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Research Article

ANTIBACTERIAL AND ANTIBIOFILM ACTIVITIES OF CRUDE EXTRACTS OF *LAWSONIA INERMIS* AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objective: The continuous rise in the prevalence of multidrug resistance pathogens globally is threatening the treatment and management of infectious diseases. Ethnomedicine plays a key role in the exploration for novel bioactive compounds. The present study evaluates the antibacterial and antibiofilm activities of the crude extracts of *Lawsonia inermis* against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA).

Methods: Shade dried and finely powdered leaves of the plant were extracted by maceration method using six solvents: Methanol, acetone, ethyl acetate, chloroform, petroleum ether, and n-hexane. Antibacterial and antibiofilm activities of the extracts against multidrug-resistant (MDR) MRSA by agar cup diffusion and tube method, respectively.

Results: Methanol extract showed the highest antibacterial activity of 18 mm compared to other extracts. Similarly, petroleum ether extract showed the highest biofilm inhibition of 84.7%. Other solvent extracts also exhibited significant biofilm inhibition [n-hexane-83.6%, ethyl acetate-79.5%, chloroform-79.2%, acetone-77%, and methanol-77%].

Conclusion: The leaf extracts of *L. inermis* have shown promising biofilm inhibitory activity and good antibacterial activity, which can be explored for the development of new drugs for the MDR pathogens.

Keywords: Antibiotic activity, Antibiofilm activity, *Lawsonia inermis,* Methicillin-resistant *Staphylococcus aureus*.

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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 MDR in bacteria causing infectious diseases is still spread worldwide. Thus, the efficacy of antimicrobial chemotherapy calls for an alternative approach to eradicating 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 grayness hair [4]. The present study evaluates the antibacterial and antibiofilm 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).

Antibiofilm activity

Antibiofilm activity was determined as using tube method described [7]. 5 ml test tubes containing 2 ml of brain heart infusion broth with 5% sucrose were inoculated with 100 µl of the culture. Simultaneously, 100 µl aliquots of crude extracts prepared from 10 mg/ml stock was added into each of the test tubes and incubated under static conditions at 37°C for 24 hrs. Then, the contents of the tubes were decanted, gently washed with phosphate buffer saline of pH 7.4 for 3-4 times, and allowed to dry. The adherent cells were stained with 1% crystal violet for 1 minute. The stained tubes were washed several times using distilled water to remove the excess stain and tubes were dried. The adhered cells on the walls of the test tubes were dissolved in 33% glacial acetic

acid, and absorbance was read at 595 nm. Only DMSO serves a control. Percentage inhibition of biofilm production was calculated as follows:

% of biofilm inhibition = (od of control-od of test)/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]. Lawsone 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, antihelminthiasis, 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.

Fig. 1: *Lawsonia inermis* (Henna plant)

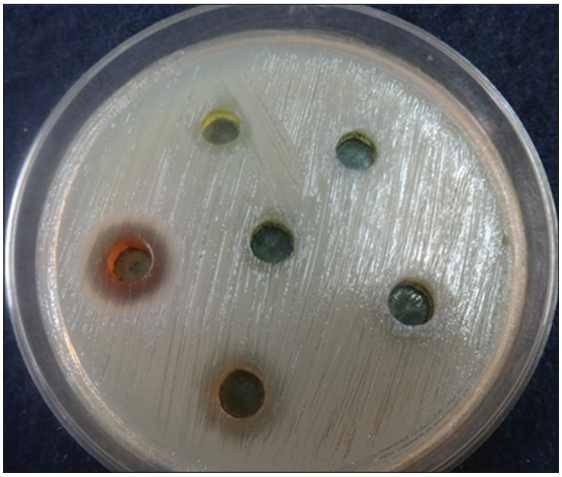

Fig. 2: Agar disc diffusion plate

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 (Kurachi) 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

SN	Solvent extract	Zone of inhibition (in mm±SD_)
1	Methanol	18±0.5
2	Acetone	8±0.7
3	Ethyl acetate	8±0.2
4	Chloroform	8±05
5	Petroleum ether	8±0.4
6	n-Hexane	8±0.5
7	Methicillin (5 mcg)	No zone
8	Vancomycin (30 mcg)	15±0.2

The data are expressed in mm±SD. sample size n=3. MRSA: Methicillin-resistant *Staphylococcus aureus*

