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Inhibition of Biofilm Forming Bacteria by Plant Extracts

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ABSTRACT

Biofilms are the main cause for increase in virulence and antibiotic resistance of microorganisms. Both gram positive and gram negative pathogens are known to produce biofilms. Due to the production of biofilm the bacteria adhere to the surface strongly and are protected from any antibiotic agents. The present study is aimed to inhibit the biofilms produced by gram negative *E. coli* isolated from UTI samples (UPEC), using plant extracts. Plants like *Magnifera indica, Punica granatum, Catharanthus roseus* and *Manikara zapota* were extracted to check for their antimicrobial as well as antibiofilm activity by well diffusion assay and biofilm assay. All the plant extracts were screened for antibiofilm activity. The result indicated that *Punica granatum* extract had a good inhibition capacity. The further scope in this field of study is to target AHL formation and hence, inhibit biofilm. The inhibition of biofilm helps in reducing many infections due to reduction in the virulence of bacteria that is mainly due to formation of biofilm, making the pathogens weak and more susceptible to antimicrobial agents.

Keywords: Biofilms, *Magnifera indica, Punica granatum, Catharanthu sroseus* and *Manikara zapota.*

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INTRODUCTION

A biofilm is a microbial determined sessile group portrayed by cells that are irreversibly appended to a substratum or interface or to each other. They are implanted in a network of extracellular polymeric substances which they have created. At long last, they display an adjusted phenotype concerning development rate and quality interpretation. The cells that attach irreversibly to surfaces (i.e., those not removed by gentle rinsing) will begin cell division, form microcolonies, and produce the extracellular polymers that define a biofilm. These extracellular polymeric substances (EPSs) consist principally of polysaccharides and can be identified minutely and by concoction examination. EPSs give the lattice or structure to the biofilm[1].

The antimicrobial activity of *Punica granatum* has been generally researched. The discoveries of a few reviews, including some identifying with hindrance of adherence, propose that the phyto therapeutic utilization of this plant may be a practical alternative in controlling diverse microbial species. The biggest segments of the *Punica granatum* natural product concentrate are tannin and polyphenolics. There is a developing enthusiasm for utilizing tannins as antimicrobial specialists in caries aversion. The activity of tannins against microbes and yeasts can be built up by a connection between their atomic structure and their danger, astringent properties or different components. The impact of tannins on microbial digestion can be measured by their activity on films.

Mango is the most imperative tropical natural products on the planet. Amid handling of mango, side effects for example, peel and kernel (seed) are created. The seed takes up around 17-22% of the natural product. The state of mango seed is a solitary level elliptical seed that can be sinewy or bushy at first glance, the inside seed coat 1 - 2 mm thick is a thin covering a solitary fetus, 4 - 7 cm long, 3 - 4 cm wide, also, 1 cm thick. Mango seed comprises of a tireless coat encasing the piece (Barreto JC, Trevisan MT et al., 18 June 2008) and also the concentrate of various parts of mango have a few natural and pharmacological properties. Sowmiya, S et.al., 2009 specified the antibacterial action for mango seeds ethanolic extricate, the concentrate demonstrated great action against pathogenic microscopic organisms, for example, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebisella pneumoniae* and *Streptococcus pyogenes*[2,3].

Catharanthus roseus is a medicinal plant belonging to the family Apocynaceae native and endemic to Madagascar. The plant is also known by the names such as *Vinca rosea*, *Ammocallis rosea* and *Lochnera rosea*. The plant has been put to traditional use for the treatment of a wide variety of ailments worldwide since ages. The plant bears active phytoconstituents and exhibits

various pharmacological activities like antidiabetic, antioxidant, anti-hypertensive, antimicrobial, cytotoxic etc.[4]. The present study is carried out to investigate the effect of crude plant extracts on inhibition of biofilms in biofilm forming bacteria.

MATERIALS AND METHOD

Plant source

For antibiofilm activity different parts of different plants were screened. For the study, leaves of *Magnifera indica, Hibiscus roseussenesis, Manilkara zapota, Catharanthusroseus; twigs of Magnifera indica, Manilkara zapota*; and fruits of *Magnifera indica, Manilkara zapota* and *Punic granatum* collected from the gardens of different residences in Bangalore were used.

Biofilm formation assay by microtiter plate method

Quantification of biofilm formation in each UPEC isolate was assayed by Microtiter plate method as described by Stepanović Set al., 2007 with some modifications. All the isolates were performed in triplets[5,6].

