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MEDICINAL USE OF PLANTS' EXTRACTS AGAINST CLINICAL ISOLATES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Abstract

Objective: *Methicillin-resistant staphylococcal infections are a global public health crisis, particularly in our region, where precise therapeutics are lacking. This research seeks effective antimicrobial drugs for methicillin-resistant S. aureus and investigates resistance patterns in clinical and asymptomatic S. aureus strains.*

Methods: *A total of 47 samples, including those from urine, pus, milk, nasal, and blood sources, were collected from various hospital facilities* of Bahawalpur region *to isolate S. aureus strains. Biochemical analysis confirmed the presence of Staphylococcus aureus in these samples. Subsequent biochemical testing revealed that 21 out of the total 47 tested samples were positive for Staphylococcus aureus. Among these, a resistance assessment demonstrated that 10 out of the 21 S. aureus isolates exhibited resistance to oxacillin. In this study, 25 medicinal plants were meticulously chosen, subjected to air drying, and subsequently extracted with Methanol. The research focused on evaluating the efficacy of these extracts of plants against oxacillin-resistant Staphylococcus aureus strains. Furthermore, the micro-dilution technique established by the CLSI-2020 was employed to determine the semi-maximum inhibitory concentration (IC50) of promising the extracts of Medicinal plants.*

Results: *Subsequent biochemical testing revealed that 21 out of the total 47 tested samples were positive for S. aureus. Oxacilin was exhibited resistance in 10(47.6%) out of the 21 samples. Ampicillin showed resistance in all 21(100%) samples. Chloramphenicol was resistant in 4(19.04%) samples. Ciprofloxacin was resistance in a 1(4.76%) sample. Sulphamethoxazole was resistance in 5(23.80%) samples. Methanolic extracts from five medicinal plants against oxacillin-resistant Staphylococcus aureus strains demonstrated diverse IC50 values (mg/ml), Punica granatum (0.499-0.530), Rubia cordifolia (0.250-0.280), Eucalyptus globulus (3.38-4.30), Lawsonia inermis (1.38-2.07), and Centratherum anthelminticum (0.946-1.20), indicating varying inhibitory efficacies with potential pharmaceutical significance. The IC50 values, indicating the concentration required for a 50% inhibition of oxacillin-resistant S. aureus strains growth, ranged from 0.250 to 4.30 mg/ml for these extracts. Conclusions: Diverse medicinal plants show promise as sources of effective antibacterial agents against multidrug-resistant S. aureus, offering valuable therapeutic prospects.*

Keywords: *Antibacterial drugs, IC_{50}, Oxacillin, Resistant, Staphylococcus aureus*

INTRODUCTION

Staphylococcal infections, predominantly caused by Staphylococcus aureus, are highly contagious diseases and a primary source of hospital-acquired infections, particularly prevalent in developing nations. This bacterium is a leading pathogen, commonly isolated from clinical specimens, notably in cases of urinary tract infections (UTIs), skin lesions, and wound infections. Globally, *S. aureus* stands as a major infectious agent responsible for both Community-associated and Hospital-acquired infections (1). S. aureus, characterized by its gram-positive nature and clustered cocci formation, lacks spore formation, motility, and is catalase-positive in a facultative anaerobic setting (2). S. *aureus* is typically located in the damp squamous epithelium of the anterior nostrils (3). Based on estimates, approximately 20% of individuals harbor *S. aureus*

in their nasal passages, while another 60 percent are categorized as carriers of this bacterium. *S. aureus* is recognized as an opportunistic pathogen capable of causing more severe suppurative infections, including conditions such as furuncles, carbuncles, abscesses, impetigo, chronic furunculosis, pneumonia, mastitis, cellulitis, deep-seated lesions, osteomyelitis, endocarditis, scalded skin syndrome, food poisoning, and toxic shock syndrome (4). *S. aureus* inhabits both the skin and mucous membranes and is primarily sourced from humans, including drug-resistant variants like MRSA (5).

Methicillin-resistant *Staphylococcus aureus* (MRSA) was initially observed in England in 1961, soon after the introduction of methicillin. Nevertheless, due to its toxicity, methicillin was subsequently largely substituted by more stable penicillins like oxacillin, flucloxacillin, and dicloxacillin. These medications are no longer available for human use (6). Infection risk escalates with MRSA colonization, with colonization strains matching the infections in 50-80% of cases. Nearly any item in contact with the skin, including white clothing, plum-adorned ties, and cell phones, can serve as a fomite for MRSA transmission. Colonization may persist for extended periods, even within the household environment, making eradication challenging. It's important to note that colonization is not a static condition, as strains can evolve within the same host and may be replaced over time (7). In the last forty years, the treatment of antimicrobial-resistant staphylococcal infections has undergone various evolutions. When MRSA first emerged, the initial strains included a complex SCC mec cassette containing multiple genes for antimicrobial resistance (3, 8).

