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ANTI-BACTERIAL AND ANTI B-LACTAMASE ACTIVITIES OF EUPHORBIA HIRTA AND TRIDAX PROCUMBENS AGAINST CLINICAL ISOLATES OF B-LACTAMASE PRODUCING SALMONELLA ENTERICA AND STAPHYLOCOCCUS AUREUS

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ABSTRACT

This study investigated the antibacterial and anti-β-lactamase activities of *Euphorbia hirta* and *Tridax procumbens* against clinical isolates of β-lactamase-producing *Salmonella enterica* and *Staphylococcus aureus*, addressing the growing challenge of antibiotic resistance to β-lactam antibiotics. A total of 200 clinical specimens, comprising 100 nasal swabs and 100 stool samples, were collected. From these, 24 isolates of *S. enterica* and 20 isolates of *S. aureus* were recovered and microbiologically characterized using standard biochemical tests. *S. aureus* was confirmed by positive catalase and coagulase reactions, while *S. enterica* showed motility, citrate utilization, and hydrogen sulfide production. Antibiotic susceptibility testing revealed high resistance levels to commonly used β-lactam antibiotics, with *S. enterica* exhibiting up to 100% resistance to cefuroxime and *S. aureus* showing up to 95% resistance. B-lactamase screening indicated high pre-exposure enzyme production in both organisms (83.3% and 85%, respectively). Phytochemical screening showed that *E. hirta* contained glycosides, tannins, saponins, and phenols, whereas *T. procumbens* contained mainly glycosides. Antibacterial assessment demonstrated that both plant extracts produced mild to moderate inhibitory effects at 600 mg/dL. Ethanolic extracts showed slightly higher activity (8–9 mm inhibition zones) than aqueous extracts (6–8 mm). However, post-exposure evaluation revealed limited anti-β-lactamase activity, with most isolates remaining β-lactamase-positive. In conclusion, although the extracts exhibited moderate antibacterial activity, their minimal anti-β-lactamase effect suggests that they may function better as supportive or complementary agents rather than standalone treatments. Further studies are recommended to isolate active compounds and assess synergistic interactions with existing antibiotics.

Keywords: Phytochemical analysis, *Staphylococcus aureus*, *Salmonella enterica*, Antimicrobial resistance, *Tridax procumbens*, β-lactamase-producing bacteria

INTRODUCTION

The escalating crisis of antimicrobial resistance (AMR) poses a significant threat to global public health, undermining the effectiveness of conventional antibiotics and complicating the treatment of infectious diseases (Lopez-Malo et al., 2020). Historically, the use of synthetic chemicals to combat infections dates back to ancient civilizations, with traditional remedies often involving natural substances now recognized for their antimicrobial properties (Adams et al., 2024). The observation that certain organisms could inhibit the growth of others laid the groundwork for modern microbiology and the discovery of antibiotics (Selwyn, 2023). Early pioneers like Sir John Burdon-Sanderson and Joseph Lister noted the antibacterial activity of Penicillium molds, foreshadowing the development of penicillin (Lawrence and Dixey, 2018; Sariguzel et al., 2023).

Bacteria, as the simplest and most abundant life forms on Earth, play crucial roles in ecosystems, from nitrogen fixation to decomposition (Helmreich, 2023). However, a small subset of these microorganisms, known as pathogens, are responsible for a vast array of human illnesses, accounting for millions of deaths annually (Dekaboruah et al., 2020). The severity of these infections is often determined by the pathogen's virulence and the host's immune response (Bergwerff and Debast, 2021). The transmission of pathogens occurs through various modes, including direct contact, indirect contact, and airborne transmission, leading to diseases such as tetanus, cholera, and tuberculosis (Meena et al., 2019; Bilash et al., 2022). Of particular concern are Gram-negative bacteria belonging to the *Enterobacteriaceae* family, such as *Salmonella*, and Gram-positive bacteria like *Staphylococcus aureus* (Moxley et al., 2022; Milani et al., 2023). While some members of *Enterobacteriaceae* are part of the normal intestinal flora, others, including *Salmonella*, are significant pathogens causing a range of infections, from gastroenteritis to septicemia (Leal et al., 2019; Tariq et al., 2022). *Staphylococcus aureus*, a common commensal organism found on skin and mucous membranes, is also a major cause of both hospital-acquired and community-acquired infections, leading to conditions like wound infections, pneumonia, and toxic shock syndrome (Tsouklidis et al., 2020; Winstel et al., 2021).

The emergence of antibiotic-resistant strains, particularly those producing β-lactamase enzymes, has rendered many conventional antibiotics ineffective against these pathogens (Chinemerem et al., 2022). β-lactamases hydrolyze the β-lactam ring of antibiotics, conferring resistance to penicillins, cephalosporins, and monobactams (Bush and Bradford, 2020). Extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases are increasingly reported in clinical isolates, leading to treatment failures, prolonged hospital stays, and increased mortality rates (Ramos et al., 2020; Kurittu et al., 2021). The widespread availability and frequent use of antibacterial products are believed to contribute to the selection and proliferation of resistant bacterial strains (Chirani et al., 2021).