Acetone extracts of plants

Fresh leaves of *Mangifera indica, Manilkara zapota, Hibiscus rosa-senesis, Catharanthus roseus,* twigs of *Mangifera indica, Manilkara zapota* and fruits of *Mangifera indica, Manilkara zapota, Punica granatum* were cleaned by washing and air dried under shade. Each of the sample were ground with clean motar-pestle to make fine powder. Ice cold acetone was added to the plant powder and ground again. Mixture were filter through Whatman No 1 filterpaper® in suction pump. Obtained plant extract were air dried to get dry powder and stored in cold condition. 1 g of coarsely powdered air dried acetone powder extract of each plant sample was suspended in 9 ml of phosphate buffer (pH 7) and stirred in magnetic stirrer for 3-5 minutes. Samples were then centrifuged at 10,000 rpm for 10 minutes. Pellets were discarded and supernatants were filtered through filterpaper®.

Antimicrobial well diffusion assay

20 mL of Muller Hinton broth was prepared and inoculated with each UPEC isolates and incubated for 24 hours at 37°C. Separately Muller Hinton agar plates were prepared under sterile conditions. 100µL of overnight grown culture was poured on to the agar plates using micropipette and was spread using sterile spreader and kept for drying. Required number of wells were punctured on the agar plates using well puncture. 10 µL of each of the plant extract were added to the wells. In one of the well standard was used as positive control. All the plates were kept for incubation for 24 hours at 37°C[7].

Biofilm inhibition by plant extract

Inhibition of biofilm was carried by modified method of biofilm inhibition spectroscopy assay in 96 microtiter plate. 100µL of UPEC cell suspension were added to the wells. Different concentration of plant extracts as 1%, 10% and 20% were added to the wells and were incubated for 24 hours at 37°C. After the incubation period, cell suspension was removed by rinsing with deionized water and 1% w/v crystal violet was added to the wells. After staining at room temperature for 30 minutes, the dye was removed and wells were washed with PBS. 95% ethanol was then added to the remaining wells and left for incubation for 15 minutes. The reaction mixture was read at 600 nm in microtiter plate reader.

RESULTS AND DISCUSSION

Primary attachment assay

Following results as illustrated in figure 1 were obtained from primary attachment assay which indicated attachment of bacteria to the microtiter plates. Different dilutions of the UPEC isolates were assayed for primary attachment of bacteria. From the graph, isolates showed positive results for the property of adhering to the wall of microtiter plate. According to the results obtained, dilution with higher concentration of *E. coli* showed higher attachment. There was a decrease in primary attachment of isolates with the decrease in concentration of the isolates. 10^8 CFU/ml dilution of sample 2 and sample 4 showed highest primary attachment, whereas 10^3 CFU/ml dilution of sample 2 and sample 4 showed lowest primary attachment. Therefore, it was concluded that the primary attachment is directly proportional to the concentration of isolates.