Various antibiotics, including vancomycin, are employed to combat *S. aureus* strains that have developed resistance. However, vancomycin is associated with significant side effects, including injection site rashes and the risk of hearing impairment (9). Throughout history, plants have served as crucial sources of medicine and alternative treatments, offering ways to alleviate symptoms and combat illnesses. In modern healthcare, the utilization of natural agents derived from plants is gaining traction due to their potential efficacy and reduced side effects (10). Medicinal plants are being explored as remedies for combating methicillin-resistant *S. aureus* infections. This underscores the importance of seeking herbal extracts with antibacterial properties to address multi-drug resistant *S. aureus*, aligning with the well-established tradition of using medicinal plants as effective remedies for various bacterial infections (11). The present study aimed to assess the antibacterial potential of extracts of medicinal plant against multi-drug resistant *S. aureus*. Local *S. aureus* strains were isolated from diverse sources, including nasal, urine, pus, milk and blood samples. Drug susceptibility was determined using the disc diffusion method (DFM). We collected and air-dried twenty-five medicinal plants from different regions, then extracted them using methanol. These plant extracts were screened for their antibacterial efficacy against multi-drug resistant *S. aureus*, with the half-maximal inhibitory concentration (IC50) determined using the micro-dilution method. The encouraging outcomes from this study suggest that our plant extracts, with their potent antibacterial properties, hold promise as potential therapeutic agents for more effective management of multi-drug resistant (MDR) *S. aureus* infections, warranting further in-depth investigation.

MATERIALS AND METHODS

SAMPLE COLLECTION

Various specimens, including nasal, blood, milk, urine, and pus, were collected for *S. aureus* isolation. Among the total 47 collected samples, there were 23 blood samples, 15 pus samples, 5 nasal samples, 3 urine samples, and 1 milk sample, all stored at -20ºC.

CONFIRMATION OF *S. AUREUS* BY BIOCHEMICAL TESTS

To isolate and identify *S. aureus*, a series of distinct biochemical tests were conducted at microbiology lab, Bahawalpur Victoria Hospital (BVH). These tests served the purpose of differentiating *S. aureus* strains from other staphylococci, such as *S. epidermidis*, and included the following assessments.

MANNITOL SALT AGAR PLATES

The procedure involved streaking the samples onto mannitol salt agar plates and incubating them at 37ºC overnight. Subsequently, a visual inspection of the plates was conducted to identify the presence of *S. aureus* colonies based on their characteristic morphology.

BIRD PARKER MEDIA

Yellow colonies were streaked on Baird Parker medium, and the plates were then incubated overnight at 37ºC. After the overnight incubation, all these plates were inspected for the presence of *S. aureus* based on colony morphology.

HEMOLYSIS ON BLOOD AGAR

Blood agar plates were utilized to assess hemolysis caused by the isolates. These specimens were streaked onto the agar plates and then incubated for 18-24 hours at 37ºC within an aerobic setting for analysis.

CATALASE TEST

In the process of distinguishing *S. aureus* from non-catalase producing bacteria, a catalase test was executed. This entailed adding bacterial cells from a colony into a 3% hydrogen peroxide droplet, and the observation of bubble formation was employed as an indicator of catalase activity.

COAGULASE TEST

For the additional characterization of *S. aureus* strains, a positive bound coagulase test was employed. This test facilitates the direct conversion of fibrinogen to fibrin through the use of rabbit plasma. In practice, bacterial cells from a colony were introduced into a test tube containing rabbit plasma, and any evident coagulation was carefully noted.

DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES (MRSA) BY PCR

For the identification of MRSA strains, a standard PCR assay was performed using the Mastercycler gradient DNA amplification instrument. Cellular DNA was extracted from Staphylococci colonies, cultivated overnight on blood agar plates, employing the DNA Extraction Kit. Amplification of the 533 base pair (bp) fragment was carried out using mecA-specific primer pairs: Forward, 5′-AAAATCGATGGTAAAGGTTGGC-3′, and Reverse, 5′-AGTTCTGGAGTACCGGATTTGC-3′. A master mix was meticulously prepared, and the PCR thermal cycling protocol involved initial denaturation at 95°C for 3 minutes, followed by 33 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension step at 72°C for 6 minutes. Visualization of the amplified products was accomplished through electrophoresis in 2% agarose gels stained with ethidium bromide (12).

ANTIMICROBIAL DISC SENSITIVITY TESTING

Subsequent steps included sub-culturing the isolates to ensure purity and confirming their identity following established criteria, along with the meticulous performance of a disc sensitivity test for oxacillin. For this purpose, antimicrobial susceptibility testing followed CLSI guidelines using Kirby-Bauer method on Mueller-Hinton agar with 0.5 McFarland standard. Incubation occurred at 35°C ± 2°C for 16-18 hours. Antibiotic discs were sourced from Oxoid, a reputable UK supplier, and systematic documentation of the results (13).

PLANT SELECTION AND EXTRACTION

Selected material of plants from 25 distinct species were collected, guided by unverified reports from various geographic regions. The fresh plant specimens or their relevant parts were methodically laid out on filter paper and subjected to a one-week, shaded drying period at room temperature (20-25°C) at the Cholistan University of Veterinary and Animal Sciences, Bahawalpur. This gradual drying approach aimed to mitigate substantial chemical changes. Subsequently, the desiccated plant material was finely chopped,

soaked in methanol, and allowed to steep overnight. Following this infusion, the resulting extracts were carefully filtered, and a fresh solvent was added for an additional overnight incubation with periodic agitation. The following day, the extracts underwent another filtration. Each plant's extracts were meticulously concentrated within a temperature range of 30 to 40°C to prevent boiling, and they were further evaporated until complete dryness was achieved (14). Selected Plants were given in Table I.