Given the escalating challenge of antibiotic resistance, there is an urgent need to explore alternative therapeutic approaches. Medicinal plants, with their rich history in traditional medicine, offer a promising avenue for the discovery of new antibacterial compounds (Howes et al., 2020). Many plants contain essential oils and bioactive compounds with proven antimicrobial properties (Jacob, 2018; Lopez-Malo et al., 2020). However, the potential of

local plants, especially in biodiverse regions, remains largely underexplored (Raven et al., 2020).

This study addresses this critical gap by evaluating the antibacterial and anti-β-lactamase activities of *Euphorbia hirta* and *Tridax procumbens* against clinical isolates of β-lactamase-producing *Salmonella enterica* and *Staphylococcus aureus*. Both *Euphorbia hirta* and *Tridax procumbens* have been traditionally used for various ailments, and this research aims to scientifically validate their potential as sources of novel antimicrobial agents. The findings of this study will contribute valuable knowledge to the field of natural product research, potentially aiding in the development of new antibiotics and providing scientifically validated natural remedies to combat the growing threat of antibiotic-resistant pathogens.

MATERIALS AND METHODS

Sample Size Determination

The sample size for this study was calculated using the Kish and Leslie (1965) formula

$$\mathbf{n} = \frac{\mathbf{Z^2 Pq}}{\mathbf{d^2}} \quad (1)$$

Where:

i. n = minimum required sample size
ii. Z = standard normal deviation at 95% confidence level (1.96)
iii. P = estimated probability that an isolate is an ESBL producer (0.5)
iv. q = 1−P (complement of the probability)
v. d = precision level at 95% confidence (0.05)

Substituting the values, the calculated minimum sample size was 384. For robustness, this was rounded up to 400 isolates. However, due to sample availability, a total of 200 samples were collected, comprising 100 nasal swabs and 100 stool specimens, which were subsequently tested.

Sampling Procedure

A multi-stage sampling technique was employed for the isolation of *Staphylococcus aureus* and *Salmonella enterica*. A total of 200 specimens were collected, consisting of 100 nasal swabs for *Staphylococcus aureus* isolation and 100 stool samples from patients with significant Widal titers for *Salmonella enterica* isolation. The detailed sampling criteria are presented in Table 1.

Table 1: Sampling Procedures

Sampling stage	Criteria
1. Isolates that were characterized as *Staphylococcus aureus* and *Salmonella enterica*	
2. Isolates showing resistance to β-lactam antibacterial agents (penicillins & cephalosporins)	
3. Isolates that produce β-lactamase at any degree	

Isolation and Microbiological Characterization of Isolates

Media Preparation

All culture media, including selenite broth, Salmonella-Shigella agar (SSA), nutrient agar, blood agar, mannitol salt agar, and Mueller-Hinton agar, were prepared according to the manufacturer's instructions.

Sample Collection

Bacterial isolates were obtained from stool and nasal swab specimens. *Staphylococcus aureus* was isolated from nasal swabs using sterile swab sticks, while *Salmonella enterica* was isolated from stool specimens collected in sterile universal bottles.

Plating of Collected Specimens

Stool specimens were inoculated into 10 mL of selenite broth and incubated at 37°C for 24 hours, followed by subculture onto Salmonella-Shigella agar (SSA). Nasal swabs were directly plated onto mannitol salt agar and blood agar. All inoculated plates were incubated at 37°C for 24 hours. Colonies exhibiting characteristic growth patterns were subcultured onto nutrient agar to obtain pure cultures. Isolates were then subjected to Gram staining and observed under a microscope (100× magnification) for preliminary identification.

Morphological Identification

Gram's Stain

A thin film of each bacterial isolate was prepared, air-dried, and heat-fixed. The smear was stained with crystal violet for 1 minute, rinsed, flooded with Lugol's iodine for 30 seconds, rinsed, decolorized with ethanol for 10 seconds, and counterstained with safranin for 1 minute. After rinsing and air-drying, samples were examined under oil immersion (100× magnification).

Biochemical Characterization of Bacterial Isolates

Rod-shaped, Gram-negative bacteria (suggestive of *Salmonellae*) and Gram-positive clusters of coccal bacteria (suggestive of *Staphylococcus aureus*) were subjected to the following biochemical tests:

Motility Test

Nutrient agar was stabbed with a sterile straight wire and incubated at 37°C for 24 hours. Motility was indicated by diffuse, hazy growth spreading throughout the medium.

Urease Test

A loopful of the test organism was inoculated onto a urea agar slant and incubated at 37°C for 24 hours.

Indole Test

A colony was inoculated into peptone water, incubated at 37°C for 24 hours, and then 0.5 mL of Kovac's reagent was added. A red ring indicated a positive result.

Triple Sugar Iron (TSI) Test

A colony was streaked onto a TSI agar slant and incubated at 37°C for 24 hours. Observations included gas production, H2S formation, and fermentation of glucose, lactose, or sucrose.

