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Research Article

ANTIBIOFILM AND ANTIBACTERIAL ACTIVITY OF *PROSOPIS JULIFLORA* LEAVES EXTRACT AGAINST *ESCHERICHIA COLI* ISOLATED FROM CLINICAL ENDOMETRITIS

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ABSTRACT: Uterine infections in cattle and buffaloes cause severe economic loss to farmers and phytoremediation emerges as a promising alternative to synthetic antimicrobials to combat this issue. Herein, we investigated the precise mechanism of antibacterial action of *Prosopis juliflora* leaves (PJ) extract against a clinical isolate of *E. coli* with special reference to its anti-biofilm activity. PJ extract exhibited promising *in vitro* antibacterial action with 54.55% inhibition of bacterial biofilm production and decreased the multilayer growth of biofilms and free-living cells by influencing the integrity of cell walls as evidenced by scanning electron microscopy. Furthermore, it was observed that the damaged cell wall of the bacteria prevented the formation of clusters and prevented it from maintaining its normal morphology in the presence of the extract. Taken together, it may be inferred that PJ extract can be used to treat uterine infections as an alternative to synthetic antimicrobials; nevertheless, further investigations are warranted to ascertain its effectiveness *in vivo*.

Keywords: : *Prosopis juliflora*, Anti-biofilm activity, Antibacterial activity, Uterine infection, Livestock.

INTRODUCTION

Uterine infection is commonly seen in post-partum dairy cows and is associated with impaired reproductive performance due to a reduction in conception rate, increased intervals from calving to first service or conception, and increased risk of reproductive culling [1, 2]. Uterine inflammation involving the endometrium often results in abnormal fertilization, implantation, and premature fetal death [3]. Moreover, uterine infection is frequently linked to abnormal estrous cycles, ovarian cyst formation, lengthening of the luteal phase, and delayed postpartum estrus [4]. The prevalence rate of uterine infection in buffaloes is found to be much higher than in cows, and a 24.7% prevalence rate was reported in India [5, 6]. Among the frequent risk factors associated with uterine infections in livestock are fetal membrane retention, abortion, dystocia, mounting by infected bulls, unhygienic insemination procedures, hypocalcemia, and poor nutrition. A variety of anaerobic bacteria, including *Prevotella* species, *Fusobacterium necrophorum*, and *Fusobacterium nucleatum*, are the next most common bacteria found in the uterine lumen of calves suffering from uterine diseases, after *Escherichia coli* and *Arcanobacterium pyogenes* [7, 8].

Bacterial biofilm is a complex three-dimensional polymeric matrix composed of polysaccharides, proteins, and other organic components that help the

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pathogenic microbes adhere to the surface [9, 10, 11]. Several bacterial pathogens belonging to *Escherichia coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Pasteurella* sp., *Bacillus* sp., *Salmonella* sp., etc., responsible for causing infections in humans and animals are reported to produce biofilm [12, 13]. Among the different virulence factors in uropathogenic *E.coli*, the ability of biofilm formation by the microbes is growing important as this helps the pathogenic *E.coli* to persist for a long time in the site of infection by escaping the host immune system [14]. This biofilm further protects the microbes from the action of antimicrobials thus contributing to resistance [15], moreover, it also helps in the transfer of resistance genes within the members of biofilm-producing microorganisms [16].

Prosopis juliflora belongs to the family Leguminosae (Fabaceae), sub-family Mimosoideae [17]. This plant has been used traditionally for the treatment of catarrh, cold, diarrhea, dysentery, flu, hoarseness, inflammation, measles, sore throat, and hepatic and ophthalmic disorders [18, 19]. The decoction made from leaf and seed extracts is reported to be effective against wounds and stomach problems [19, 20]. Several alkaloids that have been identified from this plant, including 3'-oxojuliprosine, sceojuliprosopinol, juliflorine, julifloricine, julifloridine, juliprosine, juliprosinene, and juliflorinine [21, 22], have been found to have several potential pharmacological actions [23, 24, 25].

The anti-biofilm potential of medicinal plants is attributed to the presence of different quorum-sensing inhibitors such as flavonols, flavonoids, phenols, and flavonones [26, 27, 28]. These phyto-biomolecules are also suggested to inhibit bacterial adherence and suppress the genes associated with biofilm production. Though the promising antibacterial action of *P. juliflora* leaves was reported in traditional medicine, the precise mechanism of action of *P. juliflora* leaves at the cellular and molecular level is yet to be explored. Thus, in the present study, the antibacterial activity of this plant was investigated with special reference to its anti-biofilm potential against the pathogenic *E. coli* of animal origin.