Table I. Selected medicinal plants used against multidrug resistant *S. aureus*

	Common Name	Scientific Name	Useable Part
1	Paneer Dodi	*Withania Coagulans*	Flower/Fruit
2	Jamun	*Syzygium Cumini*	Leaves
3	Charaita	*Swertia Chirata*	Roots
4	Mundi Booti	*Shaeranthus Indicus*	Leaves
5	Mako	*Solanum Nigram*	Leaves
6	Sandal	*Santalum Album*	Wood
7	Majith	*Rubia cordifolia*	Heartwood
8	Anaar	*Punica Granatus*	Annar Coat
9	Gauva	*Psidium Guajava*	Leaves
10	Black Pepper	*Piper Nigrum*	Seeds
11	Sheetal Chini	*Piper Cubeba*	Seeds
12	Hermal	*Peganum Harmala*	Leaves
13	Kalonji	*Nigella Sativa*	Seeds
14	Sahajna	*Moringa Oleifera*	Leaves
15	Mehndi	*Lawsonia inermis*	Leaves
16	Shahtra	*Fumaria Officinalis*	Whole Plant
17	Eucaluptus	*Eucalyptus globulus*	Seeds/Leaves
18	Tumma	*Citrullus Colocynthis*	Fruit
19	Kali Jeeri	*Centratherum anthelminticum*	Seeds
20	Red Chili	*Capsicum Frutescens*	Fruit
21	Sumblo	*Berberis Lycecum*	Root Bank
22	Afsanteen	*Artemisia absinthium*	Leaves
23	Aloe Vera	*Aloe Vera*	Leaves
24	Korh	*Acacia Suma*	Roots
25	Kikar	*Acacia Nilotica*	Beans/Leaves

ANTIMICROBIAL ANALYSIS OF PLANTS EXTRACTS

The investigation of antimicrobial effectiveness against multi-drug resistant *S. aureus* was carried out in microbiology lab, Cholistan University of Veterinary and Animal Sciences, Bahawalpur accordance with the methodologies delineated in the materials and methods section. Standardized inoculums were diligently prepared and applied as per the specified procedure. In a methodical manner, sterile filter paper discs, each imbued with 10µl known concentration of an extract, were judiciously positioned at the precise center of appropriately labeled plates. These plates were subsequently inverted and placed in an aerobic incubator set at 37°C. After an incubation period of 19 hours, a comprehensive examination was conducted to identify and assess any visible zones of inhibition (15).

HALF MAXIMAL INHIBITORY CONCENTRATION (IC_{50}) DETERMINATION

The IC_{50} values were ascertained employing the micro-dilution technique, following the guide lines established by the CLSI-2020. The determination of IC50 involved serial dilution (in 2-fold increments) of plant extracts, starting from an initial concentration of 40mg/ml, distributed across ten test tubes (16).

STATISTICAL ANALYSIS

Graph-Pad Prism software was used for statistical analysis, and the outcomes were expressed as the mean ± standard error (SE).

RESULTS

POSITIVE *S. AUREUS* SAMPLE

Collected from various hospitals of Bahawalpur region, a sum of 47 clinical and sub-clinical samples was examined. Notably, only 21 of these samples (44.68%) exhibited a positive presence of *S. aureus*. These positive cases encompassed, 14 from pus samples, 1 from blood samples, 1 from milk samples, 3 from urine samples, and 2 isolates from nasal samples.

BIOCHEMICAL IDENTIFICATION OF *S. AUREUS*

Out of the 47 samples, only 21 displayed yellow growth when streaked on mannitol salt agar, while the remainder did not exhibit yellow growth on this medium. In the current study, the 21 confirmed *S. aureus* isolates were subsequently streaked on Baird-Parker (BP) medium, resulting in the development of black colonies after a 24-hour incubation period. These 21 isolates demonstrated the presence of beta hemolysins by their ability to completely lyse red blood cells, as evident on the blood agar plate. Furthermore, all 21 samples were found to produce catalase. Additionally, these 21 samples exhibited the capacity to convert fibrinogens directly into fibrin when exposed to rabbit plasma, leading to the formation of clots.

ANTIMICROBIAL SUSCEPTIBILITY TESTING BY DISCS DIFFUSION METHOD

In this comprehensive analysis, a disc diffusion assay was carried out on all samples that exhibited positive results for *S. aureus*. For an enhanced antibiotic diffusion during the antibiotic sensitivity test, the choice of Mueller-Hinton Agar (MHA) as the medium was made. Furthermore, the examination of antimicrobial resistance, performed via the disc diffusion method, included the testing of each individual isolate with a panel of five distinct antibiotics shown in Fig. 1.

