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Characterization Of Biofilm Forming Bacteria From Urinary Tract Infected Patients And Its Inhibition By Plant Extracts

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Abstract

Biofilms are spatially structured communities of microbes whose function depends on a complex web of symbiotic interactions. High cell density and proximity of diverse species of microorganisms are typical of life in natural biofilms, where organisms are involved in complex social interactions that occur both within and between species and can be either competitive or cooperative. 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. The present study is aimed at characterization of biofilm forming bacteria from UTI samples. N-acylhomoserine Lactone (AHL), an autoinducer responsible for the formation of biofilms in gram negative bacteria is characterized. The capacity of the UPEC isolates to form biofilms is analysed using biofilm assay. Further on, N-acylhomoserine lactone was extracted by Liquid Liquid Extraction (LLE) and was estimated colorimetrically and spectroscopically by using Fourier Transform Infrared Spectroscopy (FT-IR). The UPEC isolates exhibited strong biofilm forming capacity. The AHL that was extracted showed a characteristic absorbance for C==O bond of lactone ring, and N==Hand C—O bond of acyl chain. 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.

Keywords: UPEC, AHL, Liquid Liquid Extraction, Biofilm, FTIR

1. Introduction

Biofilm has been portrayed in numerous frameworks since Van Leeuwenhoek analyzed the plaque on teeth all alone in the seventeenth century and discovered "animalcules". In 1683, his were among the primary perceptions on living microorganisms at any point recorded. In nature, microorganisms exist basically by binding to and growing on living and lifeless surfaces. These surfaces may take numerous forms, including those found in soil and oceanic frameworks, those on the range of indwelling therapeutic gadgets, and those of living tissues, for example, tooth veneer, heart valves, or the lung, and center ear. The regular element of this joined development state is that the cells build up a biofilm. Biofilm development is a procedure whereby microorganisms irreversibly connect to and develop on a surface and deliver extracellular polymers that encourage connection and lattice arrangement, bringing about a change in the phenotype of the life forms regarding development rate and quality translation. The defenselessness of biofilms to antimicrobial agents can't be dictated by methods for standard microdilution testing, since these tests depend upon the reaction of planktonic (suspended) instead of biofilm (surface-related) life forms. Rather, vulnerability must be resolved specifically against biofilm-related organisms, preferably under conditions that reproduce conditions in vivo. Numerous circulation system contaminations and urinary tract diseases are related with indwelling restorative gadgets and, subsequently, biofilm related. The best system for treating these diseases might be evacuation of the biofilm polluted gadget. A superior comprehension of the procedure of biofilm development may affect clinical basic leadership by influencing the way blood tests and catheter-tip tests are gathered and inspected or

by giving a clearer photo of the confinements of routine treatments for treating biofilm related contaminations [1].

The structure of the biofilm is not an insignificant homogeneous monolayer of sludge but rather is heterogeneous, both in space and after some time, with "water channels" that permit transport of basic supplements and oxygen to the cells developing inside the biofilm. Biofilms have an affinity to act nearly as filters to capture particles of different sorts, including minerals and host segments, for example, fibrin, RBCs, and platelet [1].

The inclination of microorganisms to create biofilms has been very much reported for various medical devices. This procedure is especially pertinent for the clinician because biofilm related microorganisms are a great deal more impervious to antimicrobial operators than are planktonic living beings and because colonization of a therapeutic gadget with a biofilm might be related with disease. Although the instruments of biofilm development and antimicrobial resistance have been explored by the examination group, there is yet a requirement for viable medicines against biofilm-related life forms. A clearer comprehension of the part of biofilms in contamination ought to upgrade clinical basic leadership and give the establishment to further research on novel control methodologies.

Quorum sensing is best characterized as a means of communication within a bacterial species, whereas competitive or cooperative signaling can occur between groups of bacteria or between bacteria and the host. These systems are often integrated into complex, multi-layered signal transduction networks that control numerous multicellular behaviors, including biofilm formation and other virulence traits. In addition, quorum signals, sensors, and signaling pathways are increasingly recognized as having biological properties that extend beyond cell communication. The deeper understanding of microbial cell communication promises to shed light on the complexities of the host-microbe relationship and may lead to novel therapeutic applications.