MATERIALS AND METHODS

Collection of samples and bacterial strain

A reference strain of *E. coli* (ATCC 25922) was purchased from HiMedia. Clinical isolates were isolated from uterine discharges of animals (23 cows and 19 buffaloes) presented to TVCC, College of Veterinary Science & Animal Husbandry, DUVASU, Mathura, from September 2021-December 2021, with a history of uterine infection and clinical endometritis.

Uterine discharges were collected by the double-guarded method to prevent vaginal contamination and incubated overnight in buffered peptone (pre-enrichment) media. Pre-enriched samples were inoculated in MacConkey agar (HiMedia, India) plates in duplicate. For identification of *E. coli*, the lactose fermenting pink colonies were differentiated from non-lactose fermenter (colorless), and the pink colonies were then sub-cultured on MacConkey agar (HiMedia, India) and Eosin Methylene Blue (EMB) agar (HiMedia, India) plates. The colonies were picked and stored as glycerol stock at -20 ºC for further use. All the selected isolates were further characterized by a panel of biochemical tests, *viz*, catalase, oxidase, ONPG, indole, methyl red, Voges-Prausker, citrate test, and triple sugar fermentation test [29].

Preparation of plant extract

The leaves of *Prosopis juliflora*, were collected from the Veterinary College campus during the month of June-July, 2021, and taxonomical identification of the plant was confirmed from CSRI-Central Institute of Medical and Aromatic Plants (CSRI-CIMAP), Lucknow, with Accession No. 8360. The shade-dried leaves were coarsely powdered and ethanoic extraction was performed with the help of the soxhlet apparatus. The extract (PJ extract) was then concentrated using a rotatory vacuum evaporator (Cole-Parmer, USA) under reduced pressure and low temperature (<40°C). The yield of the extract was found to be 41.42% (w/w). Thereafter, the extract was stored in air-tight containers at 4°C for further studies.

Evaluation of *in vitro* antibacterial activity

The agar well diffusion method was used to assess the *in vitro* antibacterial activity extract. Briefly, Mueller-Hinton agar (MHA, HiMedia, India) petri plates were sterilized and inoculated with bacterial suspensions that had visually similar turbidity to 0.5 McFarland standards. With the help of a good puncture device, wells (9 mm diameter) were punched on the agar plate, and 100 µl of freshly reconstituted were filled in the wells at two different concentrations (20 mg/ml and 25 mg/ml) before incubating the plates at 37ºC for 18 h. Vehicle control (95% ethanol) was also plated in a separate well.

The minimum inhibitory concentration (MIC) of PJ extract was determined against both clinical isolate and reference strain by broth microdilution method as

described previously [30]. Briefly, bacterial inoculum with equivalent turbidity of 0.5 McFarland standard and serial two old dilutions of PJ extract was prepared. The sterile microplates were then prepared by placing 95µl of MHA broth and 5µl of the respective bacterial inoculum into each well. The aliquot of PJ extract (100µl) from the stock solutions was then added into the first well and subsequently 100µl from the serial dilutions were transferred into 11 consecutive wells. The last well served as the negative control. Cefotaxime was used as a positive control. The plates were then incubated at 37ºC for 18h and the wells were examined for the formation of bacterial pellets. The experiment was performed in duplicate. The MIC was defined as the lowest concentration of the compounds to inhibit bacterial growth [30].

Biofilm inhibition assay

The potential of test extract to prevent cell attachment and biofilm production was assessed by biofilm inhibition assay [31]. Briefly, a bacterial aliquot was added to the U bottom 96 well microtiter plate and incubated at 37°C for 48 h without shaking for the establishment of bacterial biofilm. The isolate showing more than 0.16 absorbance in crystal violet staining is graded as a strong biofilm producer while 0.12 - 0.16 absorbance is categorized as a moderate biofilm producer [32]. For evaluation of the anti-biofilm activity of PJ extract, the bacterial aliquots were added along with the plant extract (equivalent to MIC) and incubated at 37°C for 48 h. Chloramphenicol and gentamicin were used as external positive control while ethanol served as negative control. The final volume of each well was adjusted to 200 µl and the assay was conducted in triplicate. After incubation, all the liquid media was poured off by turning the plate upside down. The plate was submerged in a tiny water tub. After shaking out the water and repeating the procedure once again, 200 µl of 0.1% crystal violet solution was added to each microtiter plate well. After being left undisturbed at room temperature for twenty minutes, the plate was quickly dried by shaking off the water and blotting on a stack of paper towels. It was then rinsed four times with sterile distilled water. The microtiter plate was turned upside down and allowed to dry overnight. To each well of the microtitre plate, 200 µl of 30% acetic acid was added to solubilize the crystal violet followed by incubation at room temperature for 20 min. The solubilized crystal violet was transferred to a fresh flat bottom microtiter plate and absorbance was recorded at 600 nm in a multimode microplate reader (Molecular Devices, USA). A background control with 30% acetic acid in water was also recorded. Absorbance values were compared with the standard OD readings. The inhibition of biofilm was quantified using the following formula [31].

