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## Experimental study for Anti-Bacterial and Anti-Biofilm Potential of *Prosopis juliflora*

Hemalatha R<sup>1</sup>, Nagaindu G<sup>1</sup>, Vennila S<sup>2</sup>, Subha S<sup>2</sup>

Students, Department of Biotechnology, St. Michael College of Engineering and Technology,  
Kalayarkoil, Sivaganga, Tamil Nadu, India<sup>1</sup>

Assistant Professor, Department of Biotechnology, St. Michael College of Engineering and Technology, Kalayarkoil  
Sivaganga, Tamil Nadu, India<sup>2</sup>

**ABSTRACT:** *Prosopis juliflora* is one of the most economically and ecologically important tree species in the arid and semi-arid zones of the world. *Prosopis* species are Xerophilous, aculeate, spiny and rarely unarmed. The present study evaluates the potential anti-bacterial and anti-biofilm activity of *Prosopis juliflora*. The ethanolic extracts were prepared for the present study and anti-bacterial activity was determined by Disc Diffusion method and Anti-biofilm activity was evaluated by Microtitre plate method. The present study (anti-bacterial) reported that the ethanolic extracts in various concentrations were effective against all the strains tested. The results showed that the extracts had the strongest inhibition effect against *S. aureus* and it was maximum for *staphylococcus sp.* The extracts also inhibit the *E. coli* and *P. aeruginosa* biofilm structure. Minimum inhibitory concentrations (MICs) were calculated by broth micro dilution method in Microtitre plates and studied their minimum inhibitory concentrations. The anti-biofilm activity was also measured and 50% of the biofilm reduced. The minimum inhibitory concentration of ethanolic extractions of *P. juliflora* was 30 µg/ml. Hence, the present work concluded that the plant extract was able to control the pathogenic organism and biofilm formation.

**KEYWORDS:** *Prosopis juliflora*, Anti-microbial activity, anti-biofilm, ethanol extract.

### I. INTRODUCTION

The increasing failure and side effects of popularly used chemotherapeutics and appearance of multiple drug resistance phenotypes in pathogenic bacteria has led to the search for new compounds with antibacterial activity. Worldwide as well as in the developing countries, most human deaths are due to the infections bacterial diseases. Bacterial organisms including Gram Positive and Gram negative, like different species of *Staphylococcus*, *Pseudomonas*, are the main source of severe infections in humans. These organisms have the ability to survive in harsh conditions due to their multiple environmental habitats.

*Prosopis juliflora* is one of the most economically and ecologically important tree species in the arid and semi-arid zones of the world. *Prosopis* species of all trees or shrubs of varying sizes predominantly Xerophilous, aculeate, spiny and rarely unarmed. The wood has high calorific value, and was known as mesquite has been used as traditional medicine on different continents for curing catarrh, cold, diarrhea, dysentery, flu inflammation, hoarseness, measles, sore throat and for healing wounds. *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped, motile organism (polar flagella) which characteristically produces water soluble pigments which diffuse through the medium. It is commonly found freely living in moist environments but is also a pathogen of plants, animals, and human. *Staphylococcus aureus* is a gram-positive coccid bacterium that is member of the Firmicutes and is frequently found in the nose, respiratory tract, and on the skin. *E. coli* bacteria were found in human colon. It causes food poisoning by producing the toxin shiga.

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In this paper, the crude extract of the plant *Prosopisjuliflora* and its microbial activity was investigated. Its anti-biofilm activity at various concentrations of the extract & its Minimum Inhibitory concentrations (MICs) has been evaluated.

## II. RELATED WORK

From ancient times, plants have provided tremendous support in traditional medicine systems as well as for the development of new potential drugs in modern pharmaceutical industries. To consider the importance of plants for therapeutic target, intensive studies have been performed on different plant extracts to isolate biologically active compounds. Although plants produce these chemicals to protect themselves, research provides evidence that they can treat humans against infections.

