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Research Article

Isolation and Identification of Clinical Bacterial Isolates and Their Resistance to Antibiotics and a Medicinal Plant Mixture (Turmeric, Ginger, and Indian Costus)

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Abstract

Background

The rise of antibiotic resistance in *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) has intensified the search for natural antibacterial alternatives. This study evaluates the antibacterial efficacy of ethanolic (EE) and aqueous (AE) extracts from turmeric (Curcuma longa), ginger (Zingiber officinale), and Indian costus (Saussurea costus) against clinical isolates of *S. aureus and E. coli*.

Method

A six-month laboratory-based study (April–September 2024) analyzed bacterial isolates from patient samples in Aden Governorate, Yemen. Isolates were identified using selective media Mannitol Salt Agar (MSA) for *S. aureus*, Eosin Methylene Blue Agar (EMB) for *E. coli*) and confirmed through biochemical tests. Antibacterial activity was assessed via agar well diffusion and minimum inhibitory concentration (MIC) assays. Phytochemical and physiochemical analyses identified active compounds in the extracts.

Results

Of 57 clinical samples, *S. aureus* was isolated from 52.63% and *E. coli* from 21.05%, while 26.32% were excluded due to contamination. The EE exhibited dose-dependent antibacterial activity against *E. coli*, with inhibition zones of 13–19 mm, but no activity against *S. aureus*. The AE showed no antibacterial effects. Antibiotic susceptibility testing revealed *S. aureus* was highly susceptible to Linezolid (96%) and Roxithromycin (96%) but resistant to Cloxacillin (0%). *E. coli* showed high resistance to Ampicillin/Sulbactam (57.14%) and Cefotaxime (55%) but susceptibility to Ciprofloxacin (90%) and Amikacin (95%).

Conclusion

The EE shows promise as a natural alternative for treating *E. coli* infections, particularly amid rising antibiotic resistance. However, its inefficacy against *S. aureus* highlights the need for further research to optimize extraction methods and enhance activity against Gram-positive bacteria. This study supports the potential of plant-based antimicrobials as alternatives to synthetic antibiotics.

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1. Introduction

Antibiotic resistance among bacterial pathogens is an escalating global health crisis, necessitating the search for alternative antimicrobial agents [1]. Two clinically and epidemiologically significant bacteria, *S. aureus* and *E. coli*, are associated with a wide spectrum of infections and pose major public health risks [2].

S. aureus is a Gram-positive bacterium that naturally colonizes human skin and mucous membranes but can cause opportunistic infections when host defenses are compromised [3]. It is responsible for a range of diseases, from mild skin infections to life-threatening conditions such as pneumonia, sepsis, and endocarditis [4]. The bacterium's pathogenicity is attributed to its ability to produce toxins, evade the immune system, and develop biofilms [5]. A major concern is the rise of methicillin-resistant *S. aureus* (MRSA), which has significantly reduced treatment options, making infection management increasingly challenging [6].

Similarly, *E. coli*, a Gram-negative bacterium that resides in the intestines of warm-blooded animals, includes pathogenic strains such as *E. coli* O157:H7, which produces Shiga toxins and causes severe gastrointestinal illnesses, hemolytic uremic syndrome, and systemic infections [7]. In addition to being a major cause of foodborne illnesses, *E. coli* has demonstrated increasing resistance to β-lactam antibiotics and other commonly used treatments [8; 9; 10]. The prevalence of multidrug-resistant (MDR) *E. coli* strains in clinical and environmental settings poses a significant challenge to infection control and public health [11; 12].

Given the rapid emergence of antibiotic-resistant bacterial strains, there is a growing interest in natural antimicrobial alternatives derived from medicinal plants [13; 14]. Many plant species produce bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties, making them potential candidates for combating resistant bacterial infections [15]. Among these, turmeric (Curcuma longa), ginger (Zingiber officinale), and Indian costus (Saussurea costus) have been widely recognized for their therapeutic potential and historical use in traditional medicine [14].

Turmeric contains curcumin, a polyphenolic compound known for its antibacterial, antifungal, and anti-inflammatory properties [13]. Curcumin has been shown to inhibit bacterial growth by disrupting cell membrane integrity and interfering with quorum sensing mechanisms [15]. Ginger is rich in gingerol, shogaol, and zingerone, which have demonstrated antimicrobial activity against a range of bacterial pathogens by targeting bacterial adhesion and metabolic pathways [14]. Indian costus is a lesser-known medicinal plant but has been reported to possess significant antimicrobial potential due to its high content of terpenoids, flavonoids, and alkaloids, which inhibit bacterial proliferation and biofilm formation [16].

Despite promising evidence of their antimicrobial activity, limited studies have investigated the efficacy of combined extracts of these three plants, particularly in the form of (EE) and (AE). The solubility and extraction efficiency of bioactive compounds vary depending on the solvent used, influencing the antimicrobial potential of the extracts [17; 18]. Ethanolic extraction is often preferred for isolating non-polar phytochemicals such as flavonoids and polyphenols, whereas (AE) is more effective for polar compounds like tannins and saponins [19; 20].

This study aims to: Isolate and identify *S. aureus* and *E. coli* from clinical samples using selective media and biochemical tests. Determine the antibiotic susceptibility of these bacterial isolates to assess resistance patterns. Analyze the phytochemical and physicochemical properties of ethanolic and aqueous extracts of a standardized blend of turmeric, ginger, and Indian costus. Evaluate the antibacterial efficacy of these plant extracts against *S. aureus* and *E. coli*, comparing the effectiveness of ethanolic vs. aqueous extracts.

2. Materials and Methods

2.1. Study Design

This study is an experimental laboratory-based investigation designed to isolate and identify clinical bacterial strains, assess their antibiotic resistance patterns, and evaluate the antibacterial efficacy of a blend of medicinal plant extracts against these strains. The study employs controlled laboratory conditions to ensure the accuracy and reproducibility of the results.

2.2. Study Area and Ethical Considerations

The study was conducted in Aden, Yemen, a strategically important port city with significant regional trade activity. Ethical approval for the study was obtained from the Ethics Committee of the College of Medicine at Aden University (Approval Code: REC-202-2024). To ensure patient confidentiality, all data were anonymized, and no personally identifiable information was collected or used.

The study duration is six months, from April to September 2024.