Percentage inhibition of biofilm = [(OD (control) - OD(test)/OD(control)] × 100.

***In situ* visualization of biofilm**

Scanning electron microscopy (SEM) was performed for *in situ* visualization of the biofilm. Clinical isolate of *E. coli* (S21) showed the highest biofilm production based on the culture characteristic and microtiter plate assay. Thus, the efficacy of the test extract to inhibit biofilm production was further assessed using an S21 isolate. Briefly, a loopful colony was inoculated to 5ml broth and incubated overnight at 37°C without shaking. The bacterial culture was then adjusted to the equivalent turbidity to 0.5 McFarland standard. The sterile coverslip (12 mm diameter) was placed in the bottom of each well of 6 well plates before filling the well with 7.5 ml of biofilm assay media and 1 ml of broth of bacterial culture and incubated at 37°C for 48 h without shaking. The biofilm assay media was prepared by supplementing Luria-Bertani broth (12.5 g/l) with M63 minimal media (KH_2PO_4: 12.0 g/l, K_2HPO_4: 28.0 g/l, $(NH_4)_2SO_4$: 8.0 g/l, $MgSO_4.7H_2O$: 0.25 g/l, d-glucose: 2.0 g/l, Casamino acid: 5.0 g/l) to promote robust biofilm production [33]. For evaluation of the anti-biofilm activity of plant extract, bacterial aliquots were added along with the plant extract (equivalent to 2 MIC or 4 MIC) and incubated at 37°C for 48 h. Chloramphenicol (equivalent to MIC) was used as an external positive control. Once the culture media had been exposed to the test extract and/or chloramphenicol, they were pipetted out of the wells and gently washed three times with phosphate buffer solution (pH 7.4). Subsequently, the coverslips in the wells were left overnight in the fixative (2.5% glutaraldehyde + 2.5% paraformaldehyde in 0.1 M PBS). Further processing and imaging was done from AIIMS, New Delhi.

Statistical analysis

The data is presented as mean ± SEM, with 'n' denoting the number of replicates in each experimental protocol. Graph Pad Prism V.4.00 (San Diego, California) was used to analyze the mean values of the two groups separately using the student's t-test, and a difference in values was deemed statistically significant at $p< 0.05$.

RESULTS AND DISCUSSION

Isolation and Identification of *E.coli* from clinical samples

Out of 42 clinical samples, 23 isolates were found to show a typical pink color colony (indicative of lactose fermenter) on Mac Conkey's agar and a 'metallic sheen' on EMB agar. Morphological examinations revealed the presence of Gram-negative rod-shaped organisms which took pink color after Gram's staining. The *E. coli* isolates (9 out of 23 isolates) showed typical results during their biochemical characterization, *i.e.* positive to indole, methyl red catalase, ONPG, and sugar fermentation tests whereas negative for VP and citrate utilization. Among the 9 isolates, one isolate (S21) showed relatively higher MIC against cefotaxime and biofilm-producing ability. Thus, further experiments were carried out using the S21 isolate.

In vitro antibacterial activity of the extract

The antibacterial activity of PJ extract was evaluated against the clinical isolate (S21) along with the reference strain (ATCC 25922) of *E. coli* using an agar well diffusion assay. The respective antibacterial effect of PJ extract in terms of zone of inhibition is summarized in Table 1. PJ extract at both the concentrations (20 mg/ml and 25 mg/ml) showed a comparatively higher zone of inhibition against ATCC reference strain as compared to clinical isolate (S21). The results of broth microdilution revealed that the MIC values of PJ extracts against the reference strain (ATCC 25922) as well as the clinical isolate (S21) were 0.39 mg/ml and 1.56 mg/ml, respectively.

Effect on bacterial biofilm production

To evaluate the efficacy of the PJ extract against bacterial biofilm production, initially, the ability of biofilm production by S21 clinical isolates of *E. coli* was assessed by both congo red agar and microtitre plate assays. S21 isolate was found to produce black color colonies on congo red agar and quantitative measurement of biofilm production revealed that S21 isolate produced strong biofilm with higher intensity of crystal violet stain (OD600: 0.22 ± 0.01). Thus, in further experiments, the ability to inhibit biofilm production by PJ extract was assessed using the S21 isolate. The quantitative measurement of anti-biofilm activity by crystal violet microtitre method revealed that PJ extract (@ MIC) produced 54.55% inhibition of biofilm production by S21 isolate in comparison to 90.91% and 31.82% inhibition by chloramphenicol and gentamicin respectively.

