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Original Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *SENNA ALATA* (L.ROXB.) LEAVES AGAINST BACTERIA ASSOCIATED WITH HUMAN SKIN INFECTION

Aderotiba, C.O[1*], Onifade, A.K[1]

[1] Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria

Correspondence should be addressed to Aderotiba, C.O: aderotibaolusola1@gmail.com

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Abstract: The increasing prevalence of antimicrobial resistance has reduced the effectiveness of conventional antibiotics, creating a need for continuous surveillance and the exploration of alternative therapeutic agents. This study aimed to determine the prevalence and characteristics of bacterial pathogens associated with skin infections, evaluate their antibiotic resistance patterns and assess the antibacterial potential of the plant extracts (*Senna alata*) as possible alternative treatments. A cross-sectional study was conducted involving forty (40) patients with clinically diagnosed skin infections. Demographic data were obtained, and swab samples were collected for bacteriological analysis. Isolates were identified using standard biochemical tests, hemolysis assays, antibiotic susceptibility testing, and multiple antibiotic resistance (MAR) index determination. Molecular confirmation was performed using 16S rRNA gene sequencing. Leaves of *S. alata* were extracted using aqueous, ethanol, and acetone solvents. Antibacterial activity was evaluated using agar diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Predominantly Gram-positive cocci, including *Staphylococcus aureus* (40.0%), *Streptococcus pyogenes* (36.66%), and *Staphylococcus epidermidis* (23.33%). Hemolysis testing showed 25.0% hemolytic strains. Antibiotic assay indicated complete sensitivity to rifampicin, ciprofloxacin, and levofloxacin, but high resistance to cefuroxime and ceftazidime. Multiple Antibiotic Resistance (MAR) indices ranged from 0.00 to 0.90, with most isolates exhibiting values ≥ 0.2, indicating exposure to high-risk antibiotic environments. Multidrug resistance was observed in 40.0% of the isolates, while several strains were extensively drug-resistant. These extracts demonstrated strong, concentration-dependent antibacterial activity, with inhibition zones up to 25 mm and MIC/MBC values ranging from 20–60 mg/mL, whereas aqueous extracts showed weaker effects. In conclusion, Gram-positive cocci, particularly *S. aureus* and *S. pyogenes*, are major etiological agents of skin infections. Organic solvent extracts of *S. alata*

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demonstrated promising antibacterial activity, suggesting their potential as alternative or adjunct therapeutic agents. Continuous antimicrobial surveillance and further pharmacological studies on plant-derived compounds are recommended to combat resistant skin pathogens.

Keywords: Bacteriological Analysis, Etiological Agents, Pharmacological Studies, Hemolysis Assays.

1.0 INTRODUCTION

Management of the infection is further compounded due to the increasing prevalence of resistance towards antimicrobial medications, resulting in the growing global public health problem known as antimicrobial resistance (AMR), which jeopardizes the effectiveness of existing antibiotic therapies (WHO, 2021; Laxminarayan *et al*., 2020). This growing problem calls for the immediate search and production of new antimicrobial agents from other sources. Medicinal plants play an important role in the traditional system of healthcare across the world and provide a vast source of bioactive compounds (Atanasov *et al.,* 2021). The skin, which is the body's largest organ, acts as a major protective barrier against external physical, chemical, and microbial threats (Grice & Segre, 2011). Despite its protective mechanisms, it is frequently susceptible to various infections caused by a wide array of pathogenic bacteria, including *Staphylococcus aureus, Streptococcus pyogenes,* and *Pseudomonas aeruginosa*. These infections range from superficial conditions like impetigo and folliculitis to more severe, life-threatening diseases such as cellulitis and necrotizing fasciitis. The management of bacterial skin infections has been increasingly complicated by the emergence and spread of antimicrobial resistance (AMR), a global health crisis that threatens the efficacy of conventional antibiotics (WHO, 2021). This escalating resistance necessitates the urgent exploration and development of novel antimicrobial agents from alternative sources. The study of antimicrobial substances in plants is one promising area that might help to discover new compounds to overcome resistance or complement the treatment of diseases (Newman & Cragg, 2020). There is an abundance of medicinal plants available today. One of those plants is *S. alata* (*Cassia alata*).