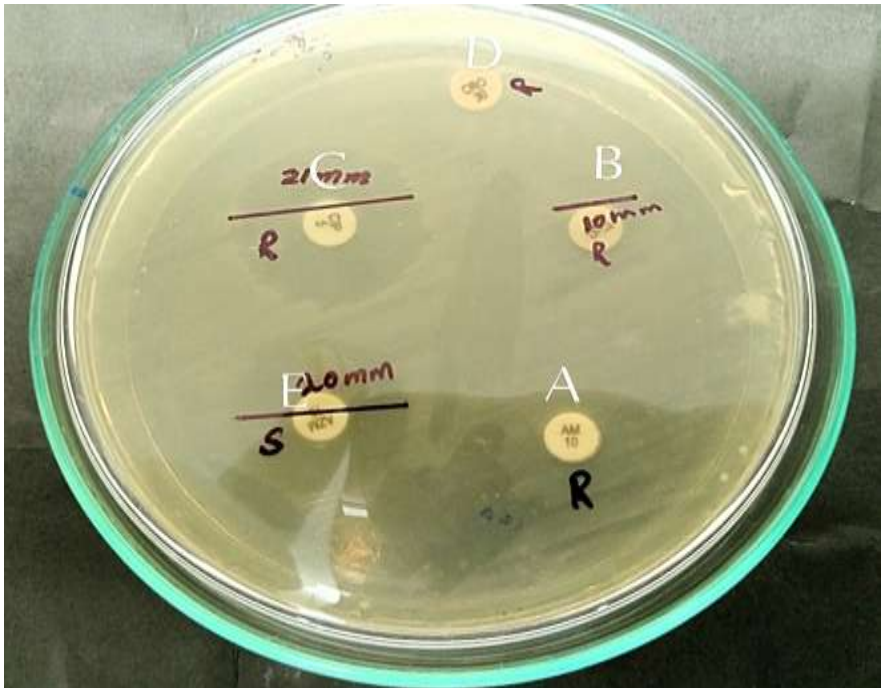

Fig. 1. Shows **(A):** Ampicillin **(B):** Oxacillin **(C):** Ciprofloxacin **(D):** Chloramphenicol **(E):** Sulphamethoxazole

Antibiotic Resistant Profiling of all samples were followed: Oxacilin was exhibited resistance in 10 samples, accounting for 47.6% of the cases. Ampicillin showed complete resistance in all 21 samples, indicating 100% resistance. Chloramphenicol was resistant in 4 samples, equivalent to 19.04%. Ciprofloxacin displayed resistance in a single sample, which constituted 4.76%. Sulphamethoxazole demonstrated resistance in 5 samples, representing 23.80% of the cases as shown in Table II.

Table II. Confirmation of Multidrug Resistant *S. aureus* strains by Discs mearing Zone (mm)

Sample No.	(1.0 µg) Oxacillin	(10.0 µg) Ampicillin	(30.0 µg) Chloramphenicol	(5.0 µg) Ciprofloxacin	(25.0 µg) Sulphamethoxazole
P.1	S 15.67±0.34	R 8.43±0.89	S 23.10±0.58	S 22.77±0.89	R 8.10±0.58
P.2	S 19.43±0.89	R 12.10±0.58	S 21.77±0.89	S 25.10±0.58	R 9.10±0.58
P.3	S 15.10±0.58	R 25.77±0.89	R 29.77±0.89	S 32.43±0.89	S 30.77±0.89
P.5	R 33.10±0.34	R 8.10±0.58	R 8.10±0.001	S 24.10±0.58	S 22.27±0.61
P.7	R 0.001±0.001	R 8.43±0.89	R 9.13±0.89	S 22.43±0.89	S 25.43±0.89
P.8	S 15.17±0.45	R 26.93±0.93	S 19.10±0.58	S 34.43±0.89	S 36.27±0.61
P.9	R 6.10±0.01	R 15.10±0.58	S 22.77±0.89	S 26.93±0.93	S 24.43±0.45
P.10	R 0.001±0.001	R 7.10±0.58	S 26.60±0.29	S 34.10±0.58	S 27.27±0.45
P.11	R 4.10±2.01	R 7.26±0.61	S 27.93±0.45	S 34.27±0.17	S 26.60±0.29
P.12	R 4.10±2.00	R 0.001±0.001	I 13.27±0.45	S 23.60±0.29	S 15.27±0.45
P.13	R 0.001±0.001	R 0.001±0.001	S 21.27±0.73	S 26.43±0.34	S 26.60±0.29
P.14	S 14.89±0.17	R 9.10±0.58	S 23.60±0.29	S 24.27±0.45	S 19.27±0.61
P.15	R 4.10±2.01	R 0.001±0.001	R 11.93±0.45	R 12.60±0.29	S 20.10±0.58
U.1	S 15.27±0.45	R 8.26±0.45	S 22.10±0.58	S 22.60±0.29	R 7.10±0.59
U.2	S 17.67±0.29	R 7.60±0.77	S 21.77±0.34	S 23.10±0.58	R 7.10±0.58
U.3	R 14.93±0.17	R 8.26±0.45	S 21.10±0.58	S 23.10±0.58	R 6.76±0.34
N.1	R 7.43±0.67	R 10.10±0.58	S 21.93±0.45	S 21.77±0.34	S 28.10±0.29
N.2	R 6.76±0.67	R 9.10±0.58	R 12.93±0.45	S 22.27±0.17	S 24.27±0.61
N.3	S 15.27±0.45	R 21.10±0.58	S 21.60±0.77	S 30.60±0.29	S 32.27±0.45
M.1	S 15.10±0.58	R 20.10±0.58	S 21.77±0.34	S 23.27±0.61	S 27.93±0.73
B.1	S 17.60±0.29	R 10.27±0.45	S 21.93±0.61	S 21.60±0.29	S 19.10±0.58

ANTIMICROBIAL ANALYSIS OF PLANTS EXTRACTS

The selection of plant materials was guided by informal reports suggesting their potential antibacterial efficacy against *S. aureus*. Various plant components were procured and subjected to a week of drying in shaded conditions. Comprehensive data, including the plant parts employed and their respective local names, was methodically collected and recorded as shown in Table I.