The key phenomenon in quorum sensing is the regulation of gene expression in response to cell density which is employed both gram positive and gram negative bacteria to regulate various physiological functions. Quorum sensing in more than 30 gram-negative bacteria is mediated by lipid signaling molecules that are chemical derivatives of acyl-homoserine lactones (AHLs). AHLs are synthesized by AHL synthases, enzymes also known as I proteins, and are sensed by the response regulator family of transcription factors known as R proteins. Intracellular accumulation of a sufficient concentration of the cell-permeable AHL generally leads to activated transcription from different promoters within the bacterial genome by induction of a transcriptionally active response regulator such as LuxR of *Vibrio fischeri* or LasR of *P. aeruginosa.* However, in several species the response regulator acts as a negative transcriptional regulator.

The present study is aimed at characterizing biofilm forming bacteria from patients suffering from urinary infection and investigate the effect of crude plant extracts on inhibition of biofilms in biofilm forming bacteria.

2. Materials And Methods

2.1 Bacterial source : Four urine samples were collected from the patients suffering with UTI and were being treated at Bhagwan Mahaveer Jain Hospital, Bengaluru. Samples were collected in sterile and clean urinary containers and cultivated for identification.

2.2 Colony Development of urine samples : All samples were inoculated by streaking on macConkey agar and incubated at 37°C for 24 hours. Bright pink coloured colonies were observed. Five different well isolated distinct colonies were observed. Pure cultures of these colonies were obtained by sub culturing on macConkey

by quadrant streaking and slant streaking. Each isolate was given a number before its identification and these numbers were maintained throughout.

2.3 Biochemical Identification of isolates : Sub cultures of isolates were further identified and characterized by various biochemical tests namely indole, MR-VP, citrate utilization, sugar fermentation test [2]. The morphology was identified using Gram's staining method by Hans Christian Gram [3].

2.4 Primary attachment assay : Primary attachment assay was carried out according to the protocol explained by Rohollah Taghadosi et. al., [4]. One loopful of identified cultures was inoculated in TSB media and were incubated for 24 hours at 37°C. Overnight grown cultures were diluted into fresh TSB media containing 1% glucose in the ratio 1:100. The optical density of the cultures was adjusted to an optical density of 0.5 at 600nm. This $1X10^8$ CFU/ml suspension of culture was diluted to 10^3CFU/ml with TSB media. 100 µl aliquots of the dilutions were added per well in a 96 wells microtiter plate. 100µl of uninoculated medium were used as negative control. Microtiter plate containing aliquots were incubated for 30 minutes at 37°C. After 30 minutes the cultures were removed from the plates and were rinsed with sterile 0.1M phosphate buffer saline (PBS). 150 µl of TSB was then added to each well and optical density was read at 600nm. Triplets were maintained for each culture.

2.5 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 S et al., with some modifications [5]. *E.coli* from UPEC isolate was inoculated into 2 ml of sterile TSB medium containing 1% W/V glucose for optimization of biofilm production. Medium containing *E.coli* was incubated to achieve an optical density of one at OD600. $1.5X10^8$ CFU/ml suspension was further diluted to achieve dilution of ~10^6 CFU/ml. To the wells of flat-bottom 96-well microtiter plate, 100µL of each of the dilutions of prepared bacterial suspension were added. For negative control, 100µL of uninoculated medium were used. Bacteria were grown and adhered to the wells of microtiter plate without agitation for 24 hours at 37°C. After the incubation, cells which were non-adherent were aseptically aspirated and was washed using water. 10µL of PBS (pH 7.2) was then added to the wells. PBS was replaced with 150µL of methanol and wells were incubated for 20 minutes at room temperature. After incubation, methanol was removed and 200µL of 1% W/V crystal violet was added to each of the well. The wells were then washed with sterile deionized water gently and plates were kept for air drying at room temperature. Once the plates were dried 200µL of 33% V/V glacial acetic acid were added to the wells and the OD was measured at 490 nm in a microtiter plate reader. All the isolates were performed in triplets.