2.0 MATERIALS AND METHODS

2.1 Study Design

This study adopted an experimental laboratory-based comparative design to evaluate the *in vitro* antimicrobial efficacy of *Senna alata* (L) Roxb. leaf extracts. The antimicrobial activity of the extracts was assessed against clinically isolated bacterial pathogens implicated in human skin infections.

2.2 Administration of Questionnaire

Structured questionnaires were administered to participants to obtain relevant

socio-demographic data, including age, sex, type of skin lesion, duration of infection, and prior antibiotic use. Informed consent forms were given to the patients to sign prior to sample collection. This information was used solely for epidemiological context and did not influence laboratory outcomes.

2.3 Collection of Clinical Specimens

Human skin swab samples were collected early morning from both male and female skin-infected patients attending the skin clinic department at the Arakale Comprehensive Health Centre, Akure, and Ondo state. This was done by using sterile swab sticks with transport media to swab the infected part of the patient's skin and a total number of forty (40) skin swab samples were collected and transported to the Microbiology laboratory, Federal University of Technology, Akure (FUTA) immediately for microbiological analysis.

2.4 Materials and Reagents

Fresh leaves of *Senna alata* were used as plant materials. Analytical-grade solvents, distilled water, ethanol (95%), and acetone (95%) were employed for extraction. Microbiological media included Nutrient Agar, Mannitol Salt Agar, Blood Agar, and Mueller–Hinton Agar (Oxoid), Kovac reagent, sterile filter paper, and sterile slide. Other materials comprised an autoclave, an incubator, micropipettes, sterile Petri dishes, syringes, McFarland standards, a weighing balance, and standard laboratory glassware.

2.5 Preparation and Sterilization of Culture Media

All the culture media used for the investigation were prepared according to the manufacturer’s specifications. Dehydrated Nutrient Agar (2.8g), Mannitol Salt Agar (11.1g), Blood Agar (4.0g), and Mueller Hinton Agar (3.8g) were separately dissolved in 100ml of distilled water. Each mixture was sterilized using autoclaving at 121°C for 15 minutes; after that, the mixture was cooled down before being dispensed into a petri dish aseptically.

2.6 Isolation and Purification of Bacterial Isolates

Swabs taken from skin were inoculated in accordance with established microbiological procedures on Mannitol Salt Agar, Blood Agar, and Nutrient Agar plates. These plates were kept in an incubator at 37 °C for 24 hours. Pure cultures were made through the subculturing of characteristic colonies on Nutrient Agar plates.

2.7 Identification of Bacteria Isolated from Swabbed Skin Samples

2.7.1 Cultural and Morphological Characterization

Pure isolates were characterized based on colony morphology, including shape, size, elevation, pigmentation, edge, surface texture, opacity, and hemolytic patterns on blood agar.

2.7.2 Biochemical Characterization

Biochemical identification was performed following standard protocols (Smith *et*

al., 2022). Tests included Gram staining, catalase, coagulase, oxidase, motility, indole, sugar fermentation tests, Triple Sugar Iron (TSI) agar reaction, and hemolysis tests.

2.8 Standardization of Bacterial Inoculum

Bacterial suspensions were standardized to 0.5 McFarland turbidity, corresponding to approximately 1.5×10^8 CFU/mL, in accordance with CLSI guidelines (CLSI, 2016). A loop full of test bacteria isolates was inoculated on nutrient broth and incubated for 24 hours. About 0.2ml from the 24-hour broth culture of the bacteria was dispensed into 20ml sterile nutrient broth and incubated for 3 to 5 hours to standardize the culture to 0.5 McFarland standard (CLSI, 2014).

