INTRODUCTION

Staphylococcus aureus is a commensal and an opportunistic human pathogen and has become one of the major etiological agents in hospital settings [1]. Methicillin-resistant S. aureus (MRSA) has caused many outbreaks across the globe and more seriously acquiring multidrug-resistant (MDR) status and posing a serious threat to the treatment options in India [2]. The prevalence of MRSA in bacteria causing infectious diseases is still spread worldwide. Thus, the efficacy of antimicrobial chemotherapy calls for an alternative approach to eradicate the pathogenicity of bacteria. Traditional medicines are used to cure various infections 3,000 B.C. Lawsonia inermis commonly known as Henna is a flowering plant, belongs to the family Lythraceae and is a glabrous branched shrub or small tree, 2-6 m in height. L. inermis produces a burgundy dye molecule, lawsone used to dye the skin, hair, fingernails, leather, silk, and wool. The dye molecule, lawsone, is mainly concentrated in the leaves [3]. L. inermis is a perennial shrub native to North Africa, Asia, and Australia and also cultivated in the tropics of America, Egypt, India and parts of the Middle East and is tolerant extreme heat and long droughts. The leaves are used in the treatments of wounds, ulcers, cough, bronchitis, lumbago, rheumatoid, inflammations, diarrhea, leukoderma, scabies, boils, anemia, hemorrhages, mental disorders, for jaundice, dermatoses, chicken pox, calculus, dysuria, bleeding diarrhea, knee pain, redness of eyes, fever, falling of hair, and graysness hair [4]. The present study evaluates the antibacterial and antifilm potential of L. inermis leaf extracts against MRSA.

METHODS

Plant collection and extraction

Leaves of L. inermis (Fig. 1) were collected around Gulbarga University of Karnataka region in the month of July 2015. The leaves were shade dried, and 1 g of dried powder was mixed with 10 of the solvent (methanol, acetone, ethyl acetate, chloroform, petroleum ether, and n-hexane) in screw-capped bottles and incubated on a rotary shaker for 3 days. The supernatant obtained was dried by evaporation at room temperature. The extract obtained was dried and weighed. Stock was prepared by dissolving 10 mg of extract in 1 ml of DMSO and only DMSO serves as control.

Microbial cultures

MDR and MRSA employed in the study are the isolates from previous studies maintained in the laboratory. Isolates have been identified and confirmed by following standard microbiological techniques [5]. Antibiotic susceptibility patterns of clinical isolates were determined out as per CLSI guidelines [2014] [6].

Antibacterial activity

Extracts of different solvents were tested for antibacterial activity by agar well diffusion method. Overnight cultures of MRSA were enriched in brain heart infusion broth to attain 0.5 McFarland turbidity. Using a sterile cotton swab, the cultures were swabbed on sterile Mueller-Hinton agar plates to obtain a near confluent lawn. The agar gel was punctured using a sterilized metal borer (5 mm diameter). 100 μl of different extracts were filled into the wells of inoculated plates. The plates were incubated at 37°C for overnight. Zone of inhibition was observed and measured (Fig. 2).
acid, and absorbance was read at 595 nm. Only DMSO serves a control. Percentage inhibition of biofilm production was calculated as follows:

\[
\% \text{ of biofilm inhibition} = \frac{(\text{od of control} - \text{od of test})}{\text{od of control}}
\]

RESULTS

Methanol extract the showed the highest zone of inhibition against MRSA of 18mm compared to vancomycin of 15 mm (Table 1). However, acetone, ethyl acetate, chloroform, petroleum ether, and n-hexane extracts did not show any antibacterial activity against MRSA isolate. Conversely, petroleum ether extract showed the highest percentage of biofilm inhibition of 84.7% as against the other extracts such as methanol - 77% (Fig. 3). Interestingly, vancomycin at MIC 4 µg/ml showed inducing of biofilm formation in MRSA (Fig. 4).

DISCUSSION

Global burden of infectious diseases caused by bacterial agents is a serious threat to public health [8]. *S. aureus* can result in array of infections in humans. *S. aureus* infections can invade through an infected wound, skin by producing hyaluronidase that destroys tissues. Adherence to the tissues by biofilm formation is one of the primary steps in the pathogenesis of *S. aureus*.

*L. inermis* (Fig. 1) is highly regarded as panacea in herbal medicine with diverse spectrum of pharmacological activity [9]. Lawson isolated from the leaves of *L. inermis* has shown a significant antifungal effect [10]. The ethanol soluble fraction of *L. inermis*

fruits displayed highly potent activity against Semliki forest virus in Swiss mice and chick embryo models [11]. *L. inermis* showed a significant effect on memory and behavior mediated via monoamine neurotransmitters [12]. Antimalarial, leishmanicidal, trypanocidal, antihelmintic, and antiscabies trypanocidal activities were also reported [13]. In the present study, we have investigated the potential antibacterial and antibiofilm properties of *L. inermis* extracts against MRSA.

Methanol extract showed a very good antibacterial activity with an inhibition zone of 18mm, whereas the other solvent extracts showed a very meager antibacterial activity. There are a few reports on the use of medicinal plants against MRSA infections includes Triphala, Camellia sinensis (tea), Azadirachta indica (neem), Holarrhena antidysenterica (Kuruchi) bark, Delonix regia (Gulmohar) flowers, Punica granatum (Pomegranate), Hemidesmus indicus (Anantamul) stem, and Plumbago zeylanica (Chitra) [14]. Antibiofilm activities of medicinal plants were poorly studied in India. However, antibiofilm activities of *Zingiber officinale* against *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* have been reported [15].

### Table 1: Antibacterial effect of *L. inermis* against clinical isolate of MRSA

<table>
<thead>
<tr>
<th>SN</th>
<th>Solvent extract</th>
<th>Zone of inhibition (in mm±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>18±0.5</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>8±0.7</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate</td>
<td>8±0.2</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform</td>
<td>8±0.5</td>
</tr>
<tr>
<td>5</td>
<td>Petroleum ether</td>
<td>8±0.4</td>
</tr>
<tr>
<td>6</td>
<td>n-Hexane</td>
<td>8±0.5</td>
</tr>
<tr>
<td>7</td>
<td>Methicillin (5 mcg)</td>
<td>No zone</td>
</tr>
<tr>
<td>8</td>
<td>Vancomycin (30 mcg)</td>
<td>15±0.2</td>
</tr>
</tbody>
</table>

The data are expressed in mm±SD. Sample size n=3. MRSA: Methicillin-resistant *Staphylococcus aureus*
Similar results obtained with different solvents of *L. inermis* [16].

However, with respect to biofilm inhibition activity, all the extracts showed promising activities ranging from 77% by acetone extract to a maximum of 84.9% by petroleum ether extract. Our study exhibits significant results for antibiofilm activities showed highest inhibition by petroleum ether of 84.7%. Phytochemical studies of *L. inermis* confirmed the presence of glycosides, phytosterol, steroids, saponins, tannins, and flavonoids.

**CONCLUSION**

The crude extracts of the leaves of *L. inermis* have shown significant biofilm inhibition activities against the MDR MRSA and methanol extract a good antibacterial activity. This calls for further exploration of the bioactive compounds from *L. inermis* available everywhere, thus promises to be an alternative for the treatment of notorious nosocomial infections.

**REFERENCES**


10. Dixit SN, Srivastava HS, Tripathi RD. Lawsona, the antifungal antibiotic from the leaves of *Lawsonia inermis* and some aspects of its mode of action. Indian Phytopathol 1980;31:131-3.