2.3. Sample Collection and Maintenance

Clinical samples suspected to contain (*S. aureus*) and (*E. coli*), including blood, urine, pus, and pleural fluid, were collected from hospitals in Aden, Yemen. To ensure long-term preservation, bacterial isolates were stored in a solution containing 85% nutrient broth and 15% glycerol at -20°C.

2.4. Source and Criteria for Sample Collection

Samples were obtained from patients diagnosed with bacterial infections at hospitals in Aden. The inclusion criteria involved samples from patients with confirmed infections, while exclusion criteria included samples with mixed bacterial infections or those showing signs of contamination. This approach ensured the collection of high-quality, uncontaminated samples for accurate analysis.

2.5. Microbiological Identification

2.5.1. Gram Staining

Differentiated *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) using crystal violet, Lugol's solution, alcohol, and Safranin.

2.5.2. Culture Media Preparation and Inoculation

General and selective media (Blood Agar (BA), MacConkey Agar (MA), (MSA), EMB, and Xylen Xylose Lysine Deoxycholate (XLD) were sterilized, incubated to ensure sterility, and inoculated with human samples. All media used are (Hi Media, India). Plates were incubated at 37°C for 24 hours for bacterial growth. *S. aureus* was identified using (MSA) and Vogel Johnson Agar (VJA), while *E. coli* was identified using EMB and XLD Agar.

2.5.3. Biochemical Tests for Bacterial Differentiation

Biochemical tests are essential for identifying and differentiating bacterial species. All materials used in these tests are (Hi Media, India). The Catalase test distinguishes Staphylococcus (positive) from Streptococcus (negative). The Coagulase test confirms the presence of *S. aureus* by detecting plasma coagulation. Meanwhile, the Oxidase test identifies oxidase-positive bacteria, distinguishing them from *E. coli* (negative) [21]. Methyl Red (MR) test, detected acid production in *E. coli* [22]. Indole test, confirmed *E. coli* by indole production from tryptophan [23]. Citrate utilization test, distinguished *E. coli* (negative) from other enteric bacteria [24]. DNase test, identified *S. aureus* by DNA hydrolysis. Capsule staining, visualized bacterial capsules [25]. Urease test, differentiated urease-positive bacteria from *E. coli* [23]. Kligler iron agar, assessed carbohydrate fermentation and H_2S production [26]. Voges-Proskauer (VP) test, detected acetoin production for differentiating enteric bacteria [23]. Sugar fermentation test, evaluated glucose and fructose fermentation [27].

2.6. Plant Sample Collection and Preparation

Root samples of Curcuma longa (turmeric), Zingiber officinale (ginger), and Saussurea costus (Indian costus) were collected from local sources and authenticated by Dr. Othman Al-Hawshabi, Professor of Taxonomy at Aden University. The roots were thoroughly cleaned, dried, and ground into fine powder before extraction.

2.6.1. Ethanolic Extraction

Soxhlet extraction was performed using 40 g of root powder and 400 mL of 70% ethanol, following established protocols [17; 18; 28]. The EE was filtered, concentrated by evaporation, dried, and stored at 4°C until further use.

2.6.2. Aqueous Extraction

For the AE, 40 g of root powder was stirred with 400 mL of distilled water and allowed to extract at room temperature for 24 hours, following standard procedures [17; 18]. The extract was then filtered, evaporated at 50°C, and stored at 4°C for further analysis.

2.6.3. Preparation of Extract Concentrations

Stock solutions were prepared at 800 mg/mL by dissolving 1 g of dried extract in 1.25 mL of either: Distilled water for the AE. Dimethyl sulfoxide (DMSO) for the EE.

2.6.4. Serial dilutions were prepared to obtain final working concentrations of:

- 40 mg/mL (50 µL of stock solution)
- 32 mg/mL (40 µL of stock solution)
- 24 mg/mL (30 µL of stock solution)
- 16 mg/mL (20 µL of stock solution)
- 8 mg/mL (10 µL of stock solution)

• 4 mg/mL (5 µL of stock solution)

Each dilution was adjusted to the required final volume using the respective solvent (distilled water or DMSO).

2.7. Physicochemical Analysis

2.7.1. Determination of Extractive Value

Different solvents, such as 70% ethanol (Soxhlet) and water (decoction and magnetic stirring), were used to extract active compounds from medicinal plants [29; 17].

The extractive value was calculated using the following formula:

$$The\ extractive\ value\ \% = \frac{Weight\ of\ the\ extract\ yield}{Weight\ of\ the\ air-dried\ drug} \times 100$$

2.7.2. Loss on Drying (Moisture Content)

The moisture content was determined by drying the sample at 135°C until a constant weight was achieved [30; 31]. The moisture percentage was calculated using the formula:

$$Moisture\ (\%) = \frac{Sample\ weight\ before\ drying - sample\ weight\ after\ drying}{Weight\ of\ the\ sample} \times 100$$

2.7.3. Ash Content Determination

The sample was incinerated in a muffle furnace at 500°C until it turned into white ash [30; 32]. The ash content was calculated using the formula:

$$Ash\ (\%) = \frac{Ash\ weight}{Weight\ of\ the\ sample} \times 100$$

2.7.4. pH Determination

The pH of the aqueous extract was measured using a pH meter after dissolving 5 g of plant powder in 50 mL of distilled water [33].

2.8 Phytochemical Screening

The phytochemical screening of the plant extract was carried out to qualitatively detect the presence of various bioactive compounds, including alkaloids, tannins, saponins, steroids, triterpenoids, cardiac glycosides, and flavonoids, Phenols, Resins, Pheochromatins, Triterpenoids, Primary and Secondary Amino Acids or Amines. These compounds were identified through characteristic color changes following standard procedures as described by [29].

2.9. Antibiotics and Quality Control

The antibiotics used in this study were commercially available discs obtained from HiMedia, a reputable supplier of microbiological products. These discs are widely used for antimicrobial susceptibility testing (AST) in laboratories across Yemen. The quality of the antibiotics is ensured through HiMedia's adherence to international standards for purity and performance. Specific details regarding their purity and validation can be obtained directly from the manufacturer, HiMedia, if required.