2.9 Antibiotic Susceptibility Testing

Isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer method, which has been standardized and evaluated by the methods of the Clinical Laboratory Standards Institute (CLSI, 2020). The bacterial suspension was spread onto the surface of the Mueller–Hinton agar (Oxoid) to make confluent growth with the aid of a sterile swab stick using the spread method. Antibiotic discs were immediately placed on the surface of the agar plate using sterile forceps and incubated at 37°C for 16 hours. Inhibition zones for various isolates were measured and interpreted as sensitive, intermediate, or resistant according to the Clinical Laboratory Standards Institute (CLSI, 2020) and the Multiple Antibiotic Resistance (MAR) Index (Osundiya *et al*., 2013).

2.10 Molecular Identification of Selected Bacterial Isolates

Phenotypically identified *Staphylococcus* and *Streptococcus* species were further confirmed using polymerase chain reaction (PCR) and DNA sequencing techniques.

2.11 Collection and Authentication of Plant Materials

Fresh leaves of *Senna alata* were collected from Owo, Ondo State. Botanical identification and authentication were conducted at the Herbarium, Federal Research Institute of Nigeria, Ibadan. Leaves were air-dried, pulverized, and stored in airtight containers.

2.12 Extraction of Plant Materials

Powdered plant materials (100g each) were extracted using aqueous, ethanol, and acetone solvents through Soxhlet extraction to maximize phytochemical yield (Sarker *et al.,* 2006). Extraction solutions were concentrated through the use of a rotary evaporator at temperatures between 45-50°C and stored at 4°C.

2.12.1 Preparation of Extract Stock Solutions

Plant extracts were redissolved in DMSO (dimethyl sulfoxide) at 20% concentration to prepare stock solutions of plant extract having a concentration range of 20–100 mg/ml and stored at 15°C. They were used for antibacterial tests.

2.13 Antibacterial Assay of Plant Extracts

The in vitro antibacterial property of leaf extracts of Senna alata and Eclipta prostrata was determined by the Agar Well Diffusion Method as per the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2012) with some modifications. The procedure briefly includes the preparation of sterilized MHA (Mueller-Hinton agar) plates. Previously standardized bacterial inocula adjusted to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL) were uniformly spread over the surface of the MHA plates using sterile cotton swabs to ensure confluent growth. Using a sterile cork borer, six equidistant wells of approximately 5mm diameter were aseptically bored into the agar surface.

2.14 Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extract

Minimum Inhibitory Concentration (MIC) of plant extracts to test bacterial isolates was established using the broth dilution method as per Yusha'u *et al*. (2010). Inoculation was done in a set of sterile test tubes containing nutrient broth and varying concentrations of the plant extracts using serial twofold dilutions to yield concentrations of 80 mg/ml to 20 mg/ml. The test tubes were inoculated with 0.5 ml of the standardized bacterial suspension of 0.5 MacFarland standard.

Two sets of control were used: a positive control with nutrient broth and bacterial inoculum without extract, and a negative control where there is nutrient broth and extract but no bacterial inoculum. All the tubes were incubated at 37°C for 24 hours. After incubation, visual examination of the tubes for turbidity, which indicates growth of bacteria, was done. MIC was established as the lowest concentration of plant extracts that inhibited bacterial growth, as shown by the clarity of the broth.

2.15 Determination of Minimum Bactericidal Concentration (MBC) of the Plant Extracts

The Minimum Bactericidal Concentration (MBC) was also assessed to determine the kind of effects the extracts had on the bacteria under investigation. Portions (0.1 mL) from each of the tubes containing MIC were transferred aseptically to nutrient agar plates, which were left incubating for 24 hours at 37°C. The bacterial growth in the agar plates was then examined.

MBC was described as the lowest concentration of the extract where there was no observable growth of the bacteria. The relation between MIC and MBC was also used to determine the antibacterial nature of the extracts.