2.6 Extraction of AHL from UPEC isolates : N-Acyl Homoserine Lactone was extracted from UPEC isolates by the method as described by Dietrich JA et al.,[6]. Loopful of bacterial isolates were inoculated in Muller Hinton Broth and incubated for 24 hours at 37°C. From the overnight grown culture, 1.5 ml of the culture was aseptically transferred to the sterile centrifuge tubes and was centrifuged at 10,000 rpm for 15 minutes. After centrifugation, the culture supernatant was retained for AHL extraction by Liquid Liquid extraction method. Supernatant was mixed with equal amount of ethyl acetate and was shaken for about 10 minutes. Mixture was allowed to stand for 10 minutes undisturbed. To form upper organic and lower aqueous layer. The upper layer was aspirated by a micro pipette and from the lower portion, extraction was repeated two more times by dissolving in ethyl acetate. The upper portion of each extracted samples were collected and dried in an oven at 40°C. after drying, the powdered extract was dissolved in ethyl acetate.

2.7 Detection of AHL by colorimetric method : For detection of AHL, High throughput detection method of quorum sensing molecule by Yang YH et.al., was performed [7]. 40µL of AHL extract of each of the sample were added into the wells of 96-well polystyrene flat-bottom microtiter plates. Mixture of 2M hydroxyl amine and 3.5M NaOH in the ration 1:1 was prepared and 50µL of this mixture was aliquoted to the wells containing samples. 50µL of 1:1 mixture of 10% ferric chloride in 4M HCL and 95% ethanol was added to the same wells containing sample. The OD was measured at 520nm.

2.8 Determination of AHL functional groups : AHL extracted were analyzed for their lactone functional groups as described by Houshang Shikh-Bardsiri et.al.,by Bruker-alpha FT-IR [8]. A drop of extracted AHL was placed in between two KBr plate and was subjected to IR.

2.9 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 *Punica granatum* collected from the gardens of different residences in Bangalore were used.

2.10 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®.

2.11 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°C7.

3. Results And Discussion

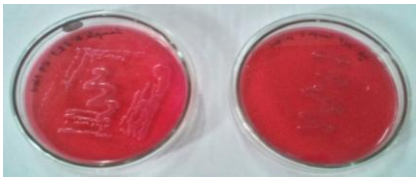
3.1 Colony Development : MacConkey media is a differential and selective culture media which is used for the growth of Gram negative and enteric bacteria. Differentiation between the bacteria is based on lactose fermentation. After 24 hours of incubation, colonies were grown on MacConkey agar plates which were subjected to characteristics and morphology. Figure 1 illustrates well isolated pink to red coloured colonies of lactose fermenting bacteria appearing on the plates and were surrounded by bile zone of precipitation. Control plate was incubated without any organisms and it represents the mac Conkey agar without any growth. Light pink colonies were obtained from sample 1 and there was no change in colour of the media. Pink colonies were obtained from sample 2. Faded pink colonies were obtained from sample 3 and sample 4 and the media colour changed to yellow around the colonies.

Control **Sample 1** **Sample 2** **Sample 3** **Sample 4**

Figure 1: Colonies from different UTI samples

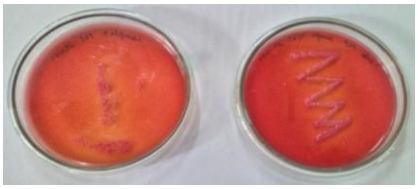
3.2 Subculture of isolated colonies : Each of the well isolated colony from the main culture were aseptically transferred on to different MacConkey agar plate to obtain pure cultures. The characteristic features of the colonies on solid agar media are then noted as shown in Table 1. These include: Shape- circular, irregular or rhizoid; Size- small, medium or large; Elevation-elevated, convex, concave or umbilicate; Surface- smooth, wavy, rough, granular or mucoid; Edges- entire, undulate, crenate, fimbriate or curled; Colour- yellow, green, pink etc; Structure- opaque, translucent or transparent; Degree of growth- scanty, moderate or profuse.