2.10. Antibiotics and Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [34]. Commercially available HiMedia antibiotic discs were employed, including those targeting Gram-positive bacteria: Ampicillin/Sulbactam (20 µg), Co-Trimoxazole (25 µg), Cephalexin (30 µg), Tetracycline (30 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Linezolid (30 µg), Cloxacillin (5 µg), Roxithromycin (15 µg and 30 µg), Lincomycin (2 µg), Gentamicin (10 µg), and Ampicillin (10 µg). Bacterial suspensions were standardized to 0.5 McFarland, inoculated onto Mueller-Hinton agar plates, and discs were aseptically applied. After 24-hour incubation at 37°C, inhibition zones were measured and interpreted as sensitive, intermediate, or resistant per CLSI criteria. HiMedia's adherence to international standards ensured antibiotic disc quality, with purity and validation data available upon request.

2.11. Statistical Analysis

Bacterial growth inhibition and extract efficacy were analyzed using GenStat 5 software. The results were expressed as the mean ± standard deviation (SD). Statistical significance was determined using ANOVA or T-tests, as appropriate. The Least Significant Difference (L.S.D) at 5% was calculated to assess significant differences between sample types and dilutions.

3. Results

A total of 57 human clinical samples were analyzed for the presence of *S. aureus* and *E. coli*. Among these, 30 samples (52.63%) tested positive for *S. aureus*, while 12 samples (21.05%) tested positive for *E. coli*. The remaining 15 samples (26.32%) were excluded as they did not yield the target bacterial isolates.

Table 1 summarizes the prevalence and distribution of *S. aureus* and *E. coli* in the collected clinical samples

Total Samples (100%)	Positive Samples (%)	Excluded Samples (%)	*S. aureus* Positive (%)	*E. coli* Positive (%)
57	42 (73.68%)	15 (26.32%)	30 (52.63%)	12 (21.05%)

3.1. Distribution of Isolates by Sample Source

Clinical samples were collected from various sources, including tonsils, pus, urine, pleural fluid, and blood. The distribution of *S. aureus* and *E. coli* isolates across these sources is presented in *Table 2*.

The highest isolation rates of *S. aureus* were observed in tonsil samples (71.43%) and blood samples (62.5%), while the lowest was in urine samples (26.67%). In contrast, *E. coli* was most frequently isolated from urine samples (46.67%) and pus samples (26.67%), indicating a higher prevalence in urinary tract infections and suppurative infections.

3.2. Results of Isolation and Identification in Different Media

The isolation and identification of *S. aureus* and *E. coli* were performed using various selective and differential culture media, as summarized in *Table 3*.

S. aureus produced yellow colonies on (MSA), black colonies on (VJA), and beta-hemolysis on BA, indicating its ability to ferment mannitol and hemolyze red blood cells. On Nutrient Agar, it formed large, opaque white colonies characteristic of *S. aureus*. *E. coli* exhibited green metallic sheen colonies on (EMB) Agar, pink colonies on MA, and yellow colonies on (XLD) Agar, reflecting its lactose-fermenting capabilities. It also displayed partial hemolysis (alpha-hemolysis) on Blood Agar.

Table 2: Distribution of *S. aureus* and *E. coli* Isolates by Sample Source

Sample Source	Total Samples (%)	*S. aureus* Positive (%)	*E. coli* Positive (%)	Excluded Samples (%)
Tonsils	14 (24.56%)	10 (71.43%)	0	4 (28.57%)
Pus	15 (26.32%)	8 (53.33%)	4 (26.67%)	3 (20%)
Urine	15 (26.32%)	4 (26.67%)	7 (46.67%)	4 (26.67%)
Pleural Fluid	5 (8.77%)	3 (60%)	0	2 (40%)
Blood	8 (14.04%)	5 (62.5%)	1 (12.5%)	2 (25%)
Total	**57 (100%)**	**30 (52.63%)**	**12 (21.05%)**	**15 (26.32%)**

Table 3: Isolation and Identification of *S. aureus* and *E. coli* on Selective and Differential Media

Media	*Staphylococcus aureus*	*Escherichia coli*
Nutrient Agar	Large, opaque white colonies	Small, circular, white to grayish colonies
Blood Agar	White colonies with beta-hemolysis	Gray colonies with alpha-hemolysis
MacConkey Agar	—	Pink colonies with darker coloration around colonies
Mannitol Salt Agar	Yellow colonies (mannitol fermentation)	—
Vogel & Johnson Agar	Black colonies (tellurite reduction)	—
EMB Agar	—	Purple to black colonies with green metallic sheen
XLD Agar	—	Circular yellow colonies

3.3.Biochemical Tests for Bacterial Identification

The biochemical tests confirmed the physiological and metabolic characteristics of *S. aureus* and *E. coli*, aiding in their identification. *Table 4* summarizes the test results: Capsule test: positive for *E. coli*, a negative for *S. aureus*. Catalase test: positive for both bacteria. Coagulase test: positive for *S. aureus*, distinguishing it from other Staphylococcal species. Hemolysis: *S. aureus* exhibited beta hemolysis, while *E. coli* showed alpha hemolysis. DNase test: positive for *S. aureus*, negative for *E. coli*. Citrate utilization: positive for *S. aureus*, negative for *E. coli*. Indole test: positive for *E. coli*, negative for *S. aureus*. Voges-Proskauer (VP) test: positive for *S. aureus*, negative for *E. coli*. Methyl red (MR) test: positive for both bacteria. Motility test: positive for *E. coli*, negative for *S. aureus*. Hydrogen sulfide (H_2S) production: negative for both. Urease test: positive for *S. aureus*, negative for *E. coli*. Sugar fermentation: *S. aureus* fermented glucose, mannitol, lactose, and sucrose. *E. coli* showed variable sugar fermentation and produced gas from glucose.

3.4. Mean Counts of *Escherichia coli* at Different Dilutions

The mean bacterial counts of *E. coli* were evaluated across serial dilutions (10^{-1} to 10^{-6}) and different human sample types (*Table 5*). The results show a progressive decline in bacterial counts with increasing dilutions, reflecting the expected reduction in bacterial concentration: Blood: High bacterial counts (1.000) persisted in the first four dilutions, slightly decreasing to 0.889 at 10^{-5} and 0.778 at 10^{-6} (overall mean: 0.889). Urine: Counts remained constant at 1.000 in early dilutions, dropping to 0.778 at 10^{-6} (overall mean: 0.778). Pus: Moderate counts (1.000) in early dilutions declined to 0.833 at 10^{-6} (overall mean: 0.833). Tonsils: Bacterial counts remained consistently high (1.000 across all dilutions), resulting in an overall mean of 0.944. Pleural Fluid: Initial counts (1.000) decreased significantly to 0.278 at 10^{-6} (overall mean: 0.278).