Catalase Test

A drop of 3% hydrogen peroxide was placed on a slide, and a colony was emulsified in it. Effervescence indicated a positive reaction.

Coagulase Test

Bacterial suspensions were mixed with citrated human plasma. Clumping of cells indicated a coagulase-positive result.

Citrate Utilization Test

The test organism was stabbed into a citrate agar slant and incubated at 37°C for 24 hours. A color change from green to blue indicated a positive result.

Antibacterial Susceptibility Test

The Kirby-Bauer disk diffusion method was used to determine antibiotic sensitivity. Agar plates were inoculated with a bacterial suspension standardized to 0.5 McFarland standard (1.5×10^8 CFU/mL). Antibiotic disks (Augmentin (30 µg), ciprofloxacin (10 µg), septrin (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), amoxicillin (30 µg), gentamicin (10 µg), perfloxacin (30 µg), tarvid (10 µg), and streptomycin (30 µg)) were applied, and plates were incubated at 37°C. Zones of inhibition were measured.

Preparation of 0.5 McFarland Standard

A 0.5 McFarland standard was prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate with 9.95 mL of 1% sulfuric acid, yielding an approximate cell density of 1.5×10^8 CFU/mL and an optical density of 0.132 at 600 nm.

Inoculation of Bacterial Isolates into the McFarland Standard

A 24-hour bacterial colony was mixed in sterile 0.89% saline, and its turbidity was adjusted to match the 0.5 McFarland standard.

Phytochemical Analysis of Plant Extracts

Aqueous and ethanolic extracts of *Tridax procumbens* and *Euphorbia hirta* were analyzed for the presence of various phytochemicals:

Test for Alkaloids

Aqueous extract was stirred with 1% HCl, and Mayer's and Wagner's reagents were added. Turbidity or precipitate indicated alkaloids.

Test for Tannins

Extract was mixed with distilled water, and a few drops of ferric chloride ($FeCl_3$) solution were added. A green-colored precipitate indicated tannins.

Test for Saponins

Extract was added to distilled water, shaken vigorously, and warmed. Stable foam indicated saponins.

Test for Flavonoids

1 cm^3 of 10% lead acetate was added to aqueous extract. A yellow precipitate indicated flavonoids.

Test for Phenols

1 cm^3 of ferric chloride ($FeCl_3$) was added to extract. A bluish-green color indicated phenols.

Test for Glycosides

i. Liebermann's Test: Acetic acid and chloroform were added to aqueous extract, heated, cooled, and then sulfuric acid was added. A green color change indicated glycosides.
ii. Keller-Killani Test: Glacial acetic acid and 2% ferric chloride were added to aqueous extract, followed by sulfuric acid. A brown ring indicated glycosides.
iii. Salkowski's Test: Sulfuric acid was added to aqueous extract. A reddish-brown color indicated glycosides.

Determination of β-Lactamase Production by Isolates

β-lactamase production was tested using the starch-iodide paper acidometric method. Filter paper strips saturated with a starch-penicillin solution and Gram's iodine were smeared with a heavy inoculum of the test organism. Decolorization of the purple strip to white indicated a positive β-lactamase test. *Staphylococcus aureus* ATCC 25923 (positive control) and *Escherichia coli* ATCC 25925 (negative control) were used.

Incubation of Isolates Positive for β-Lactamase Production with Different Concentrations of the Extracts

Concentrations of 100 mg/dL, 200 mg/dL, 300 mg/dL, and 600 mg/dL of the extracts were prepared in nutrient agar. Each mixture was inoculated with 10 mL of a 0.5 McFarland standard preparation of β-lactamase-positive isolates (1.5×10^8 CFU/mL), poured into plates, and incubated at 37°C for 48 hours.

Post-Exposure Determination of Anti-β-Lactamase Activity of the Plant Extracts

After 48 hours of incubation, organisms from plates showing microbial growth were re-tested for β-lactamase production using the same method described in Section 3.8.

Determination of Antibacterial Activity of the Extracts

Crude aqueous and ethanolic extracts of *Tridax procumbens* and *Euphorbia hirta* were prepared at concentrations of 100 mg/dL, 200 mg/dL, 300 mg/dL, and 600 mg/dL. Wells were bored into Mueller–Hinton agar plates previously inoculated with *Staphylococcus aureus* and *Salmonella enterica*. Each extract concentration was introduced into separate wells, and the plates were incubated at 37 °C for 24 hours. After incubation, zones of inhibition were measured in millimeters.

Statistical Analysis:

All experiments were carried out in triplicates. The mean inhibition zone and standard deviation (mean ± SD) were calculated for each extract and concentration. Data were analyzed using one-way ANOVA to determine significant differences among extract concentrations, followed by a post-hoc test (Tukey's test) where applicable. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

This study investigated the biochemical characteristics, antibiotic susceptibility patterns, and β-lactamase production of *Salmonella enterica* and *Staphylococcus aureus* isolates obtained from clinical specimens. A total of 200 specimens (100 stool samples and 100 nasal swabs) were collected, yielding 24 isolates of *Salmonella enterica* and 20 isolates of *Staphylococcus aureus*.