Additionally, extracts obtained from *Capsicum Frutescens, Artemisia Absinthium, Piper Nigrum, Nigella Sativa, Psidium Guajava, Acacia Nilotica, and Piper Cubeba*, *Withania Coagulans*, demonstrated evident zones of inhibition as shown in data in Table III.

Table III. Inhibitory Zone (mm) against *S. aureus* Isolates by Plant Extracts

Extracts	Bacterial Samples IDs (Zone of Inhibition in mm)									
Plant Names	**P.5**	**P.7**	**P.9**	**P.10**	**P.11**	**P.12**	**P.13**	**P.15**	**N.1**	**N.2**
Withania Coagulans	15.50 ± 0.28	16.83 ± 0.44	19.00 ± 0.57	17.17 ± 0.44	17.33 ± 0.88	16.33 ± 0.88	18.83 ± 0.44	19.00 ± 0.57	17.50 ± 0.76	15.50 ± 0.28
Syzygium Cumini	8.83 ± 0.44	10.17 ± 0.44	12.50 ± 0.28	12.00 ± 0.57	12.50 ± 0.28	11.83 ± 0.44	8.50 ± 0.28	8.83 ± 0.44	10.50 ± 0.28	12.33 ± 0.88
Swertia Chirata	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Shaeranthus Indicus	9.83 ± 0.44	10.17 ± 0.44	8.83 ± 0.44	8.50 ± 0.28	9.83 ± 0.44	12.00 ± 0.57	9.16 ± 0.44	8.50 ± 0.28	9.16 ± 0.44	11.83 ± 0.44
Solanum Nigram	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Santalum Album	12.00 ± 0.57	12.50 ± 0.28	11.83 ± 0.44	8.83 ± 0.44	9.83 ± 0.44	12.00 ± 0.57	8.50 ± 0.28	10.50 ± 0.28	12.33 ± 0.88	11.83 ± 0.44
Rubia cordifolia	16.83 ± 0.44	17.50 ± 0.76	12.50 ± 0.28	15.50 ± 0.28	18.83 ± 0.44	15.17 ± 0.72	19.00 ± 0.57	17.17 ± 0.44	18.83 ± 0.44	17.33 ± 0.88
Punica granatum	18.83 ± 0.44	16.33 ± 0.88	17.33 ± 0.88	19.00 ± 0.57	21.00 ± 0.57	16.83 ± 0.44	23.83 ± 0.16	18.83 ± 0.44	20.33 ± 0.72	17.50 ± 0.76
Psidium Guajava	12.50 ± 0.28	14.83 ± 0.44	13.67 ± 0.44	12.33 ± 0.88	12.00 ± 0.57	13.50 ± 0.28	11.83 ± 0.44	15.17 ± 0.72	13.67 ± 0.44	12.50 ± 0.28
Piper Nigrum	12.00 ± 0.57	9.83 ± 0.44	10.17 ± 0.44	12.50 ± 0.28	11.83 ± 0.44	12.00 ± 0.57	10.50 ± 0.28	12.50 ± 0.28	11.83 ± 0.44	13.50 ± 0.28
Piper Cubeba	11.83 ± 0.44	10.50 ± 0.28	9.16 ± 0.44	15.17 ± 0.72	12.33 ± 0.88	12.50 ± 0.28	13.67 ± 0.44	10.17 ± 0.44	12.50 ± 0.28	12.00 ± 0.57
Peganum Harmala	12.50 ± 0.28	11.83 ± 0.44	12.33 ± 0.88	9.16 ± 0.44	12.50 ± 0.28	10.50 ± 0.28	9.83 ± 0.44	9.16 ± 0.44	12.33 ± 0.88	8.50 ± 0.28
Nigella Sativa	12.00 ± 0.57	13.67 ± 0.44	9.83 ± 0.44	15.17 ± 0.72	12.33 ± 0.88	12.50 ± 0.28	16.33 ± 0.88	14.83 ± 0.44	15.50 ± 0.28	14.83 ± 0.44
Moringa Oleifera	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lawsonia inermis	15.50 ± 0.28	16.83 ± 0.44	13.67 ± 0.44	15.50 ± 0.28	8.83 ± 0.44	15.17 ± 0.72	22.50 ± 0.28	17.50 ± 0.76	16.33 ± 0.88	17.33 ± 0.88
Fumaria Officinalis	12.00 ± 0.57	12.50 ± 0.28	9.83 ± 0.44	10.50 ± 0.28	8.50 ± 0.28	10.17 ± 0.44	8.83 ± 0.44	8.50 ± 0.28	9.16 ± 0.44	11.83 ± 0.44
Eucalyptus globulus	9.16 ± 0.44	15.50 ± 0.28	13.50 ± 0.28	13.67 ± 0.44	8.83 ± 0.44	15.17 ± 0.72	16.83 ± 0.44	12.33 ± 0.88	16.33 ± 0.88	17.33 ± 0.88
Citrullus Colocynthis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Centratherum	17.33	16.33	14.83	14.83	8.50	9.83	20.33	18.83	17.50	16.83