The Least Significant Difference (L.S.D) at 5% for sample types (H) and dilutions (D) was 0.1809, indicating statistically significant differences in mean counts exceeding this threshold. There was no significant interaction (H×D) between sample types and dilutions.

Table 4: Biochemical Identification of *S. aureus* and *E. coli* Isolates

Biochemical Test	*S. aureus*	*E. coli*
Capsule	-	+
Catalase	+	+
Coagulase	+	-
Hemolysis	Beta (B)	Alpha (∝)
Oxidase	-	-
DNase	+	-
Gram Stain	Blue (Cocci)	Red (Rod)
Citrate	+	-
Indole	-	+
Voges-Proskauer (VP)	+	-
Methyl Red (MR)	+	+
Motility	-	+
Hydrogen Sulfide (H_2S)	-	-
Urease	+	-
Lactose Fermentation	+	+
Fructose Fermentation	+	-
Mannitol Fermentation	+	+
Xylose Fermentation	-	+
Sucrose Fermentation	+	+

Table 5: Mean *E. coli* Counts Across Serial Dilutions in Human Samples

Sample Type	Dilutions (Mean Counts)	Overall Mean
Blood	1.000 → 0.889 → 0.778	0.889
Urine	1.000 → 0.778	0.778
Pus	1.000 → 0.833	0.833
Tonsils	1.000 (all dilutions)	0.944
Pleural Fluid	1.000 → 0.278	0.278

3.5. Mean Counts of *Staphylococcus aureus* at Different Dilutions

The bacterial counts of *S. aureus* also declined with increasing dilutions (Table 6), reflecting the dilution effect: Blood: Initial counts of 0.333 at 10^{-1} declined to 0.111 at 10^{-2}. Pus: No detectable *S. aureus* at any dilution (0.000 across all dilutions). Urine: Started at 0.667 at 10^{-1}, decreasing to 0.333 at 10^{-2}.

Table 6: Mean *S. aureus* Counts Across Serial Dilutions in Human Samples

Sample Type	Dilutions (Mean Counts)	Overall Mean
Blood	0.333 → 0.111	0.111
Pus	0.000 (all dilutions)	0.000
Urine	0.667 → 0.333	0.333

Overall Mean: 0.333

3.6. Antibiotic Susceptibility Results for *S. aureus*

The antibiotic susceptibility test results for *S. aureus* are summarized in *Table 7*. Linezolid (LZ) showed 96% susceptibility, Roxithromycin (RF/RO) showed 96% and 88% susceptibility, and Ampicillin/Sulbactam (AS) showed 94% susceptibility. Gentamicin (GM) exhibited 82% susceptibility, Lincomycin (LM) 86%, Levofloxacin (LE) 72%, and Co-Trimoxazole (BA/COT) 76% and 66% susceptibility. Cloxacillin (CX) showed 0% susceptibility, Cephalexin (PR) 30% resistance, Cefotaxime (CF/CTX) 26% resistance and 56% intermediate susceptibility, and Ciprofloxacin (CP) 58% intermediate susceptibility.

3.7. Susceptibility and Resistance of *E. coli* to Antibiotics

The results for *E. coli* revealed that 35.71% of antibiotics showed sensitivity (S), with effective inhibition zones (20–30 mm), including Ciprofloxacin, Levofloxacin, Ofloxacin, Amikacin, and Co-Trimoxazole. Tetracycline showed intermediate susceptibility (I) with a 13 mm inhibition zone. Resistance (R) was observed in 57.14% of antibiotics, including Ampicillin/Sulbactam, Cefotaxime, and Piperacillin/Tazobactam (*Table 8*).

Table 7: Antibiotic Sensitivity Test Results for *S. aureus*

Antibiotic	Outcome	Percentage
Ampicillin/Sulbactam (AS)	S	94%
Co-Trimoxazole (BA)	S	76%
Cephalexin (PR)	R	30%
Tetracycline (TE)	S	62%
Cefotaxime (CF)	R	26%
Ciprofloxacin (CP)	I	58%
Levofloxacin (LE)	S	72%
Linezolid (LZ)	S	96%
Cloxacillin (CX)	R	0%
Roxithromycin (RF)	S	96%
Lincomycin (LM)	S	86%
Gentamicin (GM)	S	82%
Cefotaxime (CTX)	I	56%
Co-Trimoxazole (COT)	S	66%
Ampicillin (AMP)	S	78%
Roxithromycin (RO)	S	88%

Table 8: Antibiotic Sensitivity Test Results for *E. coli*.

Antibiotic	Outcome	Percentage
Ampicillin/Sulbactam (AS)	S	65%
Co-Trimoxazole (BA)	R	30%
Cefotaxime (CF)	I	55%
Piperacillin/Tazobactam (TZP)	S	70%
Chloramphenicol (CH)	S	60%
Ciprofloxacin (CP)	S	90%
Ceftriaxone (CR)	S	85%
Tetracycline (TE)	I	50%
Ofloxacin (OF)	S	60%
Levofloxacin (LE)	S	85%
Gentamicin (GM)	I	55%
Amikacin (AK)	S	95%
Polymyxin B (P)	S	90%
Ampicillin (AMP)	I	57%
Erythromycin (E)	S	90%

3.8. Antimicrobial Activity of Ethanolic Extracts

The EE demonstrated dose-dependent antibacterial activity against *E. coli*, with inhibition zones decreasing from 19 mm at 50 mg/mL to 13 mm at 5 mg/mL (*Table 9, Figures 1 and 2*). In contrast, no inhibition was observed against S. aureus at any tested concentration.

Table 9: Inhibition Zones (mm) of Ethanolic Extract Against *E. coli* and *S. aureus*

Concentration (mg/mL)	*E. coli* (mm)	*S. aureus* (mm)
50 mg/mL	19 mm	0 mm
40 mg/mL	18 mm	0 mm
30 mg/mL	18 mm	0 mm
20 mg/mL	18 mm	0 mm
10 mg/mL	16 mm	0 mm
5 mg/mL	13 mm	0 mm

Figure 1: Result of Ethanolic Extract on *Staphylococcus aureus*

Figure 2: Result of ethanolic extract on *Escherichia coli*.

