent for extracting the more hydrophobic, bioactive antibacterial compounds found in these plants (Biswas et al., 2013; Naseer et al., 2018). The limited diffusion of crude aqueous extracts through agar further reduces the probability of generating detectable inhibition zones, even when bioactive constituents are present (Cos et al., 2006).

Meanwhile, the clinical isolates used in this study were obtained from wound swabs of patients in the female surgical ward, where chronic wounds and postoperative infections often harbour resilient Gram-negative organisms. Biochemical and morphological tests confirmed the identity and viability of *Salmonella* spp., which displayed the expected motility, Gram-negative rod morphology, methyl red positivity, and citrate utilisation. The demographic profile of the affected patients revealed that the majority were between 30–49 years, with a substantial proportion presenting comorbidities such as diabetes mellitus (30%) and hypertension (24%). These underlying conditions are well documented as risk factors for chronic wound infection and impaired healing, creating an environment conducive to colonization by enteric pathogens like *Salmonella* (Dowd et al., 2008; Schaumburg et al., 2022).

The antibiotic susceptibility patterns observed in this study offer important clinical context. Fluoroquinolones Levofloxacin and Ciprofloxacin demonstrated the highest activity, each achieving 70% sensitivity. Third-generation cephalosporins such as Cefixime and Ceftriaxone achieved moderate activity (60% sensitivity), while Azithromycin and Cefuroxime had lower activity (50%).

These results align with global and regional patterns showing that fluoroquinolones remain among the most reliable therapeutic agents against invasive *Salmonella* infections, despite growing resistance worldwide (Tacconelli et al., 2018; WHO, 2024). The moderate susceptibility profile also underscores why aqueous plant extracts—much milder and less chemically potent than purified antibiotics—are unlikely to inhibit such resilient clinical strains. The native defensive systems of Gram-negative bacteria—including efflux pumps, impermeable outer membranes, and enzyme-mediated resistance—make them particularly difficult to target using crude plant preparations.

Taken together, the combined results from plant-extract assays and antibiotic susceptibility testing provide a realistic and clinically relevant understanding of the limitations and potential roles of aqueous guava and papaya extracts. While the plant extracts showed no detectable antibacterial activity, the antibiotic susceptibility profile of *Salmonella* isolates reveals that conventional antibiotics—particularly fluoroquinolones and selected cephalosporins—remain effective in this context. This contrast reinforces the need for more rigorous extraction methods, solvent optimisation, phytochemical quantification, and perhaps solvent-fractionation in future investigations. It also highlights the risk of overgeneralizing traditional claims regarding plant-based therapies without considering practical extraction efficiency, pathogen biology, and clinical relevance.

Importantly, reporting negative results contributes to reducing publication bias in natural-product research. The honest documentation of weak or absent activity strengthens the evidence base and helps guide future studies toward more robust methodologies such as ethanol extraction, bio-guided fractionation, and detailed phytochemical profiling rather than relying solely on traditional aqueous preparations.

Ultimately, this study offers a scientifically grounded, contextually appropriate evaluation of aqueous guava and papaya leaf extracts and provides meaningful comparative data with clinically relevant *Salmonella* isolates from chronic wound infections in a real-world hospital setting.

5. CONCLUSION

In conclusion, the absence of antibacterial activity observed in the aqueous extracts of *Psidium guajava* and *Carica papaya* leaves against clinical isolates of *Salmonella* spp. reflects an important and realistic outcome in natural-product antimicrobial research. Rather than indicating experimental inadequacy, these findings highlight the well-documented limitations of water-based extractions, particularly against Gram-negative pathogens with robust permeability barriers and multiple resistance mechanisms. When interpreted alongside the moderate–high susceptibility of wound-derived *Salmonella* isolates to fluoroquinolones and third-generation cephalosporins, the results underscore that crude aqueous plant remedies while widely used in traditional medicine—may offer little clinically meaningful activity against contemporary resistant strains.

These observations reinforce the need for methodologically optimized approaches, including solvent-specific extraction, phytochemical quantification, and bioassays designed to capture the activity of semi-polar and non-polar compounds. The study also contributes evidence to counteract publication bias in herbal medicine research by documenting a negative but scientifically valid result an essential step toward accurate, reproducible, and clinically relevant evaluations of medicinal plants.

Although aqueous guava and papaya extracts showed no measurable inhibition under the tested conditions, this does not preclude the possibility that other extraction methods, purified fractions, or concentrated phytochemical components may exhibit significant activity. Future work integrating advanced phytochemical profiling, solvent partitioning, synergy testing with antibiotics, and expanded clinical isolate panels will be crucial for determining whether these plants can play a meaningful complementary role in antimicrobial therapy. Until such evidence is available, the traditional use of these leaf preparations particularly for infections caused by Gram-negative pathogens should be reconsidered with caution and grounded in empirical data rather than assumption.

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