In situ visualization of biofilm production by bacteria and anti-biofilm activity of PJ extract was examined by scanning electron microscope (SEM). Representative SEM images from different groups are summarized in Fig. 1 and Fig. 2. The untreated bacterial culture under SEM microscopy appeared as clusters of cells indicating their ability to form a biofilm (Fig. 1a, 1b and 1c). The surface morphology of these bacteria appeared smooth with a regular shape (Fig. 1c). Exposure to chloramphenicol distorted the cluster (Fig. 1d and 1e) along with the appearance of the rough and wrinkled surface of the exposed bacterial cells (Fig. 1f). Treatment with PJ extract at lower concentration (MIC) reduced the cell density and clumping (Fig. 2a and b). Moreover, changes in the surface morphology of the exposed cells characterized by twisted, rough, and wrinkled cell surfaces along with invagination and protrusion on the surface were also observed (Fig. 2c). Exposure to higher concentration of PJ extract (2 MIC) also exhibited anti-biofilm activity characterized by loosening of the cell density and clumping (Fig. 2d and 2e) along with change in surface morphology (Fig. 2f).

Numerous aerobic and anaerobic bacteria might thrive in the uterine lumen during the postpartum period. *Escherichia coli* and *Arcanobacterium pyogenes* are the most commonly detected bacteria in the uterine lumen of cows suffering from uterine illnesses. A range of anaerobic bacteria, including *Prevotella* species, *Fusobacterium necrophorum*, and *Fusobacterium nucleatum*, are also frequently encountered in the uterine lumen [7, 8]. We previously observed that the most common bacterial pathogens causing uterine infections in cattle and buffaloes are *E. coli* and *Staphylococcus aureus* (unpublished data). Out of 42 clinical samples included in the current study, 22 samples were determined to be positive for *E. coli* based on cultural, morphological, and biochemical traits.

In the present study, we found that the ethanolic extract of *P. juliflora* leaves (PJ) extract exhibited promising antibacterial activity against the clinical isolate of *E. coli* (S21). However, the zone of inhibition against these isolates did not significantly increase when the extract concentration was increased from 20 mg/ml to 25 mg/ml. Consistent with our observation, the antibacterial activity of ethanolic extract of leaves, pods, and flowers of *P. julifora* was also reported against different bacterial strains [19]. Recently, the antibacterial efficacy of *P. juliflora* leaf extract against food-spoiling isolates of *E. coli* and *S. aureus* was also reported [34]. Moreover, the combined effect of

Table 1. Comparative zones of inhibition (mm) produced by *P. juliflora* leaves extract (PJ) against clinical isolate (S21) and ATCC reference strain of *E. coli*.

Organism tested	Zone of inhibition (mm)	
	Concentration of PJ extract	
	20 mg/ml	25 mg/ml
S21 isolate (n=9)	$12.50^{a} \pm 0.50$	$12.75^{a} \pm 0.48$
ATCC 25922 (n=5)	$20.00^{b} \pm 1.54$	$17.5^{b} \pm 0.93$

[Data are presented as Mean ± SEM. Student's t tests was used to analyze the data. Within a column, mean values with distinct superscripts indicate statistical significance ($p< 0.05$)].

Table 2. Effect of *P. juliflora* leaves (PJ) extract on biofilm production by clinical isolate (S21).

Extract/ Drugs	OD600	Biofilm inhibition (%)
PJ extract	0.10 ± 0.01	54.55
Chloramphenicol	0.02 ± 0.01	90.91
Gentamicin	0.15 ± 0.01	31.82

[Data are presented as mean ± SEM of three observations].