Biochemical Characterization of Isolates

The biochemical characteristics of the isolated *Staphylococcus aureus* and *Salmonella enterica* are summarized in Table 2. These distinct profiles were crucial for their accurate identification and differentiation.

Table 2: Biochemical Characterization of the Isolates

Biochemical Test	*Staphylococcus aureus*	*Salmonella enterica*
Blood agar plate	Negative	Negative
Catalase	Positive	Negative
Coagulase	Positive	Negative
Mannitol salt agar	Positive	Negative
Lactose fermentation	Negative	Negative
Sulfur deposition	Negative	Positive
Oxidase	Negative	Negative
Urease	Negative	Negative
Citrate	Negative	Positive
Triple sugar iron	Negative	Positive
Motility	Negative	Positive
Taxos A	Negative	Negative
Taxos P	Negative	Negative
Indole	Negative	Positive

Staphylococcus aureus isolates were consistently positive for catalase and coagulase, and demonstrated mannitol fermentation on mannitol salt agar, producing yellow colonies. They were negative for lactose fermentation, sulfur deposition, oxidase, urease, citrate utilization, triple sugar iron (TSI) reactions, motility, Taxos A (bacitracin sensitivity), Taxos P (optochin sensitivity), and indole production. Conversely, *Salmonella enterica* isolates were negative for catalase, coagulase, mannitol fermentation, lactose fermentation, oxidase, urease, Taxos A, and Taxos P. They were positive for sulfur deposition, citrate utilization, TSI reactions, motility, and indole production.

Antibiotic Susceptibility Patterns

The antibiotic susceptibility patterns of *Salmonella enterica* and *Staphylococcus aureus* isolates to various β-lactam antibiotics are presented in Table 3 and Table 4, respectively. The results indicate high levels of resistance among the tested isolates.

Table 3: Sensitivity and Resistance Patterns of *Salmonella enterica* (n=24)

Drugs	Sensitivity	Intermediate	Resistance
Ampiclox	0%	4.2%	95.8%
Amoxicillin	0%	4.2%	95.8%
Augmentin	0%	4.2%	95.8%
Cefuroxime (Z)	0%	0%	100%
Ceftazidime (R)	0%	50%	50%

For *Salmonella enterica*, resistance rates were notably high: 95.8% for Ampiclox, Amoxicillin, and Augmentin, and 100% for Cefuroxime. Ceftazidime showed 50% resistance and 50% intermediate sensitivity.

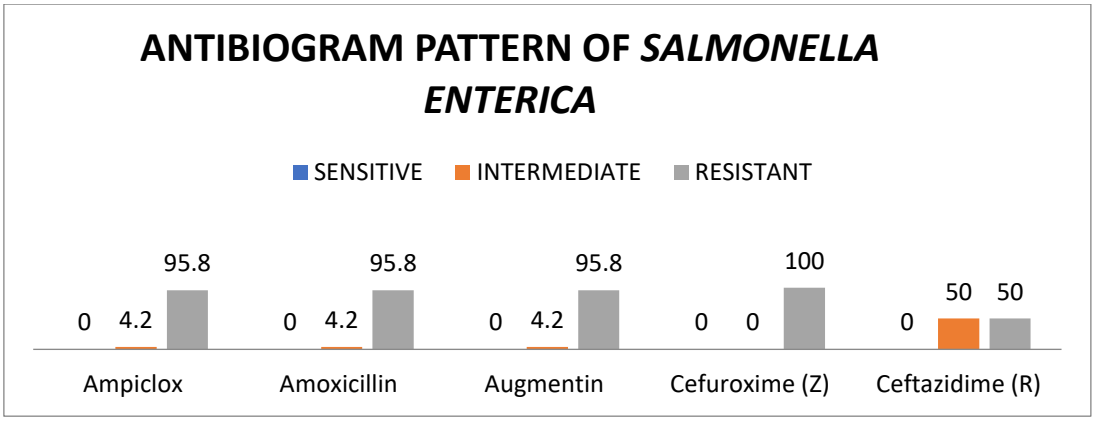

Figure 1: Bar Chart on Sensitivity and Resistance Patterns of *Salmonella enterica* in Percentage (n=24)

Table 4: Sensitivity and Resistance Patterns of *Staphylococcus aureus* (n=20)

Drugs	Sensitivity	Intermediate	Resistance
Ampiclox	0%	20%	80%
Amoxicillin	0%	20%	80%
Augmentin	0%	10%	90%
Cefuroxime (Z)	0%	5%	95%
Ceftazidime (R)	0%	60%	40%

Staphylococcus aureus isolates also exhibited significant resistance: 80% for Ampiclox and Amoxicillin, 90% for Augmentin, and 95% for Cefuroxime. Ceftazidime showed 40% resistance and 60% intermediate sensitivity.