anthelminticum	±	±	±	±	±	±	±	±	±	±
	0.88	0.88	0.44	0.44	0.28	0.44	0.72	0.44	0.76	0.44
	10.50	12.50	11.83	12.33	11.83	13.67	8.83	9.16	12.00	10.50
Capsicum Frutescens	±	±	±	±	±	±	±	±	±	±
	0.28	0.28	0.44	0.88	0.44	0.44	0.44	0.44	0.57	0.28
	9.16	10.17	8.83	8.50	7.83	9.83	8.00	8.50	9.16	7.83
Berberis Lycecum	±	±	±	±	±	±	±	±	±	±
	0.44	0.44	0.44	0.28	0.44	0.44	0.57	0.28	0.44	0.44
	12.33	15.50	9.16	13.50	10.50	12.00	15.17	8.83	14.83	11.83
Artemisia absinthium	±	±	±	±	±	±	±	±	±	±
	0.88	0.28	0.44	0.28	0.28	0.57	0.72	0.44	0.44	0.44
	10.17	12.50	12.00	12.33	9.83	12.50	8.50	11.83	8.83	10.17
Aloe Vera	±	±	±	±	±	±	±	±	±	±
	0.44	0.28	0.57	0.88	0.44	0.28	0.28	0.44	0.44	0.44
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acacia Suma	±	±	±	±	±	±	±	±	±	±
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	15.17	16.83	14.83	12.00	10.17	17.50	17.17	17.33	17.17	16.83
Acacia Nilotica	±	±	±	±	±	±	±	±	±	±
	0.72	0.44	0.44	0.57	0.44	0.76	0.44	0.88	0.44	0.44

The largest zones of inhibition were recorded for five medicinal plants: *Rubia cordifolia* (18mm), *Lawsonia inermis* (22mm), *Punica granatum* (24mm), *Eucalyptus globulus* (16mm), and Centratherum anthelmenticus (19mm) respectively have shown in Fig. 2.

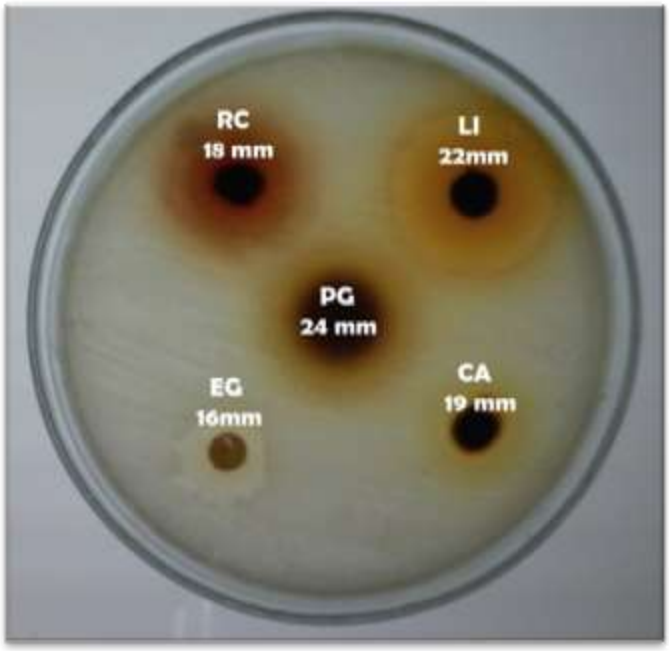

Fig. 2. Inhibitory Effect of Medicinal Plant on Multidrug resistant *S. aureus*, **(EG):** *Eucalyptus globulus*, **(CA):** *Centratherum Anthelmenticus*, **(PG):** *Punica granatum*, **(LI):** *Lawsonia inermis*, **(RC):** *Rubia cordifolia*

Out of the ten oxacillin-resistant strains, there were selected three strains for IC50 testing against five potent plant extracts *Punica Granatum, Rubia Cordifolia, Centratherum Anthelminticum, and Eucalyptus Globulus* (Seeds), *Lawsonia Inermis, and* as described in Table IV. The study results revealed that *Centratherum Anthelminticum* extract in mathanol exhibited a 50(%) inhibition of the P.7 strain of *S. aureus* at a concentration of 1.20 mg/ml. In the case of the P.10 and P.13 strains, the 50(%) inhibition was observed at concentrations of 1.20 mg/ml and 0.946 mg/ml, respectively. In a similar experimental setup, the methanolic extract of *Eucalyptus globulus* (Seeds) showed IC50 values of 4.30 mg/ml and 3.38 mg/ml for the P.7 and P.10 strains of *S. aureus*, while the P13 strain exhibited 50% inhibition at a concentration of 4.43 mg/ml. The extract of *Lawsonia Inermis* in methanol demonstrated an IC50 value of 2.07 mg/ml for the P.7 strain, and it achieved 50% inhibition for the P.10 and P.13 strains at concentrations of 1.99 mg/ml and 1.38 mg/ml, respectively. Likewise, the extract of *Punica Granatum* in methanol resulted in 50% inhibition for the P7 strain of *S. aureus* at a concentration of 0.530 mg/ml, while the P.10 and P.13 strains both displayed 50%