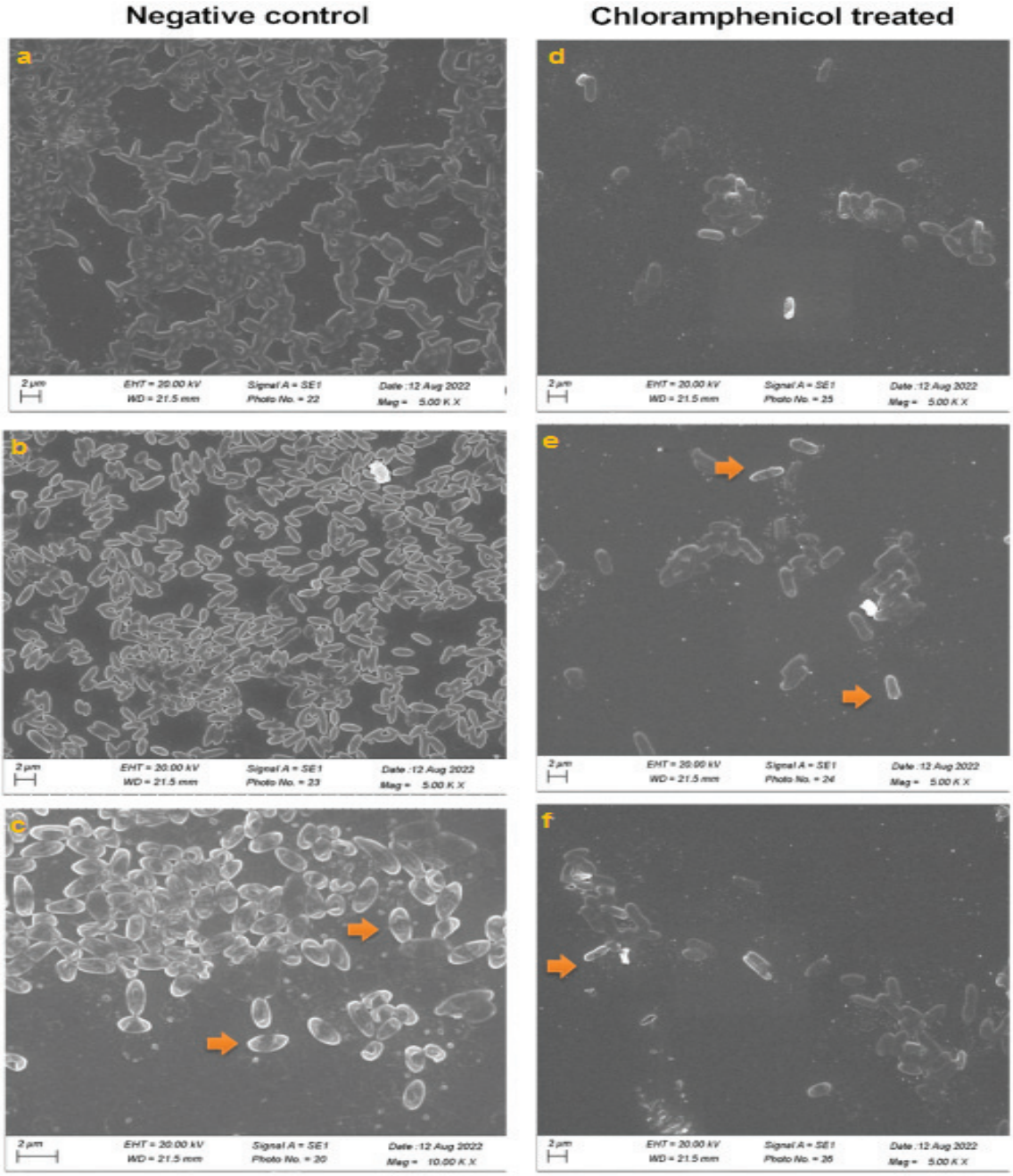

Fig. 1. Representative scanning electron microscopy (SEM). [Images of untreated bacterial cells (S21 isolates) showing clusters of cells indicating the biofilm production (a-c). The surface of the bacteria appeared smooth (c). Treatment with chloramphenicol reduced the cell density or clusters (d-f) with rough and wrinkled surface (e, f)].