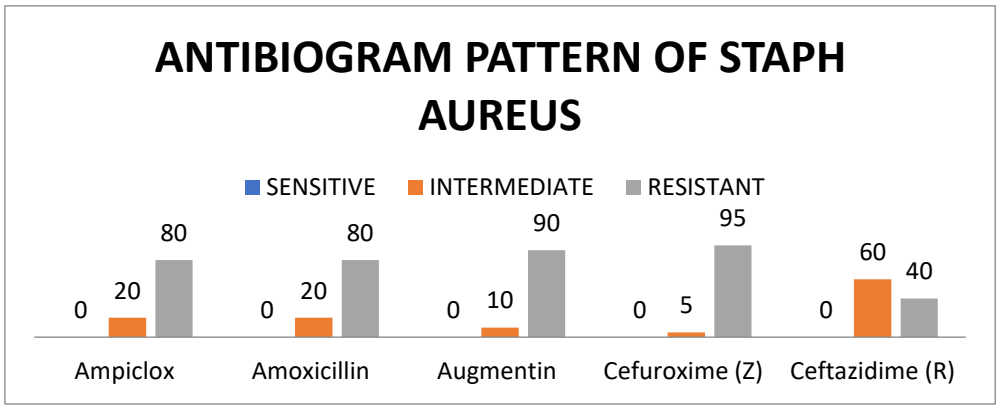

Figure 2: Bar chart on Sensitivity and Resistance Patterns of *Staphylococcus aureus* (n=20)

Qualitative Phytochemical Analysis of Tridax and Euphorbia Extracts

The phytochemical analysis of aqueous and ethanolic extracts of *Tridax procumbens* and *Euphorbia hirta* is presented in Table 5.

Table 5: Qualitative Phytochemical Analysis of Tridax and Euphorbia Extracts

Extracts	Glycoside	Alkaloids	Tannin	Saponin	Flavonoid	Phenol
Tridax aqueous	+	-	-	-	-	-
Tridax ethanol	+	-	-	-	-	-
Euphorbia aqueous	+	-	+	+	-	+
Euphorbia ethanol	-	-	+	-	-	+

Phytochemical screening revealed distinct variations in the bioactive constituents of *Tridax procumbens* and *Euphorbia hirta* extracts. In *Tridax procumbens*, glycosides were detected in both the aqueous and ethanolic extracts, while alkaloids, tannins, saponins, flavonoids, and phenols were not detected. For *Euphorbia hirta*, the aqueous extract showed that glycosides, tannins, saponins, and phenols were detected, whereas alkaloids and flavonoids were not detected. The ethanolic extract of *E. hirta* revealed that tannins and phenols were detected, while glycosides, alkaloids, saponins, and flavonoids were not detected.

These findings indicate that *E. hirta* contains a wider range of phytochemicals compared to *T. procumbens*, particularly in its aqueous extract, which may contribute to its relatively enhanced biological activity.

Pre-Exposure β-Lactamase Production by Isolates

The prevalence of β-lactamase production among *Salmonella enterica* and *Staphylococcus aureus* isolates before exposure to plant extracts is shown in Table 6 and Table 7, respectively.

Table 6: Pre-Exposure Patterns of β-Lactamase Production of *Salmonella enterica* (n=24)

Production	Number	Percentage
Positive	20	83.3%
Negative	4	16.7%

Out of 24 *Salmonella enterica* isolates, 20 (83.3%) were β-lactamase-positive, and 4 (16.7%) were β-lactamase-negative.

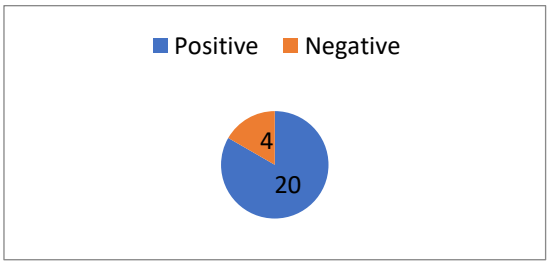

Figure 3: Pie chart: Pre-exposure Patterns of β-lactamase Production of *Salmonella enterica* in Percentage (n=24)

Table 7: Pre-Exposure Patterns of β-Lactamase Production of *Staphylococcus aureus* (n=20)

Production	Number	Percentage
Positive	17	85%
Negative	3	15%

Among 20 *Staphylococcus aureus* isolates, 17 (85%) were β-lactamase-positive, and 3 (15%) were β-lactamase-negative.

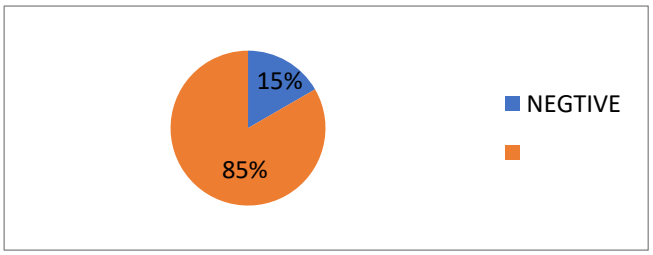

Figure 4: Pre-Exposure Patterns of β-Lactamase Production of *Staphylococcus aureus* (n=20)

Post-Exposure β-Lactamase Production by Isolates

The post-exposure β-lactamase production patterns of *Salmonella enterica* and *Staphylococcus aureus* isolates after treatment with plant extracts are presented in Table 8 and Table 9, respectively.