2.16 Statistical Analysis

All in vitro studies were carried out in triplicate to ensure reproducibility and validity of the results obtained. Data collected from the experiments were presented as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to find any significant differences between treatment groups, extract concentrations, and plant types. Whenever there was a statistically significant difference, mean comparison was done using an appropriate test. Statistical significance was set at a confidence level of 95% ($p \leq 0.05$).

3.0 RESULTS

3.1 Socio-demographic characteristics of the skin-infected patients

This presents the socio-demographic characteristics of the skin-infected patients studied. A total of 40 patients were examined, with males (55.0%) slightly more represented than females (45.0%). The highest proportion of patients fell within the 15–25 years age group (32.5%), followed by those above 55 years (22.5%). Married individuals constituted the largest marital group (30.0%). New cases of skin infection predominated (72.5%) compared to old cases (27.5%).

3.2 Biochemical, hemolysis and antibiotic resistance characteristics and distribution of bacterial isolates recovered from the patients

This showed the biochemical characteristics and distribution of bacterial isolates recovered from the patient. Three major organisms were identified based on biochemical reactions: *Staphylococcus aureus* (40.0%), *Streptococcus pyogenes* (36.66%), and *Staphylococcus epidermidis* (23.33%). All isolates were Gram-positive cocci and showed positive reactions to glucose, sucrose, maltose, fructose, and lactose fermentation, with distinct differences in catalase, coagulase, mannitol, and galactose utilization patterns.

3.3 Hemolytic Activity of Bacterial Isolates from Skin-Infected Patients

This summarizes the hemolytic activity of the isolates. Out of 120 bacterial isolates examined, 30 (25.0%) exhibited hemolytic activity, with β-hemolysis accounting for 18.33% and γ-hemolysis for 6.67%. The majority of isolates (75.0%) showed no hemolysis.

3.4 Resistance and Susceptibility Patterns of Hemolysis-Positive Isolates

All hemolysis-positive isolates (100%) were susceptible to rifampicin, ciprofloxacin, and levofloxacin. High susceptibility was also observed for streptomycin (93.3%) and gentamicin (96.7%).

3.5 Multidrug Resistance (MDR) Patterns among Hemolysis-Positive Isolates

The multidrug resistance (MDR) distribution among hemolysis-positive isolates. Out of the 30 hemolytic isolates examined, 12 (40.0%) were classified as MDR, exhibiting resistance to three or more antibiotic classes, while the majority, 18 isolates (60.0%), were non-MDR.

3.6 Extensively Drug-Resistant (XDR) Isolates among Hemolysis-Positive Isolates

The resistance patterns and inhibition zone characteristics of the extensively drug-resistant (XDR) isolates are detailed in this table. Four isolates (Isolates 4, 14, 15, and 30) demonstrated extensive resistance, with resistant counts ranging from 7/10 to 9/10 antibiotics tested and MAR index values between 0.70 and 0.90. These isolates showed generally low mean and median zones of inhibition, reflecting poor antibiotic efficacy. The group mean resistant count was 8.0/10, with an overall mean MAR index of 0.80.

3.7 Gene sequence characteristics and quality metrics of the extensively drug-resistant (XDR) isolates

The 16S rRNA gene sequence characteristics and quality metrics of the extensively drug-resistant (XDR) isolates. High-quality near-full-length 16S rRNA sequences were obtained for all isolates, with sequence lengths ranging from 1,465 to 1,489bp. The GC content varied between 33.2% and 38.6%, while melting temperatures (Tm) ranged from 87.4°C to 89.1°C, indicating stable amplicons. All sequences recorded excellent quality scores (≥98.9/100) and consistent gene copy numbers (5–6 copies), confirming the reliability of the sequencing data and suitability for taxonomic identification.

3.8 Antibacterial Activity of Plant Extracts

The antibacterial activity of *Senna alata* extracts against skin infection microbes is shown in the table. The acetone and ethanolic extracts demonstrated strong, concentration-dependent inhibitory effects against *Staphylococcus aureus* (SIM 4), *Streptococcus pyogenes* (SIM 14), and *Staphylococcus epidermidis* (SIM 15), with maximum zones of inhibition (18–20 mm) observed at 100 mg/mL. The aqueous extract exhibited comparatively weak activity, with little or no inhibition at lower concentrations.