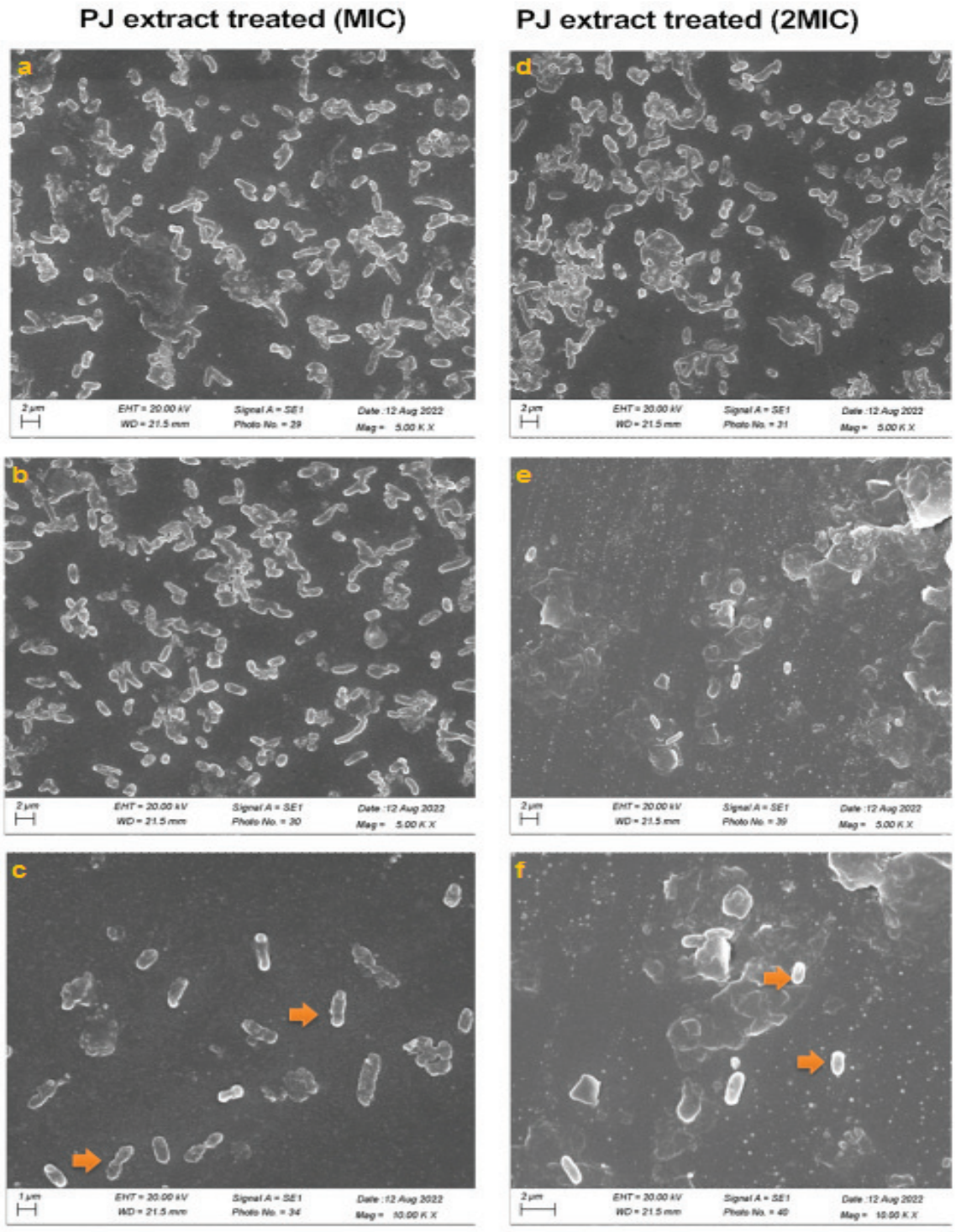

Fig. 2. Representative scanning electron microscopy (SEM). Images showing the antibiofilm activity of *P. juliflora* leaves (PJ) extract. Bacterial cells (S21 isolates) treated with lower concentration (MIC) of extract showed reduced clumping of bacterial cells (a-c) with rough surface (c). Higher concentration (2 MIC) also inhibited bacterial cluster formation (d, e) with invagination and protrusion on cell surface (f).

the aqueous extract of *P. juliflora* leaves with silver nano-particles encapsulated in chitosan showed greater antibacterial activity against *E. coli* [33, 35]. It is interesting to note that the MIC values of the PJ extract and cefotaxime against the S21 isolate were found to be relatively higher (1.56 mg/ml and 32 µg/ml, respectively). The higher virulence of the S21 isolates was attributed to its capacity to form a potent

biofilm. Many phytoconstituents, including alkaloids, tannins, phenolics, steroids, terpenes, flavonoids, proteins, sugars, and fatty acids, have been shown to have antibacterial action. These compounds were possibly playing the vital role in imparting the antibacterial activity of the PJ extract [36, 37].

Biofilm-producing ability is considered to be a pathogenic virulence factor that helps the microbes to adhere to the site of infection and protect from the host immune system and antibiotic treatment [38, 39]. Currently, natural products and plant-derived secondary metabolites are emerged as therapeutic agents against bacterial biofilms [40]. Therefore, we evaluated the anti-biofilm activity of *P. juliflora* leaf extract against a clinical isolate of *E. coli*. *P. juliflora* leaf extract exhibited promising anti-biofilm action (54.55 % inhibition) against a clinical isolate of *E. coli*. This notion was corroborated by SEM analysis, which revealed that PJ extract changed the integrity of cell walls, thereby reducing the multilayer formation of biofilms and free-living cells. It was also noted that the bacterium's disrupted cell wall caused failure in cluster emergence and the inability to retain their characteristic morphology in the presence of the extract. As quercetin has been shown to have strong antibiofilm properties against bacteria including *E. coli*, *S. aureus, S. Typhimurium*, and others, the anti-biofilm action of PJ extract was ascribed to the flavonoid and phenolic acid content of the extract [41]. In a similar study, natural compounds like eugenol, thymol, and carvacol were reported to inhibit biofilm formation by *S. Typhimurium* by more than 50% [42]. Nevertheless, our findings provide substantial evidences of the anti-biofilm activity of PJ extract which have the promising potential for the development of innovative therapeutic approaches to treat uterine infection.