Biofilm formation has been described as an important factor having significant implications in pathogen virulence. Biofilms protect the pathogens from inhibitory effect of antibiotics and immune cells, because it prevents their effective penetration [1]. Biofilm can be described as a structured community of the microorganism encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface [2]. The presence of biofilm in the food processing environments is a potential source of contamination that may lead to food spoilage and disease transmission [3]. Bacteria including biofilm structure are generally more resistant to anti-microbial agents than planktonic cells. The effects of plant extracts to prevent biofilm formation and adherence have been showed in earlier studies [4]. Several efficient qualitative and quantitative techniques have been described for rapid and efficient detection of the biofilms [5] and simultaneously screening of different agents for their anti-biofilm potential, where many of these anti-biofilm agents were reported are synthetic and chemical of origin [6].

## III. MATERIALS AND METHODS

### Plant Matter

The leaves of the whole plant *Prosopisjuliflora* were collected from natural populations in Madurai. The leaves of the plant were washed with distilled water and air-dried at room temperature for 48 hours.

### Preparation of the Plant Extract

The air dried plant materials are powdered using mortar and pestle. This plant material was successfully extracted with ethanol using Microwave assisted extraction method at various concentrations. It was used as an alternative method for Soxhlet apparatus.

### Test Organisms

Microorganisms used for current study include the following, which were collected from clinical laboratory Shenbhagam Pioneer Diagnostic center, Madurai. The organisms used are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

### Preparation of the Sub-Culture

The nutrient Agar (Hi -media) was used to culture the test bacteria. The prepared Agar medium the prepared medium was sterilized by autoclaving & poured in to the petri plates and allowed to solidify. They are used stored in refrigerator for further use. The test organism was sub cultured by streaking the plates and they are incubated at 37°C for 24 hr.

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## Determination of Anti-Bacterial Activity

The antimicrobial potential of the plant extract against various organisms was examined by Disk Diffusion method [7]. Briefly, *E.coli*, *P.aeruginosa*, *S.aureus* culture was inoculated in nutrient broth at incubated at 37°C for 24 hours. After the incubation, microbial lawn was prepared by pouring 100µl of culture on the Nutrient agar plate. Sterile paper discs of diameter 50mm were prepared. Disks soaked with various concentrations of plant extracts were placed in the plates. And the plates were incubated at 37°C for 24 hours. Anti-microbial potential of the plant extract were evaluated on the basis of appearance and size of the zone of inhibition.

## Determination Anti-Biofilm Activity

The ability bacteria to form biofilms were assayed as described [8] with some modification. In well plate containing 50 µl of Nutrient broth per well, a 50 µl of fresh bacterial suspension was added. After incubation at 37 °C for 48 h, the content of each well was gently removed by tapping the plates. The wells were washed with 200 µl of sterile saline to remove free-floating bacteria. Biofilms formed by adherent cells in plate were stained with 0.1% crystal violet and incubated at the room temperature for 20 minutes.

Briefly 1% of overnight cultures (OD adjusted to 0.4 at 600 nm) of test pathogens were added into 200µl of the fresh nutrient broth medium and cultivated in the presence and absence of extracts without agitation for 48 hours at 37°C. The wells containing only broth served as control. After incubation, the wells were washed with water to remove planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 minutes at room temperature. Wells were washed once again to remove the crystal violet solution that is not specifically staining the adherent bacteria. Micro plates inverted and vigorously tap on paper towels to remove any excess liquid and air dried. 200µl of 95% ethanol and 33% glacial acetic acid poured in Gram-negative bacteria (*E.coli* and *P.aeruginosa*) and Gram-positive bacteria wells, respectively. Biofilm stains solubilized at room temperature. The stained biofilms were suspended in 200µl phosphate buffer saline (PBS) and OD 550nm was measured by calorimeter. Percentage of inhibition of the tested extracts was calculated.

## Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentrations (MICs) of test samples found to be active by the diffusion test were determined based on the macro dilution method. The ethanolic extract of various concentrations ranging from 0.01ml to 3ml and nutrient broth supplemented with 10% glucose and 0.05% phenol red (color indicator). These were later inoculated with 0.2ml suspension of the mixed culture of the test Organisms. A tube containing nutrient broth only was seeded with the test organism as described above to serve as control. The microbial growth was determined by observing for colour change in the tube, (red to yellow when there is growth). The lowest concentration that showed no change of colour was considered as the MIC [9].