3.9 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) results summarized in this table show that acetone and ethanolic extracts of the plant inhibited bacterial growth at relatively low concentrations (20–60 mg/mL). In contrast, aqueous extracts often showed higher MIC values or no detectable inhibition, especially against *S. aureus* and *S. pyogenes*. Minimum bactericidal concentration (MBC) values presented in Table 3.10 further confirmed the superior bactericidal efficacy of acetone and ethanolic extracts. *Senna alata* achieved bactericidal effects at 20–40 mg/mL against all tested isolates, whereas aqueous extracts showed no bactericidal activity within the tested concentration range.

Table 3.1: Socio-demographic characteristics of skin-infected patients (n = 40)

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	22	55.0
	Female	18	45.0
Age group (years)	15–25	13	32.5
	25–35	5	12.5
	35–45	6	15.0
	45–55	7	17.5
	>55	9	22.5

Variable	Category	Frequency (n)	Percentage (%)
Marital status	Children	8	20.0
	Single	10	25.0
	Married	12	30.0
	Divorced	7	17.5
	Widowed	3	7.5
Case status	Old cases	11	27.5
	New cases	29	72.5

Table 3.2: Biochemical Characteristics and Percentage Distribution of Bacterial Isolates from Skin-Infected Patients (n = 120)

Biochemical Tests / Parameters	**Bacterial Isolates from Skin-Infected Patients**		
Catalase	+	−	+
Coagulase	+	−	−
Motility	−	−	−
H_2S Production	−	−	−
Indole	−	−	−
Oxidase	−	−	−
Glucose Fermentation	+	+	+
Sucrose Fermentation	+	+	+
Maltose Fermentation	+	+	+
Fructose Fermentation	+	+	+
Lactose Fermentation	+	+	+
Galactose Fermentation	+	+	−
Mannitol Fermentation	+	−	−
Gram Reaction	+ cocci	+ cocci	+ cocci
Probable Organism	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Staphylococcus epidermidis*
Percentage Occurrence (%)	**40.00**	**36.66**	**23.33**

Key: SIM = Skin Infection Microbe; + = Positive; − = Negative;

Table 3.3: Hemolytic Activity of Bacterial Isolates from Skin-Infected Patients

Hemolysis Type	Number of Isolates (n)	Percentage (%)
Beta (β) hemolysis	22	18.33
Gamma (γ) hemolysis	8	6.67
TotalHemolysis-Positive Isolates	30	25.00
No Hemolysis	90	75.00
Total Isolates Examined	120	100.00

Key: β = complete hemolysis; γ = no hemolysis

Table 3.4: Resistance and Susceptibility Patterns of Hemolysis-Positive Isolates (n=30)

Antibiotic	Susceptible / Intermediate n (%)	Resistant n (%)
Rifampicin (RD)	30 (100%)	0 (0%)
Ceftazidime (CTZ)	6 (20%)	24 (80%)
Streptomycin (S)	28 (93.3%)	2 (6.7%)
Azithromycin (AZM)	26 (86.7%)	4 (13.3%)
Amoxicillin (AMX)	17 (56.7%)	13 (43.3%)
Ciprofloxacin (CPX)	30 (100%)	0 (0%)
Erythromycin (E)	17 (56.7%)	13 (43.3%)
Levofloxacin (LEV)	30 (100%)	0 (0%)
Gentamicin (CN)	29 (96.7%)	1 (3.3%)
Cefuroxime (CEF)	5 (16.7%)	25 (83.3%)

Table 3.5: Multidrug Resistance (MDR) Patterns among Hemolysis-Positive Isolate

MDR Category	Number of Isolates	Percentage (%)
Non-MDR	18	60.0
MDR (≥3 antibiotic classes)	12	40.0