Subculture 1



Subculture 2

Subculture 3



Subculture 4

Figure 2 : Subcultures of the UTI Samples

Table 1: Colony characteristics of microrganisms isolated from urine samples

Isolate No.	Shape	Elevation	Margin	Colour
1	Circular	Elevated	Undulated	Pink
2	Circular	Elevated	Smooth	Pink
3	Circular	Flat	Smooth	Pink
4	Circular	Elevated	Undulated	Pink

3.3 Biochemical identification of isolates : The identification of bacteria is a careful and systemic process that uses many different techniques to narrow down the types of bacteria that are present in an unknown bacterial culture. Multiple tests were performed to provide the fermentation abilities, presence of certain enzymes and certain biochemical reactions. Qualitative observations were made based on the tests, which were compared as the bacteria identification key to aid identification process.

Indole test: Indole test is based on the presence of an enzyme called tryptophanase which determines the ability of bacteria to split amino acid Tryptophan. Indole production is determined by Kovac's Reagent which contain 4-p diethylamino benzaldehyde which reacts with indole to produce a red coloured compound. All the samples showed positive result for Indole [9].

Methyl red test: Methyl red test is based on the ability of bacteria to perform mixed acid fermentation when supplied with glucose. Enteric bacteria metabolize glucose to produce pyruvic acid. Some enteric bacteria then use mixed acid pathway to metabolize pyruvic acid to other acidic products such as acetic, lactic and succinic acid. Large amount of acid production reduces the pH to 4 or below. This lower pH is then determined by methyl red pH indicator. Acidic pH (below 4.4) gives red colour and basic pH (above 7) gives yellow colour after the addition of pH indicator. All the samples showed positive result for methyl red test.

Voges Proskauer Test: In some organisms, the end products of glucose metabolism, pyruvic acid, is further metabolized by using Butylene glucol pathway to produce neutral end products such as acetoin and 2,3 butanediol. Presence of acetoin is determined using Barrit's Reagent A(40%KOH) and Barrit's Reagent B (5% solution of alpha naphthol). Acetoin in presence of oxygen and Barrit's Reagent is oxidized to diacetyl, where alpha naphthol acts as catalyst. Diacetyl then reacts with guanidine components of peptone to produce a cherry red colour. All the samples showed negative result for VogesProskauer test [9].

Citrate utilization test: This test determines the ability of microorganisms to utilize citrate. Some bacteria have capability to convert salts of organic acids such as sodium citrate to alkaline carbonates. Sodium citrate is one the important metabolite of Krebs cycle. Certain bacteria use citrate as the sole carbon source. Citrate is converted to oxaloacetic acid by citrate lyase and oxaloacetate decarboxylase activity will convert oxaloacetate to pyruvate with the release of carbon dioxide. The carbon dioxide reacts with sodium and water to form sodium carbonate. Sample 2 and Sample 3 were citrate negative whereas Sample 1 and Sample 4 showed positive result for citrate test. For further confirmation samples were tested for Metallic Sheen on EMB plates [9].

Sugar fermentation test: Sugar fermentation test is performed to check for the ability of a microorganism to ferment the carbohydrate which is indicated by presence of gas. Basal medium containing 1% of single carbohydrate source with an inverted Durham's tube is used for the test. Production of hydrogen or carbon dioxide due to fermentation process forms air bubble in Durham's tube, which indicates positive result for the test. All the samples showed positive result for sugar fermentation test [2].

Gram Staining: This is a differential staining process developed by Hans Christian Gram 1884, which separates microorganisms based on their cell wall composition. Microorganisms which stains purple are gram positive bacteria because they have thick layer of peptidoglycan (90% of cell wall) which takes up the primary stain (crystal violet). Whereas Gram negative bacteria has thin layer of peptidoglycan (10% of cell wall) and high lipid content and are stained pink by counterstain [3].

Four UPEC isolates were subjected to above biochemical tests for identification of microorganisms. Following results were obtained as tabulated in table 2.