## IV. EXPERIMENTAL RESULTS

### Anti-Bacterial Activity

The organisms were subcultured in the Nutrient agar plate and the isolated colonies are used for the antibacterial activity determination.

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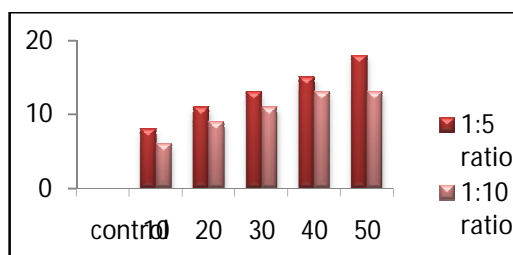
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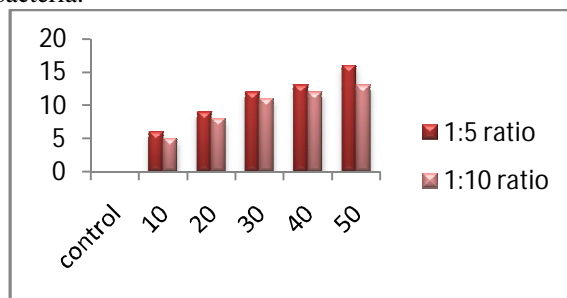
**Fig 1: Anti-microbial activity of plant extract against *Staphylococcus aureus***

The results of the *in vitro* study of the anti- bacterial activity of the plant extract *Prosopisjuliflora* showed a high anti-microbial activity against the pathogenic bacteria like *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonasaeruginosa*. The maximum activity was seen for *staphylococcus sp.*



**Fig 2A: Plant extracts at various Concentrations showing Anti-Bacterial activity against *Pseudomonas***

The ethanolic extracts of *P.juliflora* demonstrated moderate to strong anti-bacterial activity. The above graph indicates the action of the plant extract against *Pseudomonas*. Its methanolic extracts from seed pods also showed a good anti-bacterial activity against all the bacteria.



**Fig 2B: Plant extracts at various Concentrations showing Anti-Bacterial activity against *staphylococcus aureus***

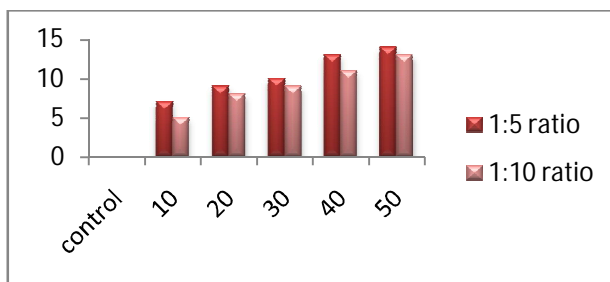
The ethanolic extracts of *P.juliflora* demonstrated moderate to strong anti-bacterial activity. The result indicates that the most sensitive bacteria were *Staphylococcus aureus*.

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**Fig 2C: Plant extracts at various Concentrations showing Anti-Bacterial activity against E.coli**

The above graph shows the antibacterial activity against *E.Coli*. The ethanolic extracts of *P.juliflora* demonstrated moderate anti-bacterial activity.

### Minimum inhibitory concentrations:

The minimum inhibitory concentrations of ethanol Extractions of *P.juliflora* was 30µg/ml. The ethanolic extract of this plant shows the minimum inhibitory concentration against bacteria ranged from 25µg/ml -50µg/ml [10]. The Minimum Inhibitory Concentrations (MICs) of ethanolic extracts of *Prosopis juliflora* was determined for Gram positive and Gram Negative bacteria using the standard broth micro dilution susceptibility test. MICs were determined by observing the colour change (from red to yellow).