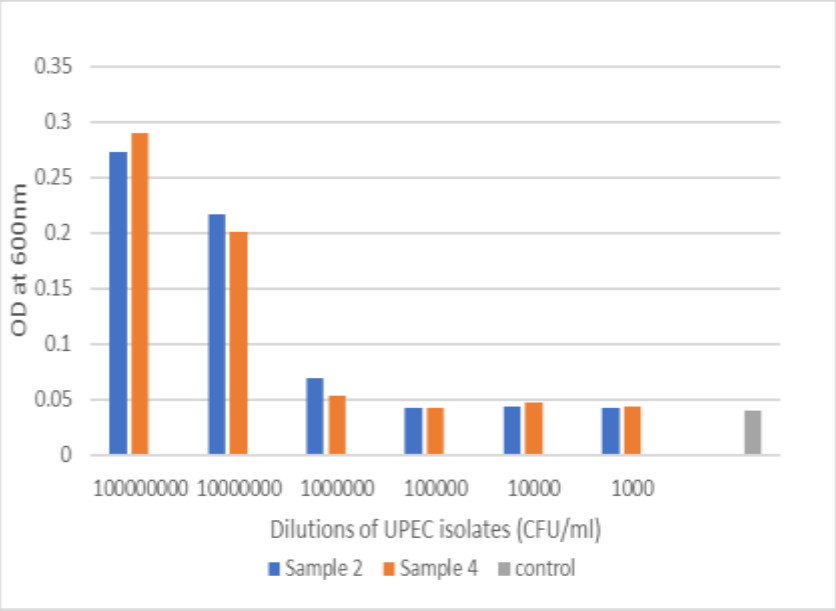

Figure 1: Primary Attachment assay

Biofilm formation assay

Figure 2 explains the result obtained from biofilm formation assay which included incubation period of 24 hours. The isolates exhibited high biofilm forming capacity. Large incubation period helps in more attachment of bacteria and hence better is the biofilm formation. According to this assay the biofilm formation of the bacteria depends on the number of bacteria remaining in the well. The two isolates displayed a highly positive biofilm forming strain. These highly positive biofilm forming property of bacteria help them to persist on the wall of microtiter well or on the wall of urogenital tract causing virulence[8].

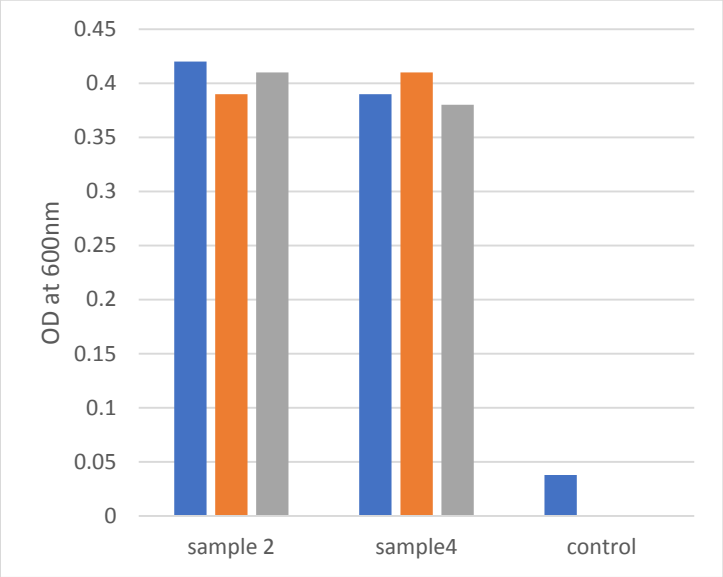

Figure 2: Biofilm forming capacity of the isolates

Well diffusion assay

Each of the plant extract of concentration 0.1g/ml were added to the well on the plates containing overnight grown cultures. Antibacterial activities of all the plant extract against selected bacterial strains were recorded in the form of inhibition zone illustrated in figure 3 and figure 4. Amongst all the samples, pericarp of *Punica granatum* showed zone of inhibition. Figure 3 depicts the results for well diffusion assay of sample 4 and figure 4 indicates the results for well diffusion assay of sample 2. This method provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant. However, since the bacterial growth inhibition does not mean the bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects[9].