Table 2: Biochemical characteristics, Gram staining and morphology of UPEC Isolates

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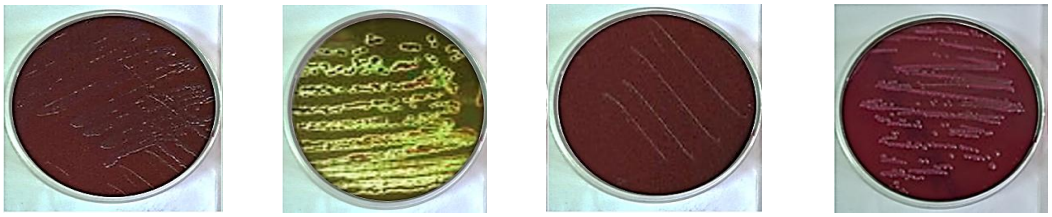
Isolate No.	Indole test	MR test	VP test	Citrate utilization test	Sugar fermentation test			Morphology	Gram Stain
					glucose	lactose	Sucrose		
1	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Rods	Negative
2	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Rods	Negative
3	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Rods	Negative
4	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Rods	Negative

Metallic sheen on EMB agar: Due to positive results for citrate utilization test, EMB agar test was performed which differentiate between Enterobacter and *E.coli*. Most of the strains of *E.coli* colonies develops a characteristic green sheen when cultured on EMB agar plate. Rapid fermentation of lactose and production of strong acids**,** reduces the pH of the EMB agar and forms a green metallic sheen with *E.coli*. Lactose non-fermenters are either colorless or light lavender. EMB media assists visual distinction of *E.coli* from other pathogenic enteric bacteria as illustrated in Figure 3. Metallic sheen were obtained for sample 2, whereas for Sample 1, Sample 3 and Sample 4 there were no development of metallic sheen and hence negative for EMB test. Hence for further analysis, Sample 2 was used.



Sample 1 **Sample 2** **Sample 3** **Sample 4**

Figure 3: Colonies on EMB Agar

3.4 Primary attachment assay

The result of primary attachment assay is depicted in Figure 4, which indicates the attachment of bacteria to the microtiter plates. This test was performed to ensure the attachment of the bacteria to the wells of the microtiter plate. 10^8 showed maximum biofilm forming capacity due to more number of colonies, 10^7 showed comparatively lesser biofilm forming capacity due to reduced number of colonies. As dilution increases, the biofilm forming capacity reduces.

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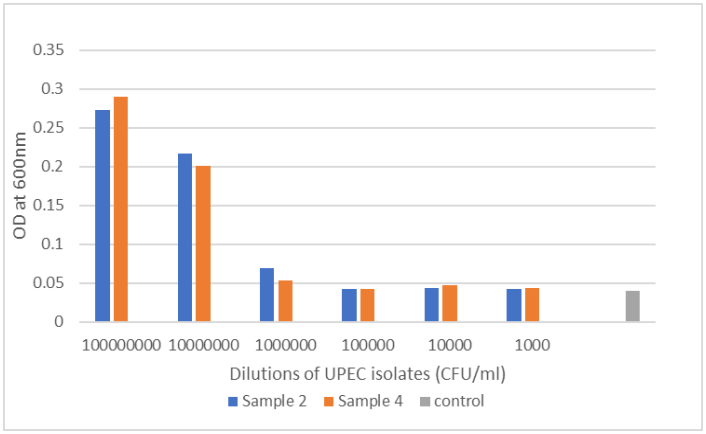

Figure 4: Primary Attachment assay

3.5 Biofilm formation assay : Illustrated below in the Figure 5 is the results 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. 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 helps them to persist on the wall of microtiter well or on the wall of urogenital tract causing virulence [10].