Table 8: Post-Exposure Patterns of β-Lactamase Production of *Salmonella enterica* (n=24)

Production	Number	Percentage
Positive	20	83.3%
Negative	4	16.7%

After exposure, 20 (83.3%) of *Salmonella enterica* isolates remained β-lactamase-positive, while 4 (16.7%) became β-lactamase-negative.

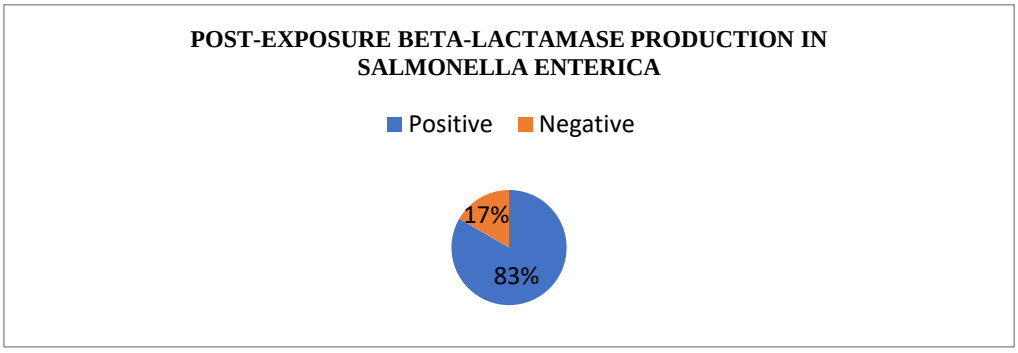

Figure 5: Pie Chart Post-exposure Patterns of β-lactamase Production of *Salmonella enterica* in Percentage (n=24)

Table 9: Post-Exposure Patterns of β-Lactamase Production of *Staphylococcus aureus* (n=20)

Production	Number	Percentage
Positive	17	85%
Negative	3	15%

Similarly, 17 (85%) of *Staphylococcus aureus* isolates remained β-lactamase-positive post-exposure, with 3 (15%) becoming β-lactamase-negative.

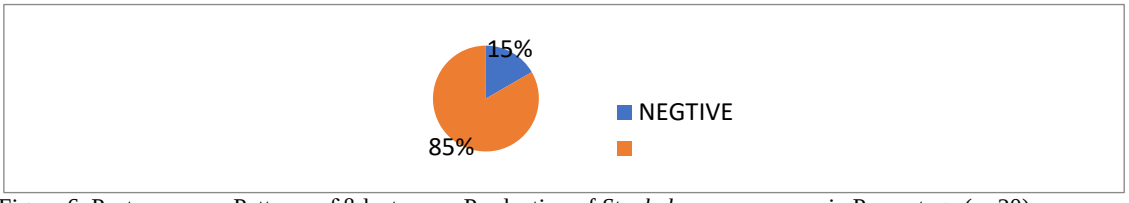

Figure 6: Post-exposure Patterns of β-lactamase Production of *Staphylococcus aureus* in Percentage (n=20)

Antibacterial Activity of *Tridax procumbens* and *Euphorbia hirta* Extracts

The antibacterial activity of *Tridax procumbens* and *Euphorbia hirta* extracts against *Salmonella enterica* and *Staphylococcus aureus* was evaluated at various concentrations. The results are summarized in Table 10 and Table 11.

Table 10: Antibacterial Activity of *Tridax procumbens* Extracts (Inhibition Zone in mm)

Extract Type	Isolate	100 mg/dL	200 mg/dL	300 mg/dL	600 mg/dL	Negative Control
Ethanolic	SE 01	0	0	0	8	0
	SE 02	0	0	0	8	0
	SA 01	0	0	0	8	0

Extract Type	Isolate	100 mg/dL	200 mg/dL	300 mg/dL	600 mg/dL	Negative Control
	SA 04	0	0	0	9	0
Aqueous	SE 01	0	0	0	6	0
	SE 02	0	0	0	6	0
	SA 01	0	0	0	6	0
	SA 04	0	0	0	6	0

Table 11: Antibacterial Activity of *Euphorbia hirta* Extracts (Inhibition Zone in mm)

Extract Type	Isolate	100 mg/dL	200 mg/dL	300 mg/dL	600 mg/dL	Negative Control
Ethanolic	SE 01	0	0	0	8	0
	SE 02	0	0	0	8	0
	SA 01	0	0	0	8	0
	SA 04	0	0	0	8	0
Aqueous	SE 01	0	0	0	6	0
	SE 02	0	0	0	6	0
	SA 01	0	0	0	8	0
	SA 04	0	0	0	8	0

Both *Tridax procumbens* and *Euphorbia hirta* extracts exhibited antibacterial activity only at the highest concentration tested (600 mg/dL). The ethanolic extracts of *Tridax procumbens* showed inhibition zones of 8 mm for *Salmonella enterica* and 8-9 mm for *Staphylococcus aureus*. Its aqueous extracts showed 6 mm inhibition zones for both pathogens. Similarly, *Euphorbia hirta* ethanolic extracts yielded 8 mm inhibition zones for both pathogens, while its aqueous extracts showed 6 mm for *Salmonella enterica* and 8 mm for *Staphylococcus aureus*.