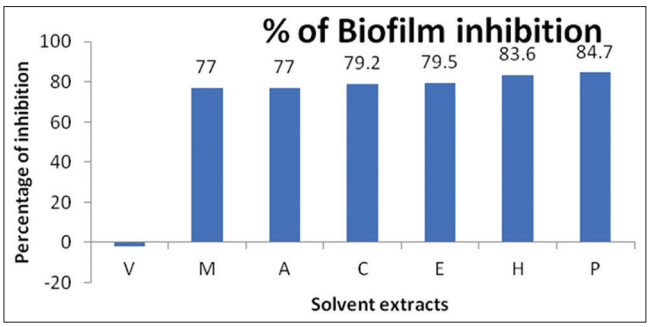

Fig. 3: Antibiofilm effect of *L.inermis* against clinical isolate of methicillin-resistant *Staphylococcus aureus*. V-vancomycin, M-methanol, A-Acetone, C-Chloroform, E-ethylacetate, H-n-Hexane, P-petroleum ether

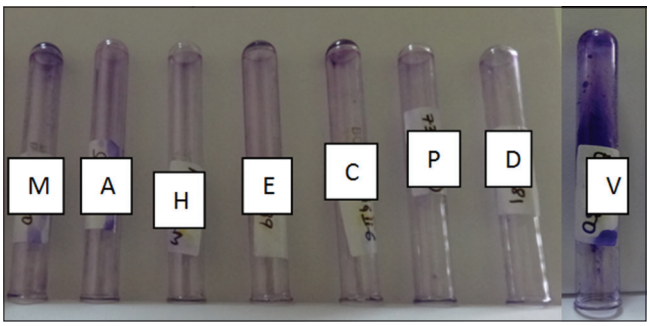

Fig. 4: Tube method-M-methanol, A-acetone, H-hexane, E-ethyl acetate, C-chloroform, P-petroleum ether, D-Dimethyl sulphoxide, V-vancomycin

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% inhibition by petroleum ether extract. Our study exhibits significant results for antibiofilm activities showed highest in 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

1. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23(3):616-87.
2. Mendem SK, Triveni AG, Shivannavar CT, Gaddad SM. Antibiotic resistance patterns of *Staphylococcus aureus*: A multi-center study from India. Microb Pathog 2016;98:167-70. Available from: http://dx.doi.org/10.1016/j.micpath.2016.07.010.
3. Al-Rubiay KK, Jaber NN, Al-Mhaawe BH, Alrubaiy LK. Antimicrobial efficacy of henna extracts. Oman Med J 2008;23(4):253-6.
4. Kidanemariam TK, Tesema TK, Asressu KH, Boru AD. Chemical investigation of *Lawsonia inermis* L. Leaves from Afar region, Ethiopia. Orient J Chem 2013;29(3):1129-34.
5. Bautista-Trujillo GU, Solorio-Rivera JL, Renterı´a-Solo´rzano I, Carranza-German SI, Bustos-Martınez JA, Arteaga-Garibay RI, *et al*. Performance of culture media for the isolation and identification of *Staphylococcus aureus* from bovine mastitis. J Med Microbiol 2013;62:369-76.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Document M100-S24. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2014.
7. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, *et al*. Adherence of coagulase negative *Staphylococci* to plastic tissue cultures: A quantitative model for the adherence of Staphylococci to medical devices. J Clin Microbiol 1985;22(6):996-1006.
8. Lister JL, Horswill AR. *Staphylococcus aureus* biofilms: Recent developments in biofilm dispersal. Front Cell Infect Microbiol 2014;4:178.
9. Chaudhary G, Goyal S, Poonia P. *Lawsonia inermis* linnaeus: A phytopharmacological review. Int J Pharm Sci Drug Res 2010;2(2):91-8.
10. Dixit SN, Srivastava HS, Tripathi RD. Lawsone, the antifungal antibiotic from the leaves of *Lawsonia inermis* and some aspects of its mode of action. Indian Phytopathol 1980;31:131-3.
11. Khan MM, Ali A, Jain DC, Bhakuni RS, Zaim M, Thakur RS. Occurrence of some antiviral sterols in *Artemisia annua*. Plant Sci 1991;75(2):161-5.
12. Iyer MR, Pal SC, Kasture VS, Kasture SB. Effect of *Lawsonia inermis* on memory and behavior mediated via monoamine neurotransmitters. Indian J Pharmacol 1998;30(3):181-5.
13. Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, Bories C, *et al*. Antiparasitic activities of medicinal plants used in Ivory Coast. J Ethanopharmacol 2004;90(1):91-7.
14. Kali A. Antibiotics and bioactive natural products in treatment of methicillin resistant *Staphylococcus aureus*: A brief review. Pharmacogn Rev 2015;9(17):29-34.
15. Nikolić M, Vasić S, Đurđević J, Stefanović O, Čomić L. Antibacterial and anti-biofilm activity of ginger (*Zingiber officinale* (Roscoe) ethanolic extract. Kragujevac J Sci 2014;36:129-36.
16. Santhanamari TH, Meenakshi PR, Sreekala V. *In vitro* antibacterial activity of extracts of *Lawsonia inermis* and *Punica granatum* against clinically isolated antibiotic resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Asian J Pharm Clin Res. 2011;4(1):62-5.