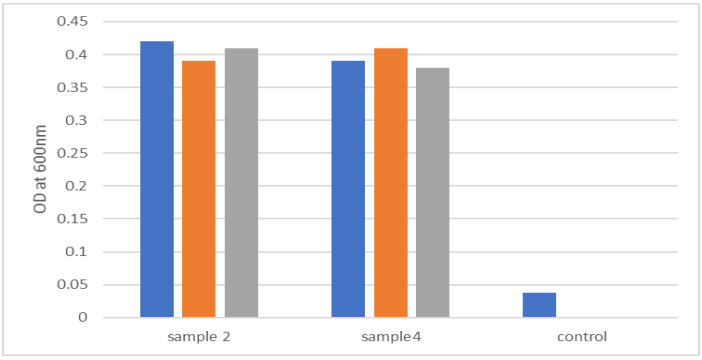

Figure 5: Capacity of biofilm formation by different isolates, series 1, 2 and 3 represents three different duplicates of a sample

3.6 Estimation of AHL by colorimetry method : Standard curve for AHL was obtained using standard lactone compound phthalic anhydride [7]. Under alkaline conditions AHLs are rapidly inactivated by pH-dependent lactonolysis, in which the homoserine lactone ring is hydrolyzed to open ring form corresponding to N-acylhomoserine. This reaction can be reversed by acidification; therefore, we monitored pH throughout the experiment to verify that an acidic pH was maintained.

From the standard graph of phthalic anhydride($y=0.0727x+0.0269$, $R^2=0.9251$), total amount of AHL in sample 2 was found to be 2.325±0.02µg/ml. Phthalic anhydride is a lactone compound and shows a similar reaction under alkaline conditions and hence was taken as a standard.

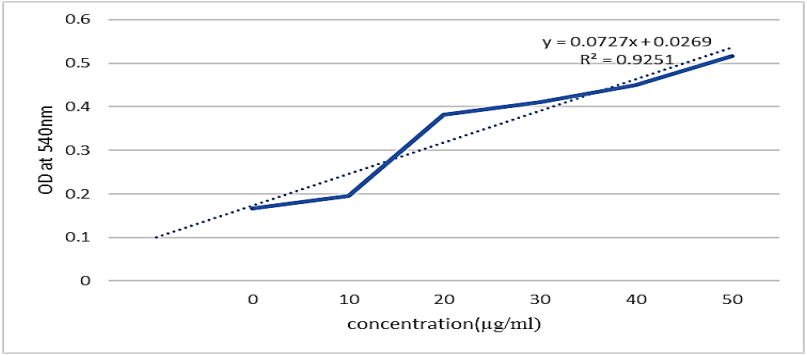

Figure 6: Standard curve for estimation of AHL

Table 3: Absorbance showed by AHL at 540 nm

Sample	OD at 540nm	
Sample 2	0.217	0.219

3.7 Determination of AHL functional group : Fourier Transform Infra-Red Spectrophotometer of the extracted AHL sample, along with standard molecule was performed to identify the organic solvent present. It relies on the principle that most molecules absorb light in infrared region (4000-600cm^{-1}) of electromagnetic spectrum. The absorption peaks correspond directly to the bond present in the sample to be identified. For determination of AHL, strong peaks in the range 1750-1735 and 1300-1000 cm^{-1} correspond to the C=O bond of lactone ring and the C-O bond, respectively should be obtained [4]. Following are the FT-IR plots for standard and unknown sample which were extracted from the bacterial samples. Phthalic anhydride was taken as a standard and peaks were obtained at 1739.40, 1372.99, 1222.14 and 1042.82 cm^{-1} and these peaks correspond to C=O of lactone ring, N=H bond, C—O and N—H respectively. Similar peaks were obtained for unknown sample 2.