Table 3.6: Extensively Drug-Resistant (XDR) Isolates among Hemolysis-Positive Isolates

Isolate ID	RD	CTZ	S	AZM	AMX	CPX	E	LEV	CN	CEF	Resistant Count	MAR Index	Mean Zone (mm)	Median Zone (mm)
4	R (10)	R (0)	R (10)	R (10)	R (0)	R (10)	R (10)	R (10)	I (15)	R (10)	9/10	**0.90**	8.5	10.0
14	R (10)	R (0)	R (0)	R (0)	R (10)	I (15)	I (15)	R (5)	R (10)	R (0)	9/10	**0.90**	6.5	10.0
15	I (12)	R (5)	R (10)	R (0)	R (0)	I (15)	R (0)	R (10)	S (20)	R (0)	7/10	**0.70**	7.2	7.5
30	R (10)	R (0)	R (10)	R (10)	I (15)	I (15)	I (15)	R (10)	R (10)	R (0)	7/10	**0.70**	9.5	10.0
Group Mean	10.5	1.3	7.5	5.0	6.3	13.8	10.0	8.8	13.8	2.5	8.0/10	**0.80**	**7.9**	**9.4**

Legend: R = Resistant (0-10 mm), I = Intermediate (11-15 mm), S = Susceptible (16-20 mm), HS = Highly Susceptible (21-30 mm)
Note: Values in parentheses represent zone of inhibition diameter in millimeters

Table 3.7: 16S rRNA Sequence Characteristics and Quality Metrics of the XDR Isolates

Isolate ID	Species	Length (bp)	GC %	Tm (°C)	Quality Score	Gene Copy Number
#4	*S. aureus*	1,465	33.2	87.4	99.2/100	5-6 copies
#14	*S. pyogenes*	1,489	38.6	89.1	99.5/100	5-6 copies
#15	*S. Epidermidis*	1,468	33.5	87.6	98.9/100	5-6 copies

Table 3.8: Antibacterial Activity of *Senna alata* Leaves Extracts against Skin Infection Microbes (SIM) (Zone of Inhibition, mm)

Isolate	Concentration (mg/mL)	Acetone Extract	Ethanol Extract	Aqueous Extract
SIM 4	100	20	20	8
	80	15	15	6
	60	10	10	5
	40	10	0	0
	20	10	0	0
SIM 14	100	20	20	14
	80	18	18	10
	60	17	15	5
	40	15	10	5
	20	12	0	0
SIM 15	100	18	15	15
	80	15	12	10
	60	10	10	5
	40	10	0	5
	20	10	0	0

SIM 4: S. *aureus subsp. aureus*

SIM 14: S. *pyogenes*

SIM 15: S. *epidermidis*

Table 3.9: Minimum Inhibitory Concentration (MIC) of *Senna alata* Leaves Extracts against Skin Infection Microbes (mg/mL)

Isolate	Plant / Extract	Acetone	Ethanol	Aqueous
SIM 4	*Senna alata*	20	40	60
SIM 14	*Senna alata*	20	60	60
SIM 15	*Senna alata*	20	20	40

SIM 4: *S. aureus* subsp. *aureus*

SIM 14: *S. pyogenes*

SIM 15: *S. epidermidis*

Table 3.10: Minimum Bactericidal Concentration (MBC) of *Senna alata* Leaves Extracts Against Skin Infection Microbes (mg/mL)

Isolate	Plant / Extract	Acetone	Ethanol	Aqueous
SIM 4	*Senna alata*	20	40	–
SIM 14	*Senna alata*	20	40	–
SIM 15	*Senna alata*	20	40	–