Percentage of inhibition of the tested extracts was calculated using the following formula. Percentage of inhibition of the tested extracts was calculated using the following formula

$$\text{PERCENTAGE OF INHIBITION} = 1 - \left( \frac{\text{OD } 550 \text{ nm SAMPLE}}{\text{OD } 550 \text{ nm CONTROL}} \right) \times 100$$

### Anti-Biofilm Activity

The results of *in vitro* activity of anti-biofilm of ethanolic extract of *P.juliflora* were presented; the bacteria used in this part of investigation have been selected from anti-bacterial activity depending on their biofilm forming potential. The results of *in vitro* activity of anti-biofilm of ethanolic extract of *P.juliflora* were presented in the following tables.

S.NO	TSB(ml)	Organism (µl)	Plant extract (µl)	Extract ratio 1:5 OD(580 nm)	Percentage of removal %	Extract ratio 1:10 OD(580 nm)	Percentage of removal %
C	0.2	10	0	0.15	0	0.15	0
1	0.2	10	10	0.13	13.34	0.14	6.67
2	0.2	10	20	0.12	20	0.14	6.67
3	0.2	10	30	0.11	26.67	0.13	13.33
4	0.2	10	40	0.11	26.67	0.12	20
5	0.2	10	50	0.09	40	0.10	33.33
6	0.2	10	60	0.08	46.67	0.09	40
7	0.2	10	70	0.07	53.34	0.08	46.67
8	0.2	10	80	0.05	66.67	0.06	60
9	0.2	10	90	0.14	0	0.14	0
10	0.2	10	100	0.15	0	0.14	0

**Table 1A: Organism used was *Pseudomonas aeruginosa***

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Anti-biofilm activity was produced from three types of results. *Pseudomonas aeruginosa* is the (thickest layer) strong biofilm forming bacteria and the other two are moderate biofilm forming bacteria.

S.NO	TSB(ml)	Organism (µl)	Plant extract (µl)	Extract ratio 1:5 OD(580 nm)	Percentage of removal %	Extract ratio 1:10 OD(580 nm)	Percentage of removal %
C	0.2	10	0	0.09	0	0.09	0
1	0.2	10	10	0.08	11	0.09	0
2	0.2	10	20	0.07	22	0.08	11
3	0.2	10	30	0.06	33	0.08	11
4	0.2	10	40	0.05	44	0.07	22
5	0.2	10	50	0.05	44	0.07	22
6	0.2	10	60	0.04	56	0.06	33
7	0.2	10	70	0.04	56	0.06	33
8	0.2	10	80	0.03	67	0.05	44
9	0.2	10	90	0.02	78	0.04	55
10	0.2	10	100	0.01	89	0.03	66

**Table 1B: Organism used was *Staphylococcus aureus***

The extract demonstrated moderate anti-biofilm activity was at 0.1µl. The percentage of *Pseudomonas aeruginosa* at 50% was 60µl.

S.NO	TSB(ml)	Organism (µl)	Plant extract (µl)	Extract ratio 1:5 OD(580 nm)	Percentage of removal %	Extract ratio 1:10 OD(580 nm)	Percentage of removal %
C	0.2	10	0	0.06	0	0.06	0
1	0.2	10	10	0.06	0	0.06	0
2	0.2	10	20	0.05	16	0.06	0
3	0.2	10	30	0.05	16	0.05	16
4	0.2	10	40	0.04	33	0.05	16
5	0.2	10	50	0.04	33	0.05	16
6	0.2	10	60	0.04	33	0.04	33
7	0.2	10	70	0.03	50	0.04	33
8	0.2	10	80	0.03	50	0.04	33
9	0.2	10	90	0.0	66	0.03	50
10	0.2	10	100	0.07	0	0.07	0

**Table 2C: Organism used was *E.coli***

### V. DISCUSSION

Many reports are available on the antiviral, anti-bacterial, antifungal, anti-inflammatory properties of plants. The extent of antimicrobial activity of the extracts based on the inhibition zone diameter has been described as low (12-18mm), moderate (19-22mm) and strong activity (23-38mm)[11]. In order to identify the plant *Prosopis juliflora* with having antibacterial activity against various bacteria using the disc diffusion method, it was established that the plant possess the potential to inhibit the growth of various pathogens. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent [12]. Ethanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity.