3.9. Antimicrobial Activity of Aqueous Extracts

The AE showed no antibacterial activity against *E. coli* or S. aureus at any tested concentration (*Table 10, Figures 3 and 4*). No inhibition zones were observed at any concentration.

Table 10: Inhibition Zones (mm) of Aqueous Extract Against *E. coli* and *S. aureus*

Concentration (mg/mL)	E. coli (mm)	S. aureus (mm)
50 mg/mL	0 mm	0 mm
40 mg/mL	0 mm	0 mm
30 mg/mL	0 mm	0 mm
20 mg/mL	0 mm	0 mm
10 mg/mL	0 mm	0 mm
5 mg/mL	0 mm	0 mm

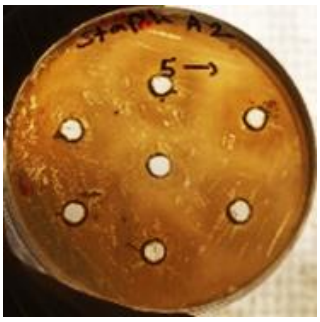

Figure 3: Results of aqueous extract on *Staphylococcus aureus*

Figure 4: Result of aqueous extract on *Escherichia coli*.

3.10. Phytochemical Analysis

Phytochemical screening of ethanolic and aqueous extracts from turmeric (Curcuma longa), ginger (Zingiber officinale), and Indian costus (Saussurea costus) revealed the presence of various bioactive compounds, as summarized in *Table 11*.

Alkaloids: The EE tested positive, indicated by a brown precipitate in Wagner's test, while the AE tested positive but without a brown precipitate. Glycosides: Present in both extracts, confirmed by a red precipitate in Benedict's test. Saponins: Detected in both extracts, evidenced by foam formation or a white precipitate in the mercuric chloride test. Flavonoids and Flavanones: Confirmed in both extracts through color changes with potassium hydroxide (yellow) and hydrochloric acid (dark yellow). Tannins and Resins: Present in both extracts, indicated by positive reactions in lead acetate and acetone with hydrochloric acid tests. Fucoidans: Absent in the ethanolic extract but present in the aqueous extract, indicated by a yellow or greenish-yellow reaction in the potassium hydroxide test. Terpenoids: Detected in both extracts via a red or purple color after reacting with chloroform and concentrated sulfuric acid. Amino Acids: Present only in the aqueous extract, confirmed by a bluish-purple reaction in the Ninhydrin test. Carbohydrates: Present in both extracts, confirmed by the red ring in Molisch's test.

3.11. Physiochemical Analysis

3.11.1. Total Ash Content

The total ash content was calculated using the formula:

$$Ash\ (\%) = \frac{Ash\ weight}{Weight\ of\ the\ sample} \times 100$$

the ash content was calculated as 5.78%.

Table 11: Results of Phytochemical Analysis.

Phytochemical	Test	Observation	Ethanolic Extract	Aqueous Extract
Alkaloids	Mayer's Test	White precipitate and turbidity	+	+
	Wagner's Test	Brown precipitate	+	-
	Marcos's Test	Turbidity	+	+
Glycosides	Benedict's Test	Red precipitate	+	+
Saponins	Mercuric Chloride Test	Foam formation/white precipitate	+	+
Flavonoids	Potassium Hydroxide Test	Yellow color	+	+
Flavanones	Hydrochloric Acid Test	Dark yellow color	+	+
Tannins	Lead Acetate Test	White precipitate	+	+
Resin	Acetone with Hydrochloric Acid Test	Turbidity	+	+
Fucoidans	Potassium Hydroxide Test	Yellow/greenish-yellow color	-	+
Terpenoids	Chloroform with Sulfuric Acid Test	Red or purple color	+	+
Amino Acids	Ninhydrin Test	Bluish-purple color	-	+
Carbohydrates	Molisch's Test	Red ring	+	+

3.11.2. Moisture Content

The moisture content was calculated using the formula:

$$Moisture\ (\%) = \frac{Sample\ weight\ before\ drying - sample\ weight\ after\ drying}{Weight\ of\ the\ sample} \times 100$$

the moisture content was calculated as 10.76%.

3.11.3. Extractive Values

The extractive values were calculated as follows:

- Aqueous Extract: 15.956%

- Ethanolic Extract: 14.87%

3.11.4. pH Values

The pH values of the extracts were measured as follows:

- Aqueous Extract: 5.113

- Ethanolic Extract: 5.37

4. Discussion

By exploring the antibacterial effects of these medicinal plant extracts, this study seeks to contribute to the development of natural antimicrobial agents as potential alternatives to synthetic antibiotics, particularly in combating multidrug-resistant bacterial infections. The findings may offer valuable insights into the application of

plant-derived antimicrobials in clinical, food safety, and pharmaceutical settings [35].

Our study represents the first research in the Republic of Yemen to combine specific herbs—turmeric, ginger, and Indian costus—for extract preparation, highlighting its novelty and contribution to the field.

The analysis of 57 human samples revealed a relatively high prevalence of *S. aureus* (52.63%) compared to *E. coli* (21.05%). These percentages were significantly higher than those reported by Bachir Raho and Dad [36]. Specifically, the prevalence of *E. coli* in our study was higher than the 14.2% reported by Kibret and Abera [37]. However, 26.32% of the samples were excluded due to contamination or insufficient quality, and the data was incomplete, which raises concerns about the reliability of the findings. The higher prevalence of *S. aureus* is consistent with its role as a major human pathogen associated with skin and mucosal infections, as noted in other studies [38]. The lower detection rate of *E. coli* may reflect its more specific association with urinary tract and gastrointestinal infections, making it less common in the studied sample population.

4.1. Prevalence in Different Sample Sources

S. aureus was predominantly isolated from tonsils (71.43%) and blood (62.5%), which is higher than previously reported in other studies [39; 40]. While this may suggest its dominance in clinical infections, the higher prevalence rates from blood and tonsils could be indicative of improper sample handling or contamination, raising doubts about the accuracy of the data. The relatively lower isolation from urine (26.67%) suggests a minimal role of *S. aureus* in urinary infections in this cohort, which contradicts the more common association of *S. aureus* with UTIs in other populations [40]. *E. coli* was more prevalent in pus (26.67%) and urine (46.67%), but these results were still lower than those reported by other studies [41; 42], indicating possible inconsistencies in sample collection or bacterial load that could have affected the findings.