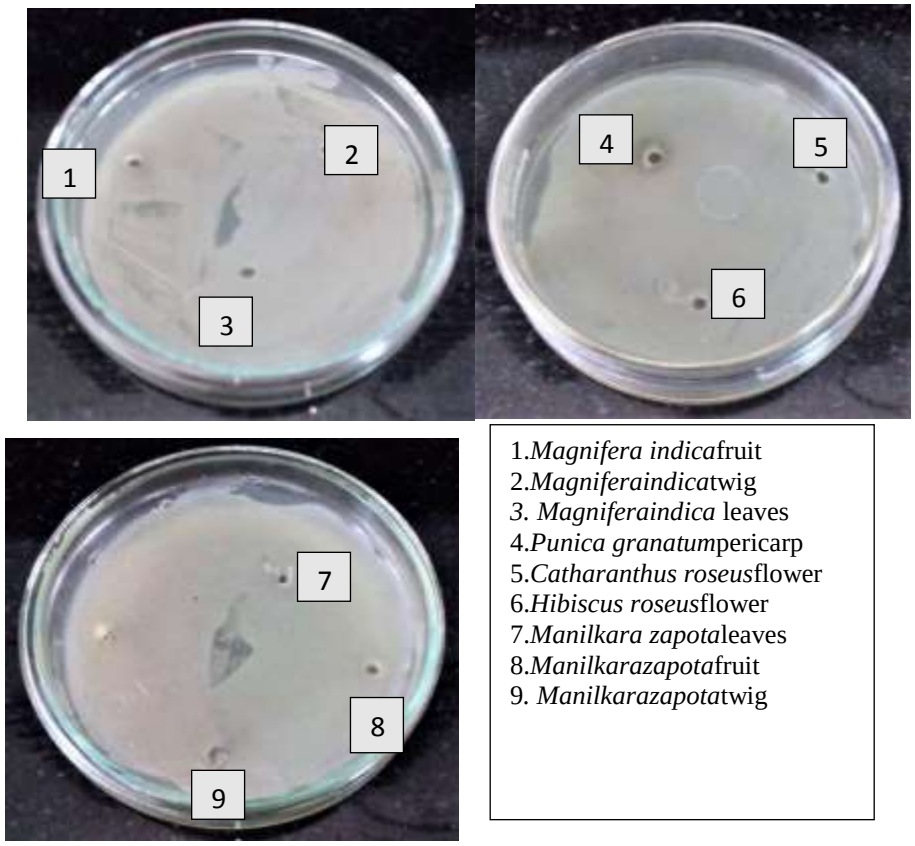

Figure 3: Well diffusion assay for sample 4

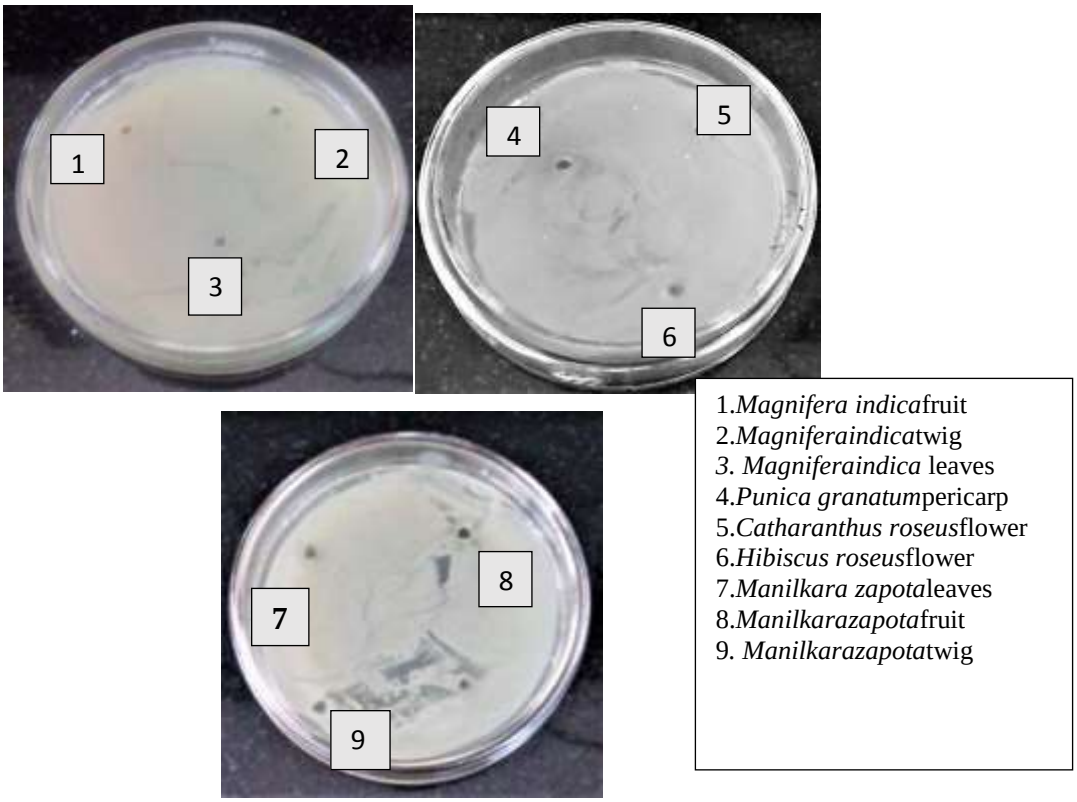

Figure 4: Well diffusion assay for sample 2

Biofilm inhibition by plant extract

Since *Punica granatum* indicated antimicrobial property it was further tested to check biofilm inhibition. Inhibition mediated reduction of biofilm formation was calculated by the following formula

% of inhibition = OD in control –OD in treatment × 100/ OD in control

Table 1: Absorbance of the samples after treated with plant extracts

Sample	OD at 540nm			
	Control	1% Extract	5% Extract	10% Extract
Sample 2	0.42	0.047	0.044	0.039
Sample 4	0.39	0.073	0.056	0.051