Discussion

The present study aimed to evaluate the antibacterial and anti-β-lactamase activities of *Tridax procumbens* and *Euphorbia hirta* extracts against clinical isolates of β-lactamase-producing *Salmonella enterica* and *Staphylococcus aureus*. The findings provide crucial insights into the biochemical characteristics, antibiotic resistance profiles, β-lactamase production, and the antibacterial efficacy of these plant extracts, contributing to the ongoing global effort to combat antimicrobial resistance.

Biochemical Characterization and Antibiotic Resistance

The biochemical characterization of *Salmonella enterica* and *Staphylococcus aureus* isolates (Table 2) confirmed their identities based on established microbiological criteria. *Staphylococcus aureus* isolates consistently tested positive for catalase and coagulase, and fermented mannitol, aligning with their known biochemical profile. Conversely, *Salmonella enterica* isolates demonstrated motility, citrate utilization, and sulfur deposition, which are characteristic features of this Gram-negative bacterium. Accurate identification of these pathogens is a foundational step for effective clinical management and targeted therapeutic interventions.

The antibiotic susceptibility patterns revealed an alarming prevalence of resistance to β-lactam antibiotics among both *Salmonella enterica* and *Staphylococcus aureus* isolates (Tables 3 and 4). *Salmonella enterica* exhibited resistance rates as high as 95.8% for Ampiclox, Amoxicillin, and Augmentin, and a striking 100% resistance to Cefuroxime. Similarly, *Staphylococcus aureus* isolates showed significant resistance, with 80% to 95% resistance to the same antibiotics. These findings are consistent with the global trend of increasing antimicrobial resistance, particularly to β-lactam antibiotics, which are frequently used in clinical practice (Lopez-Malo et al., 2020; Chinemerem et al., 2022). The high resistance rates observed underscore the urgent need for alternative treatment strategies and emphasize the critical role of antimicrobial stewardship programs to curb the overuse and misuse of these vital drugs (Ramos et al., 2020).

β-Lactamase Production and Inhibition

The high prevalence of β-lactamase production among the isolates was a key finding, with 83.3% of *Salmonella enterica* and 85% of *Staphylococcus aureus* isolates testing positive for β-lactamase before exposure to the plant extracts (Tables 6 and 7). This confirms that β-lactamase enzymes are a primary mechanism of resistance in these clinical isolates, enabling them to hydrolyze the β-lactam ring of antibiotics and render them ineffective (Bush and Bradford, 2020; Kurittu et al., 2021). The widespread presence of these enzymes poses a significant challenge to the efficacy of conventional β-lactam antibiotics.

Following exposure to the plant extracts, the majority of isolates retained their β-lactamase-positive status (83.3% for *Salmonella enterica* and 85% for *Staphylococcus aureus*) (Tables 8 and 9). This suggests that the crude extracts of *Tridax procumbens* and *Euphorbia hirta*, at the tested concentrations, had limited direct inhibitory effects on β-lactamase production or activity in most isolates. However, a small but notable proportion of isolates (16.7% of *Salmonella enterica* and 15% of *Staphylococcus aureus*) became β-lactamase-negative after exposure. This promising observation warrants further investigation to identify the specific compounds responsible for this partial inhibition and to elucidate their mechanisms of action. Such compounds could potentially serve as novel β-lactamase inhibitors or sensitizers, enhancing the efficacy of existing antibiotics.

Antibacterial Activity of Plant Extracts

Both *Tridax procumbens* and *Euphorbia hirta* extracts demonstrated antibacterial activity against *Salmonella enterica* and *Staphylococcus aureus*, albeit only at the highest concentration tested (600 mg/dL) (Tables 10 and 11). The ethanolic extracts generally exhibited slightly higher efficacy compared to their aqueous counterparts, with inhibition zones ranging from 8 mm to 9 mm, while aqueous extracts showed milder activity (6 mm to 8 mm). This difference in efficacy between extraction solvents highlights the importance of optimizing extraction methods to maximize the yield and potency of bioactive compounds. The lack of activity at lower concentrations suggests that the concentration of active

compounds in these crude extracts may be insufficient to exert significant antibacterial effects at lower doses.

The qualitative phytochemical analysis (Table 5) revealed the presence of glycosides in both *Tridax procumbens* extracts. *Euphorbia hirta* extracts, on the other hand, contained a richer profile of bioactive compounds, including glycosides, tannins, saponins, and phenols. These phytochemicals are well-known for their diverse biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (Lopez-Malo et al., 2020). The observed antibacterial activity of the plant extracts can be attributed to the synergistic or additive effects of these compounds. For instance, tannins are known to exert antimicrobial effects by binding to bacterial cell walls and enzymes, while phenols can disrupt bacterial membranes. The presence of these compounds supports the traditional medicinal uses of these plants and provides a scientific basis for their observed antibacterial properties.