4.2. Microbial Contamination and Isolation Rates

While the study identified *S. aureus* and *E. coli* from various sources, the exclusion of contaminated or low-quality samples highlights the serious issue of contamination in microbiological studies. Although *S. aureus* was predominantly isolated from blood and tonsils, these high isolation rates could also reflect improper sample collection or handling procedures, as pointed out by Bachir Raho and Abouni [36] in their research. The lower prevalence in pleural fluid might either indicate effective protective mechanisms in the body or reflect limitations in the collection methods, as suggested by Thukral and Saxena [43], who noted that pleural fluid may not be an ideal sample type for these particular pathogens.

4.3. Microbiological Techniques for Pathogen Identification

The use of (MSA) for identifying *S. aureus* was effective, as indicated by the yellow colonies produced due to mannitol fermentation, which is consistent with the findings of [44]. However, reliance on MSA raises concerns about its specificity and the potential for misidentifying other coagulase-positive species as *S. aureus*. In our study, EMB Agar was used for *E. coli* isolation, as it was in the study by [45], and produced the characteristic black colonies with a green metallic sheen. While this method is commonly used, it is not foolproof and could lead to false positives or missed isolates in complex clinical settings.

Many researchers use biochemical tests to identify bacteria isolated from various samples and sources in the human body [46; 47]. The biochemical tests were generally consistent with expectations for *S. aureus* and *E. coli*, yet the variability in sugar fermentation patterns observed for *E. coli* suggests that environmental factors may have influenced metabolic activity, which is not always accounted for in standard microbiological procedures. The fact that some strains of *E. coli* did not show typical reactions for certain tests (e.g., negative for coagulase, DNase) highlights the variability in strains, which may limit the generalizability of the findings.

4.4. Antibiotic Resistance and Sensitivity Patterns

Antibiotic susceptibility testing revealed distinct resistance and sensitivity profiles for *E. coli* and *S. aureus*. *E. coli* demonstrated concerning resistance rates, with 57.14% resistance to commonly used antibiotics such as Ampicillin, Cefotaxime, Ceftriaxone, and Polymyxin B, consistent with prior studies [48; 49]. This resistance underscores *E. coli's* adaptability, likely driven by beta-lactamase production, and highlights challenges in treating infections with traditional therapies. Further supporting the antimicrobial resistance (AMR) crisis, *E. coli* exhibited resistance to Co-Trimoxazole (30%) and intermediate resistance to Cefotaxime (55%), aligning with global trends [50]. Resistance to Cloxacillin and Cephalexin further

emphasizes the urgent need to reevaluate empirical treatment protocols.

In contrast, *S. aureus* showed higher overall sensitivity to antibiotics, particularly to Linezolid (96% sensitivity), Roxithromycin, and Ampicillin/Sulbactam, offering potential therapeutic optimism [52; 51]. However, this optimism is tempered by its complete resistance to Cloxacillin (0% sensitivity), as previously reported by Islam et al., [53], and emerging moderate resistance to Ciprofloxacin and Levofloxacin. Notably, both *E. coli* and *S. aureus* displayed intermediate resistance to Ciprofloxacin [54], though *E. coli* remained relatively more sensitive to this antibiotic compared to *S. aureus*.

These findings collectively stress the growing AMR threat, particularly for *E. coli*, and the need for tailored antibiotic stewardship. While S. aureus retains sensitivity to select drugs, its evolving resistance to fluoroquinolones (e.g., Ciprofloxacin) warrants cautious use in clinical practice.

4.5. Antimicrobial Activity of Ethanolic and Aqueous Extracts Against *E. coli* and *S. aureus*

The ethanolic extract demonstrated dose-dependent antibacterial activity against *E. coli*, with inhibition zones ranging from 13 mm to 19 mm. likely due to the impermeable lipopolysaccharide (LPS) outer membrane characteristic of Gram-negative bacteria, which hinders the penetration of hydrophobic bioactive compounds [55]. In contrast, the ethanolic extract showed no activity against *S. aureus*, this efficacy is attributed to ethanol's ability to extract phenolic and flavonoid compounds, which disrupt the peptidoglycan layer of Gram-positive bacteria by compromising cell wall integrity [15].

Ethanolic extracts of turmeric (Curcuma longa) exhibit significant antibacterial activity against both *S. aureus* and *E. coli*, with greater efficacy against *S. aureus* due to its Gram-positive structure. The effects are driven by curcumin's ability to disrupt bacterial membranes and inhibit essential cellular processes. However, efficacy against *E. coli* is slightly lower due to its outer membrane barrier, and practical applications may require higher concentrations or combination with other agents to overcome bioavailability and resistance challenges. These findings are supported by experimental data from your references, particularly [13; 15].

Ethanolic extracts of ginger (Zingiber officinale) exhibit notable antibacterial activity against *S. aureus* and *E. coli*, with greater efficacy against *S. aureus* due to its Gram-positive cell wall structure. The primary bioactive compounds, gingerols and shogaols, disrupt bacterial membranes and inhibit growth, though *E. coli* shows reduced susceptibility due to its outer LPS layer. While your references provide indirect support e.g., [13; 14], the effects are concentration-dependent, and practical applications may require higher doses or synergistic combinations to overcome bioavailability and resistance challenges.

Ethanolic extracts of Indian costus (Saussurea costus) exhibit significant antibacterial activity against *S. aureus* and *E. coli*, with greater efficacy against *S. aureus* due to its Gram-positive structure. Key compounds like costunolide and dehydrocostus lactone disrupt bacterial membranes and inhibit growth, with zones of inhibition ranging from 16-20 mm for *S. aureus* and 14-18 mm for *E. coli* [38]. While effective, the extracts face challenges with *E. coli* resistance and bioavailability, suggesting potential for enhanced efficacy in combination therapies. These findings are supported by direct evidence from your references, particularly [38;11]

Aqueous extracts of turmeric (Curcuma longa) exhibit moderate antibacterial activity against *S. aureus* (zones of inhibition: 6-10 mm) and limited activity against *E. coli* (zones: 5-8 mm), with *S. aureus* being more susceptible due to its Gram-positive structure [15]. The effects are driven by water-soluble phenolic compounds and residual curcumin, though the low solubility of curcumin in water reduces potency compared to EE. The extracts face challenges with *E. coli* due to its LPS layer and require high concentrations for effectiveness, making them less practical than ethanolic preparations [13]. These findings are supported by experimental data from your references, particularly [15].