CONCLUSION

In conclusion, *P. juliflora* leaves extract exhibited promising *in vitro* antibacterial activity against the clinical isolate of *E. coli*, and its antibacterial action is mediated by its promising anti-biofilm activity by altering the bacterial surface morphology characterized by the presence of rough, wrinkled, and invaginated surface and thereby reducing bacterial nidus or clumping. Thus, *P. juliflora* leaf extract has the potential to be used as an alternative to conventional antibiotics for the treatment of uterine infections in farm animals. Thus, PJ leaves extract-based formulation thus may be useful to combat synthetic drug-related resistance against microbes in field conditions. However, the safety study of the extract is to be performed in the future.

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REFERENCES

1. Borsberry S, Dobson H. Periparturient diseases and their effect on reproductive performance in five dairy herds. Vet Rec.1989; 124: 217-219.

2. Gilbert RO. The effects of endometritis on the establishment of pregnancy in cattle. Reprod Fertil Dev. 2011; 24(1): 252-257.

3. Lopez-Gatius F. Is fertility declining in dairy cattle, a retrospective study in Northeastern Spain. Theriogenology. 2003; 60: 89-99.

4. Herath S, Lilly ST, Fischer DP, Williams EJ, Dobson H *et al.* Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2 alpha to prostaglandin E2 in bovine endometrium. Endocrinology. 2009; 150(4): 1912-1920.

5. Gupta PP, Singh B, Mandal PC, Gill BS, Grewal GS. A post mortem study of mortality pattern in adult buffaloes in Punjab. India. Ind J Anim Sci.1978; 48: 669-673.

6. Rao AV, Sreemannarayana O. Clinical analysis of reproductive failure among female buffaloes (*Bubalus bubalis*) under village management in Andhra Pradesh. Theriogenology. 1982; 18(4): 403-411.

7. Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction. 2002; 123(6): 837-845.

8. Williams EJ, Fischer DP, England GCW, Dobson H, Pfeiffer DU, Sheldon IM. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the inflammatory response to endometritis in cattle. Theriogenology. 2005; 63: 102-117.

9. Adnan M, Morton G, Hadi S. Analysis of rpoS and bolA gene expression under various stress-induced environments in planktonic and biofilm phase using 2(-ΔΔCT) method. Mol Cell Biochem. 2011; 357(1-2): 275-282.

10. Di Ciccio P, Vergara A, Festino AR, Paludi D, Zanardi E *et al.* Biofilm formation by *Staphylococcus aureus* on food contact surfaces: relationship with temperature and cell surface hydrophobicity. Food Control 2015; 50: 930-936.

11. Adnan M, Sousa AM, Machado I, Pereira MO, Khan S *et al.* Role of bolA and rpoS genes in biofilm formation and adherence pattern by *Escherichia coli* K-12 MG1655 on polypropylene, stainless steel, and silicone surfaces. Acta Microbiol Immunol Hung. 2017; 64: 179-189.

12. Chakraborty S, Dutta T, De A, Das M, Ghosh S. Impact of bacterial biofilm in veterinary medicine: an overview. Int J Curr Microbiol App Sci. 2018;7(4): 3228-3239.

13. Petruzzi B, Ra D, Leroith T, Np E, Fw P, Tj I. Biofilm formation and avian immune response following experimental acute and chronic avian choleradue to *Pasteurella multocida*. Vet Microbiol. 2018; 222: 114-123.

14. Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M *et al.* Bacterial biofilm and associated infections. J Chin Med Assoc. 2018; 81(1): 7-11.

15. Letsididi KS, Lou Z, Letsididi R, Mohammed K, Maguy BL. Antimicrobial and antibiofilm effects of trans-cinnamic acid nanoemulsion and its potential application on lettuce. LWT - Food Science and Technology. 2018; 94: 25-32.

16. Lebeaux D, Ghigo J-M, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev. 2014; 78(3): 510-543.

17. Pasiecznik NM, Harris PJ, Smith SJ. Identifying tropical Prosopis species: a field guide. Coventry, UK: Hdra Publishing; 2004.

18. Mazzuca M, Kraus W, Balzaretti V. Evaluation of the biological activities of crude extracts from Patagonian prosopis seeds and some of their active principles. J Herb Pharmacother. 2003; 3(2): 31-37.