Implications and Future Directions

The findings of this study underscore the potential of *Tridax procumbens* and *Euphorbia hirta* as sources of natural antimicrobial agents, particularly in the context of rising antibiotic resistance. While the crude extracts showed promising antibacterial activity at higher concentrations, their limited anti-β-lactamase activity suggests that they may be more effective as complementary therapies rather than standalone treatments for β-lactamase-producing bacteria. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for the observed antibacterial and partial anti-β-lactamase effects. This would involve advanced analytical techniques to identify the chemical structures of these compounds and further *in vitro* and *in vivo* studies to elucidate their precise mechanisms of action. Additionally, exploring synergistic combinations of these plant extracts with conventional antibiotics or known β-lactamase inhibitors could offer novel strategies to overcome drug resistance and enhance therapeutic outcomes.

β-Lactamase Production and Inhibition

The high prevalence of β-lactamase production among the isolates was a key finding, with 83.3% of *Salmonella enterica* and 85% of *Staphylococcus aureus* isolates testing positive before exposure to the plant extracts (Tables 6 and 7). This confirms that β-lactamase enzymes remain a dominant mechanism of resistance in these organisms, enabling them to hydrolyze the β-lactam ring and deactivate commonly used antibiotics.

After exposure to the plant extracts, most isolates retained their β-lactamase–positive status (83.3% for *S. enterica* and 85% for *S. aureus*) (Tables 8 and 9). This outcome indicates that the crude extracts of *Tridax procumbens* and *Euphorbia hirta*, at the tested concentrations, had **limited direct inhibitory effects** on β-lactamase production or activity.

Why the Extracts Were Ineffective against β-Lactamase in Most Isolates

Several factors may explain why the extracts did not significantly inhibit β-lactamase activity:

Enzyme Diversity and Robustness

The β-lactamases produced by clinical isolates, particularly TEM, SHV, and CTX-M types are structurally diverse and often highly efficient. Crude plant extracts may lack compounds with adequate affinity or specificity to bind and inactivate these enzymes. Some β-lactamases (e.g., ESBLs and AmpC variants) are inherently more resistant to inhibition, even by established synthetic inhibitors.

Insufficient Concentration of Active Compounds

Although the extracts showed antibacterial activity at 600 mg/dL, the concentration of specific β-lactamase-inhibiting phytochemicals within the crude extracts may have been too low to exert measurable effects on enzyme activity. Plant extracts often contain a complex mixture of compounds, many of which may be pharmacologically inert.

Compound Specificity and Structural Limitations

β-lactamase inhibition requires molecules that structurally mimic β-lactam antibiotics or possess strong reactive groups capable of binding the active site. The phytochemicals detected, such as glycosides, tannins, saponins, and phenols—may not possess the structural characteristics needed to competitively or irreversibly inhibit β-lactamase enzymes.

Complexity of Crude Extracts

Crude extracts contain numerous compounds that may interfere with one another, reducing the potency of potential β-lactamase inhibitors. Some constituents may even hinder penetration into bacterial cells, limiting interaction with intracellular β-lactamases.

Possible Induction of β-Lactamase Expression

Exposure to plant-derived stress compounds may trigger bacterial defense mechanisms, including the inducible expression of β-lactamase genes, thereby masking or counteracting any inhibitory effects of the extracts.

Despite the limited inhibition, a small number of isolates (16.7% of *S. enterica* and 15% of *S. aureus*) converted to β-lactamase-negative following exposure. This suggests that certain phytochemicals may possess weak or selective inhibitory activity and highlights the need for further fractionation and purification to isolate compounds with true β-lactamase-modifying potential.

CONCLUSION

This study provides valuable insights into the antibacterial and anti-β-lactamase activities of *Euphorbia hirta* and *Tridax procumbens* against clinical isolates of β-lactamase-producing *Salmonella enterica* and *Staphylococcus aureus*. The findings highlight the alarming prevalence of β-lactam resistance among these pathogens, underscoring the urgent need for novel therapeutic strategies.

While the crude extracts of *Euphorbia hirta* and *Tridax procumbens* demonstrated mild to moderate antibacterial activity, particularly at higher concentrations, their anti-β-lactamase effects were limited. This suggests that these plant extracts may serve as complementary agents in combating bacterial infections rather than standalone treatments for β-lactamase-producing strains. The presence of various phytochemicals, such as glycosides, tannins, saponins, and phenols, in these plants likely contributes to their observed antibacterial properties.

Future research should focus on the isolation and characterization of specific bioactive compounds from these plants, followed by detailed studies to elucidate their mechanisms of action. Furthermore, investigating the synergistic potential of these plant-derived compounds in combination with conventional antibiotics or known β-lactamase inhibitors could pave the way for the development of new and effective strategies to overcome the growing challenge of antimicrobial resistance.

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