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## VI. CONCLUSION

*Prosopisjuliflora* showed a wide range of influence on bacteria. The aqueous plant extract of this plant also showed maximum inhibitory effect [13]. It was seen that the ethanolic extracts of this plant showed maximum inhibitory effects against many bacteria. This may be due to the presence of ingredients such as alkaloids, terpenoids and mesiquitanol. This investigation has opened up the possibility of the use of this plant in drug development and human consumption possibly for the treatment of gastro intestinal tract, skin, and mouth infections. However the compounds responsible for anti-bacterial activity of this plant extract is currently unclear. The effect of this plant on more pathogenic organism and toxicological investigation and purification therefore, needs to be carried out. In vivo evaluation anti-microbial activity along with toxicity studies of the extracts from *Prosopisjuliflora* are suggested for further studies.

## REFERENCES

- [1] Ojha A, Anand M, Bhatt A, Kremer L, Jacobs WR Jr, et al. (2005) GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. *Cell* 123: 861–873.
- [2] Donlan, R.M. "Biofilms: microbial life on surfaces". *Emerg. Infect. Dis.* 8: 881- 890. (2002)
- [3] Hood S.K, Zootola Z.E, 1995, *Biofilms in food processing*, *Food Control* 6:9,18
- [4] Quave, C.L., Plano L.R.W., Pantuso, T., Bennett, B.C." Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*", *J. Ethnopharmacol.* 118: 418-428, (2008).
- [5] Hanning, C., M. Follo et al. "Visualization of adherent micro-organism using different techniques", *J Med Microbiol* (2009)59 (Pt1): 1-7.
- [6] Chen, X. and Stewart, P.S."Biofilms removal caused by chemical treatments." (2000), *Water Research* 34(17), pg.: 4229-4233
- [7] Inette, E.H. (1985). *Manual of clinical microbiology*, 4th ed. American Association for Microbiology, Washington, DC, pp:978-987.
- [8] O'toole G.A, Kolter R. "Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: a genetic analysis". *Mol. Microbiol.* 28, 449-461, (1998).
- [9] Misbakh, Showkat Ahmad Ganie, Ishfak Hussainwani, "Anti-microbial potentiality of *Elsholtziadensa* against pathogenic Bacterial and Fungal strains", *Journal of Pure and Applied Microbiology*, 2013, vol. 7(2), pg.: 1019-1026.
- [10] Shachi Singh, Swapnil, S.K.Verma "Anti-bacterial properties of Alkaloid rich fractions obtained from various parts of *Prosopisjuliflora*" *International Journal of Pharma Sciences and Research (IJPSR)* Vol.2 (3), 114-120(2011).
- [11] Ahmad, I., Z. Mehmood and F. Mohammad, 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.*, 62: 183-193.
- [12] Doughari. *Trop J Pharm Res*, December 2006; 5 (2) 597. *Tropical Journal of Pharmaceutical Research*, December 2006; 5 (2): 597-603.
- [13] Sathiy, M. and Muthuchelian, K." Investigation of Phytochemical profile and Anti-Bacterial Potential of Ethanolic leaf Extract of *Prosopisjuliflora* DC" (2008), 1240-45.
- [14] Andrews, J.M." BSAC standardized disc susceptibility testing method (version 4)". *J. Antimicrob. Chemother.* 56: 60-76(2005).
- [15] Lewis, K." *Riddle of biofilm resistance. Antimicrobial Agents*". *Chemother.* 45: 999- 1007(2001).
- [16] Sarker, S.D., Nahar, L., Kumarasamy, Y."Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals *Methods* "42: 321- 324(2007).
- [17] Pragnesh N.Dave, Jeya Bhandari, "Prosopisjuliflora: A review" *International Journal of chemical studies*, vol.1 no.3 pg181-196,(2013)
- [18] Begun, J., Gaiani, J.M., et al" *Staphylococcal biofilm exopolysaccharide protects against caenorhabditiselegans immune defenses*" (2007), *PLoS Pathog.* vol.3.p.e57
- [19] Hall-Stoodley, L. and Stoodley P."Biofilm formation and dispersal and the transmission of human Pathogens", *Trends Microbiol* 13(1):pg. 7-10.