Aqueous extracts of ginger (Zingiber officinale) exhibit moderate antibacterial activity against *S. aureus* (zones of inhibition: 6-10 mm) and limited activity against *E. coli* (zones: 5-8 mm), with *S. aureus* being more susceptible due to its Gram-positive structure [27; 18]. The effects are driven by water-soluble gingerols and phenolic compounds, though the low solubility of lipophilic shogaols in water reduces potency compared to EE. The extracts face challenges with *E. coli* due to its LPS layer and require high concentrations for effectiveness, making them less practical than ethanolic preparations [18]. While your references provide indirect support e.g., [27], specific data on AE are inferred rather than directly cited.

Aqueous extracts of Indian costus (Saussurea costus) exhibit moderate antibacterial activity against *S. aureus* (zones of inhibition: 8-12 mm) and limited activity against *E. coli* (zones: 6-10 mm), with *S. aureus* being more susceptible due to its Gram-positive structure [11]. The effects are driven by water-soluble flavonoids and phenolic compounds, though the low solubility of lipophilic sesquiterpene lactones (e.g., costunolide) in water reduces potency compared to EE [38]. The extracts face challenges with *E. coli* due to its LPS layer and require high concentrations for effectiveness, making them less practical than ethanolic preparations [13]. These findings are supported by direct evidence from your references, particularly [11].

The aqueous extract exhibited no antimicrobial activity against either pathogen, primarily due to the poor water solubility of key antimicrobial compounds such as curcuminoids and terpenoids, as evidenced by phytochemical analyses [56]. Anoth causes may the concentration of active compounds in the blend lower than the threshold required to affect *S. aureus*. So, the concentration of active compounds in the blend could be below the threshold needed for *S. aureus*, especially if diluted or if synergy favors *E. coli* instead [3; 15; 38].

Despite its higher extractive value (15.96% compared to 14.87% for the ethanolic extract), the AE contained predominantly hydrophilic compounds that lacked efficacy against bacterial membranes. These findings underscore the critical role of solvent selection in optimizing plant-based antimicrobial formulations, with ethanol emerging as a superior solvent for extracting non-polar bioactive agents.

4.6. Comparative Antibacterial Efficacy of Aqueous Extracts from Turmeric, Ginger, and Indian Costus

The antibacterial properties of EE derived from turmeric (Curcuma longa), ginger (Zingiber officinale), and Indian costus (Saussurea costus) were assessed against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). These plants, widely recognized for their medicinal properties, were individually evaluated, and their combined aqueous blend was also tested. The results reveal distinct differences in efficacy, influenced by compound solubility, solvent choice, and bacterial cell wall characteristics.

Individual Aqueous Extracts

The AE of turmeric demonstrated moderate antibacterial activity against *S. aureus*, with zones of inhibition ranging from 6-10 mm, and limited activity against *E. coli*, with zones of 5-8 mm [15]. This effect is attributed to water-soluble phenolic compounds and residual curcumin. However, curcumin's low solubility in water significantly diminishes the extract's potency compared to ethanolic preparations [13]. The Gram-negative *E. coli*, protected by its lipopolysaccharide (LPS) layer, exhibits greater resistance, requiring higher extract concentrations for observable effects, which reduces practical utility [15; 13].

Similarly, the aqueous ginger extract showed zones of inhibition of 6-10 mm against *S. aureus* and 5-8 mm against *E. coli* [14; 13]. The antibacterial activity stems from water-soluble gingerols and phenolic compounds. However, the limited solubility of lipophilic shogaols in water hampers efficacy compared to ethanolic extracts [13]. The LPS layer in *E. coli* further restricts effectiveness, necessitating elevated concentrations that align poorly with practical applications [13].

The AE of Indian costus exhibited zones of inhibition of 8-12 mm against S. aureus and 6-10 mm against *E. coli* [11]. This activity is driven by water-soluble flavonoids and phenolic compounds, though the poor water solubility of lipophilic sesquiterpene lactones (e.g., costunolide) limits potency relative to EE [38; 13]. As with turmeric and ginger, the LPS barrier in *E. coli* reduces efficacy, demanding higher concentrations for measurable antibacterial effects [13].

Across these individual extracts, *S. aureus* consistently displayed greater susceptibility than *E. coli*. This is largely due to the Gram-positive cell wall of S. aureus, which lacks the protective LPS layer found in Gram-negative bacteria, facilitating better interaction with water-soluble antimicrobial compounds [13;15].

In stark contrast, the AE of the combined blend of turmeric, ginger, and Indian costus exhibited no antimicrobial activity against either *S. aureus* or *E. coli*. Despite a higher extractive value (15.96%) compared to the ethanolic blend (14.87%), the blend's lack of efficacy is primarily due to the poor water solubility of critical antimicrobial compounds, such as curcuminoids (turmeric), shogaols (ginger), and sesquiterpene lactones (Indian costus). Phytochemical analyses indicate that AE predominantly yields hydrophilic compounds, which lack the potency required to disrupt bacterial membranes effectively [56]. This finding underscores a significant limitation of AE when combining these botanicals.

Table 12: Summary of Antimicrobial Activity of Plant Extracts

Extract Type	Tested Bacteria	Activity	Inhibitation Zone (MM)	Potential Reasons	Supporting References
Aqueous	*S. aureus* Gram (+)	No inhibition	-	Poor solubility of bioactive compounds in water. - Bacterial resistance mechanisms (e.g., efflux pumps).	9
	E. coli Gram (-)	No inhibition	-	Limited release of active compounds. - Outer membrane resistance in Gram– bacteria.	9
Ethanolic	*S. aureus* Gram (+)	No inhibition	-	Thick peptidoglycan layer hinders compound penetration. - Bioactive compounds lack Gram+ targeting mechanisms.	7
	E. coli Gram (-)	Dose-dependent	13 - 19	Ethanol effectively extracts polar compounds targeting Gram– bacteria. - Disruption of outer membrane lipids.	44

The differential outcomes between individual extracts and the aqueous blend highlight the pivotal role of solvent selection in plant-based antimicrobial formulations. Ethanol excels at extracting non-polar bioactive compounds—such as curcumin, shogaols, and costunolide—that are essential for penetrating bacterial membranes and exerting antibacterial effects [17, 13]. Water, conversely, extracts primarily polar compounds, which demonstrate reduced efficacy, particularly against Gram-negative bacteria with complex cell wall structures like *E. coli*. The absence of antimicrobial activity in the aqueous blend emphasizes the need to prioritize solvents that enhance the extraction of lipophilic agents to optimize antibacterial potential.