Table 2: Percentage inhibition showed by *Punica granatum*

	Percentage inhibition		
	1% Extract	**5% Extract**	**10% Extract**
Sample 2	88	89	90
Sample 4	81	85	86

Table 1 shows the absorbance for biofilm production after treating the UPEC isolates with *Punica granatum* pericarp extract. Different dilutions of extract were used for this assay. Absorbance for sample 2 with 1%, 5% and 10% extract of plant extract were 0.039, 0.044 and 0.039 OD respectively. Absorbance for sample 4 with 1%, 5% and 10% extract of plant extract were 0.073, 0.056 and 0.051 OD respectively. Table 2 shows percentage inhibition by *Punica granatum* extract. Different dilutions of plant extract were used. Sample 2 showed 88, 89 and 90 percent inhibition against 1%, 2% and 5% plant extract respectively. Sample 4 showed 81, 85 and 86 percent inhibition against 1%, 2% and 5% plant extract respectively. Obtained results showed that pericarp of *Punica Granatum* extract displayed potent biofilm inhibition and eradication activity against *E. coli*. Though the extract used in this study was a crude extract, further isolation and detection of phytochemical for their antibiofilm property is required. The finding from this study will help to establish effective phototherapeutics to be exploited in pharmaceutical industry[10].

CONCLUSION

Antibiofilm potential of different plant extracts were performed based on zone of inhibition and colorimetric biofilm estimation of corresponding antimicrobial extract. Zone of inhibition was obtained against *Punica granatum* pericarp. Antibiofilm assay was performed for 10% of 0.1g/ml pomegranate acetone extract. Percentage inhibition by pomegranate against UPEC

isolate sample 2 was found to be 88%, 89%, 90% for 1%, 5% and 10% extracts of the sample and sample 4 was found to be 81%, 85% and 86% for 1%, 5% and 10% extracts of the sample. Hence, the plant extract was found to possess antibiofilm activity due to the presence of phytochemical group present. Due to the inability to isolate and purify individual phytochemical compounds, individual compounds were not assayed for antibiofilm activity. The specific compound(s) responsible for this antibiofilm activity are hence unknown. Anyhow further research has to be carried out to ascertain the exact mechanism of action of the specific compounds responsible for the observed pharmacological activities.

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REFERENCES

1. Rodney M. Donlan, Biofilm Formation: A Clinically Relevant Microbiological Process, Biofilm Laboratory, Centers for Disease Control and Prevention 2001; 33 (8): 1387-1392
2. Sowmiya S, Soundarapandian P, Rajan S. Bioactive studies of Mangifera indica against bacteria isolated from urine samples. Current Research Journal of Biological Sciences 2009; 1(3):139-143.
3. Entissar Faroun Ahmed, Antimicrobial and Antibiofilm Activity of Mango Seeds ExtractIraqi Journal of Science, 2015;56(4B): 3121-3129
4. Cassandra L. Quave, Lisa R.W. Plano, Traci Pantuso, and Bradley C. Bennett, Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin resistant Staphylococcus aureus, HHS Public Access 2008; 6(3): 444-450
5. Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić S, Ćirković I. & Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm, US National Library of Medicine 2007; 115(8):891-9.
6. Rohollah Taghadosi, Mohammad Reza Shakibaie, Shalaleh Masoumi, Biochemical detection of N-Acyl homoserine lactone from biofilm-forming Uropathogenic Escherichia coli isolated from urinary tract infection samples, Reports of Biochemistry and Molecular Biology 2015; 3(2): 56–61

7. Antara Sen and Amla Batra, Evaluation of Antimicrobial Activity of different Solvent Extracts of Medicinal Plant: Melia azedarach 2012; 5(1): 47-50
8. Ponnusamy P, Natarajan V, Sevanan M.; In vitro biofilm formation by uropathogenic Eschericia coli and their antimicrobial susceptibility pattern; Asian Pac J Trop Med. 2012;5(3):210-3
9. Mounyr Balouiri,' Moulay Sadiki, Saad Koraichi Ibnsouda,; Methods for in vitro evaluating antimicrobial activity: A review; Journal of Pharmaceutical Analysis**,** 2016; 6(2) : 71–79
10. Shanmugapriya Perumal and Roziahanim Mahmud; Chemical analysis, inhibition of biofilm formation and biofilm eradication potential of *Euphorbia hirta* L. against clinical isolates and standard strains; BMC Complement Alternative Medicine 2013; 13: 346.