inhibition at a concentration of 0.499 mg/ml each. Remarkably, the study's findings indicated that the *Rubia Cordifolia* extract in methanol induced 50% inhibition for both the P.7 and P.10 *S. aureus* strains at concentrations of 0.280 mg/ml each, with the P.13 strain exhibiting an IC50 at a concentration of 0.250 mg/ml.

Table IV. IC50 of Medicinal Plant against *S. aureus* Isolates

Sample No.	IC_{50} of Medicinal Plants in mg/ml				
	Punica granatum	*Rubia cordifolia*	*Eucalyptus globulus*	*Lawsonia inermis*	*Centratherum anthelminticum*
P.7	0.530	0.280	4.30	2.07	1.02
P.10	0.499	0.280	3.38	1.99	1.20
P.13	0.499	0.250	4.43	1.38	0.946

DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES (MRSA) BY PCR

The identification of MRSA strains was conducted through a PCR assay targeting the mecA gene, which was present in all *S. aureus* strains. The results unveiled that 42.1% of the Staphylococci isolates harbored the mecA gene. Fig. 3 illustrates the presence of the mecA gene in five specifically chosen *S. aureus* isolates.

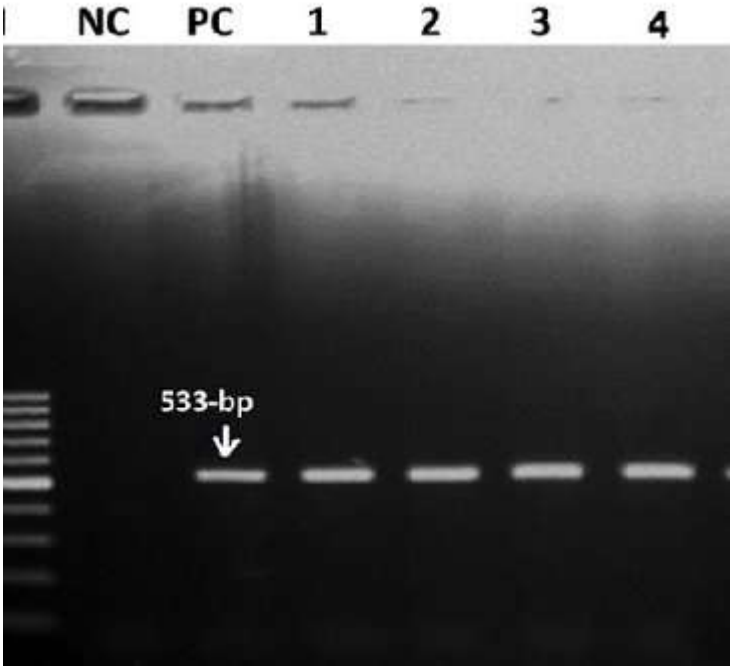

Fig 3. Five selected *S. aureus* Isolates mecA gene

MIC DETERMINATION

The distribution of Oxacillin Minimum Inhibitory Concentration (MIC) readings for MRSA is as follows: all isolates exhibited MIC values below 4 µg/ml, specifically, 54.5% of the isolates displayed MIC values below 2 µg/ml, 35.8% fell within the range of 2-3 µg/ml, and 9.6% had MIC values in the 3-4 µg/ml range.

DISCUSSION

The treatment of *S. aureus* infections primarily depends on the nature of the infection and whether drug-resistant strains are present. When antimicrobial treatment is deemed necessary, the duration and approach to therapy are largely determined by the specific infection and various associated factors (17). *S. aureus* serves as the causative agent for a diverse spectrum of infections, encompassing soft tissue infections, benign colonization, sepsis, biofilm-associated prosthetic infections, deep wound infections, severe life-