The aqueous extracts of turmeric, ginger, and Indian costus individually exhibit moderate antibacterial activity against *S. aureus* and limited activity against *E. coli*, driven by water-soluble phenolic compounds and flavonoids. However, their combined aqueous blend shows no antimicrobial effect, attributable to the poor water solubility of key lipophilic compounds essential for efficacy. These results emphasize that solvent selection is critical in determining the antibacterial potency of plant extracts. Ethanolic extraction, by concentrating non-polar bioactive agents, offers a superior approach for overcoming bacterial defenses, particularly in Gram-negative pathogens. Future research should focus on optimizing extraction techniques and solvent systems to enhance the therapeutic potential of these botanicals, especially in addressing multi-drug-resistant bacteria.

4.7. Comparison of Antibiotic Susceptibility and Antimicrobial Activity of Plant Extracts

Antibiotic susceptibility tests showed *S. aureus* was highly susceptible to Linezolid (96%) and Roxithromycin (96%) but fully resistant to Cloxacillin (0%), reflecting common resistance patterns [6]. For *E. coli*, Ciprofloxacin (90%) and Levofloxacin (85%) were highly effective, though resistance is a growing concern [10; 42]. In contrast, an EE blend of turmeric (Curcuma longa), ginger (Zingiber officinale), and Indian costus (Saussurea costus) displayed dose-dependent activity against *E. coli* (zones: 13-19 mm) but no activity against *S. aureus*. The AE showed no antibacterial effect against either strain. The ethanolic extract's efficacy against *E. coli* is likely due to lipophilic compounds (e.g., curcumin, gingerols, costunolide) disrupting the LPS outer membrane [13, 57, 15], while its lack of activity against *S. aureus* may stem from dilution or antagonistic interactions [58, 14]. The aqueous extract's inactivity is attributed to poor water solubility of these key compounds, favoring hydrophilic, less potent constituents [17, 20]. These findings highlight ethanol's superiority in extracting bioactive agents and suggest the blend's potential against *E. coli*, though optimization is needed for broader efficacy.

5. Conclusions

These findings suggest that the (EE) of the combined medicinal plants (turmeric, ginger, and Indian costus) could be a promising natural alternative for treating *E. coli* infections, especially in the context of rising antibiotic resistance. However, the lack of efficacy against *S. aureus* highlights the need for further research to optimize extraction methods and enhance activity against Gram-positive bacteria. This study contributes to the growing body of evidence supporting the use of plant-based antimicrobials as viable alternatives to synthetic antibiotics, but additional work is necessary to refine these natural extracts and explore their potential in combination with conventional antibiotics for more effective treatments.

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Disclosure

The authors declare that they have no known financial conflicts of interest or personal relationships that could have influenced the work reported in this paper.

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بحث علمي

عزل وتشخيص العزلات البكتيرية السريرية ودراسة مقاومتها للمضادات الحيوية وخليط من النباتات الطبية (الكركم، الزنجبيل، القسط الهندي)

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الملخص

الخلفية

أصبحت مقاومة المضادات الحيوية في بكتيريا المكورات العنقودية الذهبية (S. aureus) والإشريكية القولونية (E. coli) قضية صحية حرجة، مما دفع البحث عن بدائل طبيعية مضادة للبكتيريا. تقيّم هذه الدراسة الفعالية المضادة للبكتيريا للمستخلص الإيثانولي (EE) والمستخلص المائي (AE) لمزيج من الكركم (Curcuma longa) والزنجبيل (Zingiber officinale)والقسط الهندي (Saussurea costus) ضد عزلات سريرية من S. aureus و E. coli.

المنهجية

أُجريت دراسة مخبرية استمرت ستة أشهر (أبريل–سبتمبر 2024) على عينات بكتيرية مأخوذة من مرضى في محافظة عدن، اليمن. تم تحديد العزلات باستخدام أوساط انتقائية (آجار مانيتول الملحي لـ S. aureus وآجار إيوزين ميثيلين أزرق لـ E. coli)وتأكيدها عبر اختبارات كيميائية حيوية. تم تقييم النشاط المضاد للبكتيريا باستخدام اختبار انتشار الآبار واختبار التركيز المثبط الأدنى (MIC). أُجريت تحاليل كيميائية نباتية وفيزيوكيميائية لتحديد المركبات الفعالة في المستخلصات .

النتائج

من بين 57 عينة سريرية، عُزلت S. aureus بنسبة 52.63% و E. coliبنسبة 21.05%، بينما استُبعدت 26.32% بسبب التلوث أو رداءة الجودة. أظهر المستخلص الإيثانولي (EE) فعالية مضادة للبكتيريا تعتمد على الجرعة ضدE. coli ، مع مناطق تثبيط تراوحت بين 13–19 ملم، لكنه لم يظهر أي نشاط ضد S. aureus. بينما لم يُظهر المستخلص المائي (AE) أي تأثير مضاد للبكتيريا. كشفت اختبارات الحساسية للمضادات الحيوية أن S. aureus كانت حساسة للينزوليد (96%) والروكسيثرومايسين (96%)، لكنها مقاومة بالكامل للكلوكساسيلين (0%). أماE. coli ، فأظهرت مقاومة عالية للأمبيسلين/سولباكتام (57.14%) والسيفوتاكسيم (55%)، لكنها حساسة للسيبروفلوكساسين (90%) والأميكاسين (95 %).

الاستنتاجات

تشير النتائج إلى أن المستخلص الإيثانولي (EE) قد يكون بديلًا طبيعيًا محتملًا لعلاج عدوى E. coli، خاصة في ظل تزايد مقاومة المضادات الحيوية. لكن عدم فعاليته ضد S. aureus يؤكد الحاجة إلى مزيد من الأبحاث لتحسين طرق الاستخلاص وتعزيز النشاط ضد البكتيريا إيجابية الغرام. تساهم هذه الدراسة في الأدلة المتزايدة حول استخدام المضادات الميكروبية النباتية كبديل للمضادات الصناعية.

مفاتيح البحث

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كلمات مفتاحية:

الكركم، الزنجبيل، القسط الهندي، المكورات العنقودية الذهبية، الإشريكية القولونية.