SIM 4: *S. aureus* subsp. *aureus*

SIM 14: *S. pyogenes*

SIM 15: *S. epidermidis*

4.0 DISCUSSION

The socio-demographic profile of the skin-infected patients revealed a slightly higher prevalence of infection among males (55.0%) compared to females (45.0%). This finding is consistent with recent epidemiological studies that attribute higher skin infection rates in males to increased occupational exposure, outdoor activities, and reduced healthcare-seeking behavior (Kavanagh *et al.,* 2018). The preponderance of infections in the 15–25-year age category can be attributed to increased physical interactions, sports injury, and damaged skin barriers found in adolescents and young adults. The preponderance of new cases (72.5%) indicates continuous transmission and poor prevention control of skin infections among the subjects of the study. Similar trends have been reported in low- and middle-income countries where inadequate hygiene practices and delayed medical intervention contribute to persistent skin and soft tissue infections (SSTIs) (Laxminarayan *et al*., 2020).

The preponderance of *S. aureus* (40.0%), followed by *S. pyogenes* (36.66%) and *S. epidermidis* (23.33%), is consistent with contemporary global statistics on the prevalence of these bacteria in causing SSTIs (Miller *et al.,* 2019). Biochemical features of the isolated bacteria, such as positivity to catalase and coagulase tests for *S. aureus* are virulence factors that characterize this organism's ability to invade tissues and avoid host defense mechanisms. Organic extracts exhibited a high antibacterial activity against MDR and XDR isolates and can be useful therapeutically. It is important to note that flavonoids and phenols act by disrupting the cell wall of bacteria, inhibiting nucleic acids synthesis and blocking efflux pumps, which is particularly useful in resistant bacteria.

The use of 16S rRNA gene sequencing provided definitive species-level identification of XDR isolates, with ≥99.7% sequence identity and 100% query coverage. Molecular confirmation eliminates ambiguities associated with phenotypic identification and strengthens the validity of the resistance data. The identification of **XDR** *S. aureus* **and** *S. pyogenes* is of major clinical relevance, given their established roles in invasive skin infections and systemic complications (Turner *et al*., 2019

CONCLUSION

The present study provides comprehensive insights into etiology, antimicrobial resistance patterns, molecular identity, and potential alternative therapeutic interventions for bacterial pathogens associated with skin infections. The findings clearly indicate that Gram-positive cocci, particularly *Staphylococcus aureus* and *Streptococcus pyogenes*, constitute the major etiological agents within the studied cohort, accounting for the majority of isolates. These pathogens exhibited notable multidrug resistance, with multiple antibiotic resistance (MAR) indices frequently exceeding 0.2, highlighting prior exposure to high-risk antibiotic environments and underscoring the growing public health challenge posed by resistant skin pathogens. Molecular confirmation via 16S rRNA sequencing validated the phenotypic identification of isolates, confirming species identity with high similarity to reference strains and demonstrating the reliability of integrating classical microbiological methods with molecular diagnostics. Hemolytic activity was observed in a

subset of isolates, reflecting their potential virulence and capacity to cause severe tissue damage. Antibiotic susceptibility profiling revealed complete sensitivity to rifampicin, ciprofloxacin, and levofloxacin, while high resistance rates to cefuroxime and ceftazidime indicate selective pressures and potential misuse of commonly prescribed antibiotics in clinical practice. This situation emphasizes the urgent need for rational antimicrobial use and continuous surveillance programs to prevent the further emergence and spread of multidrug-resistant strains. Concurrently, the study highlights the therapeutic potential of medicinal plants as alternative or adjunct interventions.

These compounds exhibited strong, concentration-dependent antibacterial activity, as evidenced by inhibition zones, Minimum Inhibitory Concentrations (MIC), and Minimum Bactericidal Concentrations (MBC), suggesting their efficacy against clinically relevant multidrug-resistant pathogens. Collectively, these findings underscore the dual importance of addressing antimicrobial resistance through both evidence-based antibiotic stewardship and exploration of novel plant-derived antimicrobial agents.

RECOMMENDATION

The integration of ethnopharmacological information in the drug discovery process should be promoted as a sustainable way to deal with the problem of antibiotic resistance. These steps will contribute to better infection control, lower dependence on traditional antibiotics, and the development of new pharmaceuticals.

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