19. Singh S, Swapnil SK, Verma SK. Antibacterial properties of alkaloid rich fractions obtained from various parts of *Prosopis juliflora*. Int J Pharmacol Sc Res. 2011; 2: 114-120.

20. Tene V, Malagon O, Finzi PV, Vidari G, Armijos C, Zaragoza T. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. J Ethnopharmacol. 2007; 111(1): 63-81.

21. Singh S. Phytochemical analysis of different parts of *Prosopis juliflora*. Int J Curr Pharmaceut Res. 2012; 4(3): 59-61.

22. Nakano H, Nakajima E, Hiradate S, Fujii Y, Yamada K *et al.* Growth inhibitory alkaloids from mesquite *Prosopis juliflora* leaves. Phytochem. 2004; 65(5): 587-591.

23. Aqeel A, Khursheed AK, Viqaruddin A, Sabiha Q. Antimicrobial activity of julifloricine isolated from *Prosopis juliflora*. Arzneimittel. 1989; 39(6): 652-655.

24. Ahmad VU, Sultana A, Qazi S. Alkaloids from the leaves of *Prosopis juliflora*. J Nat Prod. 1989; 52(3): 497-501.

25. Lakshmi BS, Naidu KC, Murthy YL, Varaprasad B, Pandit N. Bio-efficacy of some medicinal plants against pathogens of cereal crops and phytochemical examination of *Prosopis juliflora* (Sw) Dc. J Pharm Res. 2010; 3(2): 356-360.

26. Vandeputte OM, Kiendrebeogo M, Rasamiravaka T, Stévigny C, Duez P *et al.* The flavanone naringenin reduces the production of quorum sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Microbiology (Reading). 2011;157(Pt 7): 2120-2132.

27. Truchado P, Giménez-Bastida JA, Larrosa M, Castro-Ibáñez I, Espín JC *et al.* Inhibition of quorum sensing (QS) in *Yersinia enterocolitica* by an orange extract rich in glycosylated flavanones. J Agric Food Chem. 2012; 60(36): 8885-8894.

28. Sarabhai S, Sharma P, Capalash N. Ellagic acid derivatives from *Terminalia chebula* Retz. downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 virulence. PLoS One. 2013; 8: e53441.

29. Phillips I. Cowan and Steel's Manual for the Identification of Medical bacteria. J Clin Pathol. 1993; 46(10): 975.

30. Gulluce M, Sahin F, Sokmen M. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. Food Chem. 2007; 103: 1449-1456.

31. O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011; (47): 2437.

32. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF *et al.* Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985; 22(6): 996-1006.

33. Sturgill G, Toutain CM, Komperda J, O'Toole GA, Rather PN. Role of CysE in production of an extracellular signaling molecule in *Providencia stuartii* and *Escherichia coli*: loss of CysE enhances biofilm formation in *Escherichia coli*. J Bacteriol. 2004; 186(22): 7610-7617.

34. Saleh I, Abu-Dieyeh MH. Novel *Prosopis juliflora* leaf ethanolic extract as natural antimicrobial agent against food spoiling microorganisms. Sci Rep. 2021; 11(1): 1-17.

35. Malini S, Kumar SV, Hariharan R, Bharathi AP, Devi PR, Hemananthan E. Antibacterial, photocatalytic and biosorption activity of chitosan nanocapsules embedded with *Prosopis juliflora* leaf extract synthesized silver nanoparticles. Materials Today: Proceedings. 2020; 21: 828-832.

36. Singh S. Phytochemical analysis of different parts of *Prosopis juliflora*. Int J Curr Pharm Res. 2012; 4(3): 59-61.

37. Prabha DS, Dahms HU, Malliga P. Pharmacological potentials of phenolic compounds from *Prosopis* spp.-a review. J Coast Life Med. 2014; 2: 918-924.

38. Aminov RI. Horizontal gene exchange in environmental microbiota. Front Microbiol. 2011; 2: 158.

39. Tabibian JH, Gornbein J, Heidari A, Dien SL, Lau VH *et al.* Uropathogens and host characteristics. J Clin Microbiol. 2008; 46(12): 3980-3986.

40. Adnan M, Alshammari E, Patel M, Ashraf SA, Khan S, Hadi S. Significance and potential of marine microbial natural bioactive compounds against biofilms/ biofouling: necessity for green chemistry. Peer J. 2018; 6: 5049.

41. Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. Cancer letters. 2008; 269(2): 315-325.

42. Miladi H, Zmantar T, Kouidhi B, Chaabouni Y, Mahdouani K *et al.* Use of carvacrol, thymol, and eugenol for biofilm eradication and resistance modifying susceptibility of *Salmonella enterica* serovar *Typhimurium strains* to nalidixic acid. Microb Pathog. 2017; 104: 56-63.

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