threatening conditions and superficial skin ailments. This highly opportunistic pathogen is capable of inciting more severe and challenging infections, spanning conditions such as carbuncles, deep-seated lesions, boils, furuncles, impetigo, cellulitis, abscesses, endocarditis, and pneumonia (18). *S. aureus* is undoubtedly the most clinically relevant pathogen. It is a prevalent component of the commensal microbiota in the nasal mucosa of approximately 20–40% of the general population (19). In cases such as chronic skin conditions, wounds, or surgical procedures that compromise the cutaneous and mucosal defenses, *S. aureus* can exploit these vulnerabilities to initiate infections (20). The escalating drug resistance observed in *S. aureus* strains has amplified the global challenge. Various strategies are being implemented to curtail the proliferation of MRSA through the judicious application of antimicrobial agents and vigilant reservoir monitoring. Preventive measures are typically employed to mitigate MRSA transmissions and infections. *S. aureus* has developed mechanisms of resistance against methicillin, penicillin, vancomycin, tetracycline, quinolone, and erythromycin as an evasion tactic against the human immune system (21). *S. aureus* is progressively acquiring resistance to antibiotics across all categories (22). In this current investigation, 47.6% of the isolates exhibited oxacillin resistance, with 23.80% displaying resistance to Sulphamethoxazole, 19.04% to chloramphenicol, 4.7% to ciprofloxacin, and 100% resistance to ampicillin. The confirmation of resistance to Oxacillin and other antibiotics was conducted through the disc diffusion assay (23). Given the increasing resistance of *S. aureus* to antibiotics across all categories, it becomes imperative to formulate effective strategies to combat this resistance issue. Medicinal plants are being explored as potential remedies for the treatment of methicillin-resistant *S. aureus* infections (24). Information on traditional medicinal plant usage and their antimicrobial properties, extracted from various plant parts, is crucial due to the rise in drug-resistant pathogens. These natural sources may offer solutions to combat multi-drug resistant infections (25). Out of the twenty-five medicinal plants tested, five exhibited antibacterial activity against *S. aureus*, while extracts from plants like *Artemisia Absinthium, Withania Coagulans,* and *Piper Nigrum, Capsicum Frutescens, Nigella Sativa, Psidium Guajava, Piper Cubeba Acacia Nilotica,* did not show such antibacterial activity (Table 3). *Centratherum Anthelmintic*um, or Kaligiri, traditionally treats leucoderma and skin issues. Its seeds have applications ranging from purgative to anti-inflammatory remedies, with scientific studies exploring various health benefits, including antibacterial, antifungal, and antioxidant properties (26). No scientific evidence currently supports the notion of drug resistance in *S. aureus* against *Centratherum Anthelmintic*um. Consequently, we conducted in vitro experiments to evaluate the antibacterial activity of *Centratherum Anthelmintic*um against multidrug-resistant *S. aureus* isolates from clinical and sub-clinical samples, specifically strains P.7, P.10, and P.13. The results demonstrated an inhibitory effect at a concentration of 40µg/ml, with zone sizes of 15.0 mm, 16.0 mm, and 19.0 mm for clinical isolates P.7, P.10, and P.13, respectively. The extract of *Centratherum Anthelmintic*um exhibited an IC50 range between 0.946 and 1.20. *Eucalyptus Globulus*, on the other hand, is employed for controlling a broad spectrum of diseases stemming from bacterial infections (27). While information on its effectiveness remains limited, this study demonstrated that multi-drug resistant *S. aureus* isolates were effectively inhibited at 40µg/ml. For clinically isolated *S. aureus* (P.7, P.10, and P.13), the zone of inhibition ranged from 13 mm to 16 mm, and the IC50 value fell between 3.38 and 4.43 mg/ml. Additionally, the leaf extract of *Lawsonia Inermis* is associated with sedative and nootropic effects in pharmacological activities (28). Sukanya et al. (2009) reported a 6.0 mm zone of inhibition against clinical *S. aureus* using leaf extracts of *Lawsonia Inermis*, employing a well-established dilution method (29). The concentration of antimicrobial activity was evident in the *Lawsonia Inermis* extract, with zone sizes of 16.0 mm, 15.0 mm, and 22.0 mm for clinically isolated *S. aureus* strains P.7, P.10, and P.13, respectively. This extract displayed an IC50 value ranging from 1.38 to 2.07 mg/ml. Notably, *Punica Granatum* rind exhibits significant antimicrobial activity (30). Additionally, rind served as an astringent agent (31). As reported by Khan et al. (2014), the methanolic extract derived from the fruit peels of *P. Granatum* exhibited efficacy against *S. aureus* (32). Our study aimed to evaluate the antimicrobial potential of *P. Granatum*. The results from the disc diffusion assay demonstrated inhibition zones of 6mm for multi-drug resistant *S. aureus* isolates, with clinical isolates (P.7, P.10, and P.13) showing zones of 15.0 mm, 18.0 mm, and 24.0 mm, respectively. *Rubia Cordifolia*, a significant plant with potential for treating various

diseases and inhibiting lipid peroxidation, was used as a methanolic root extract against oxacillin and other drug-resistant *S. aureus*. The inhibition zones for clinically isolated *S. aureus* (P.7, P10, and P.13) were 17.0 mm, 15.0 mm, and 18.0 mm, respectively, with IC50 values ranging from 0.250 to 0.280. These findings suggest that the extracts of *Eucalyptus Globules* (seeds), *Centratherum Anthelmintic*um (seeds), *Lawsonia Inermis* (leaves), *Rubia Cordifolia* (root) and *Punica Granatum* (peel), have promising potential in the treatment of *S. aureus* infections.

CONCLUSION

This article has detailed the resistance profiles of various drugs against *S. aureus* while also assessing the antimicrobial potential of medicinal plants against this bacterium. The findings strongly suggest the potential application of these plant extracts as pharmaceutical agents. This research offers valuable insights into the identification of active compounds within these extracts, demonstrating robust antibacterial efficacy against MRSA, a formidable pathogen. These active compounds hold promise for future drug development targeting MRSA.

Conflict of Interest:

Authors have no conflict of interest.

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