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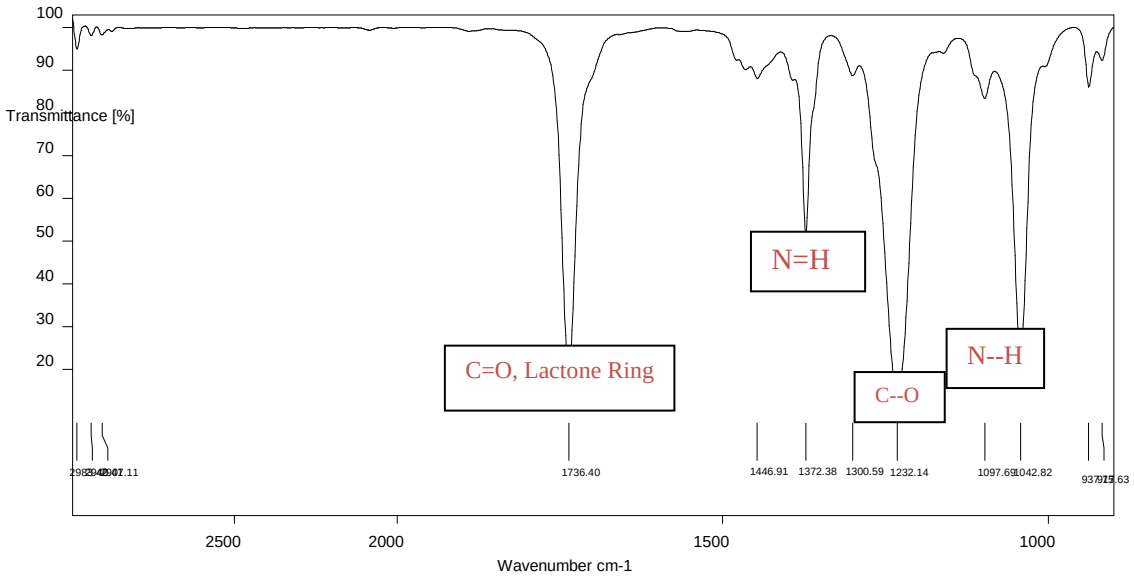
Standard



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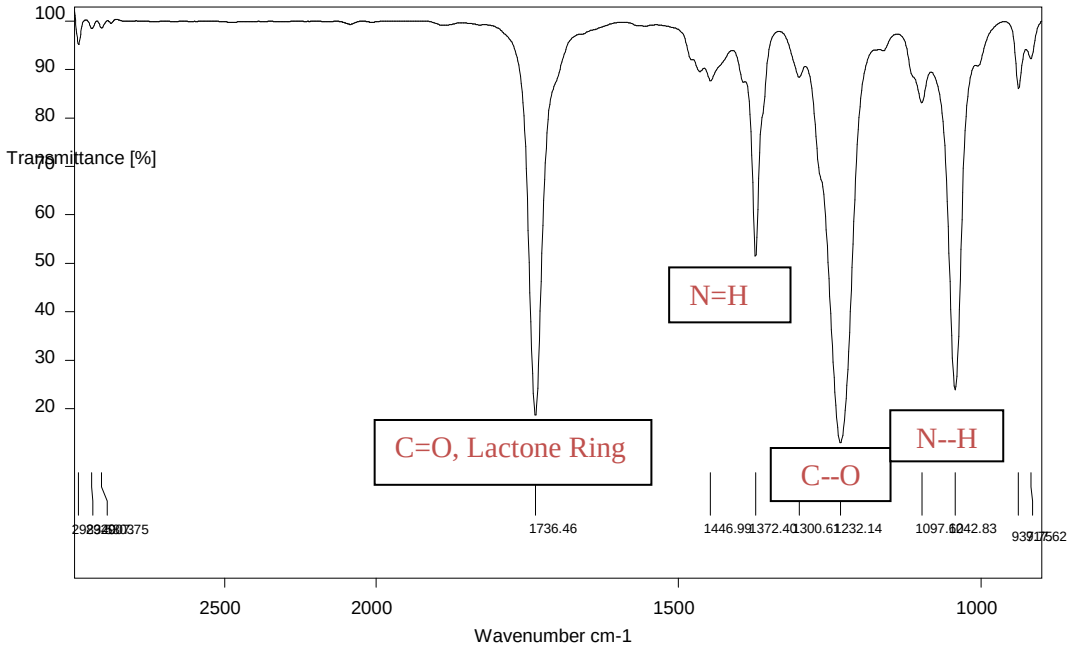

Sample 2

Figure 7 : FT-IR analysis

3.8 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. Amongst all the samples, pericarp of *Punica granatum* showed zone of inhibition. Figure 8 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.

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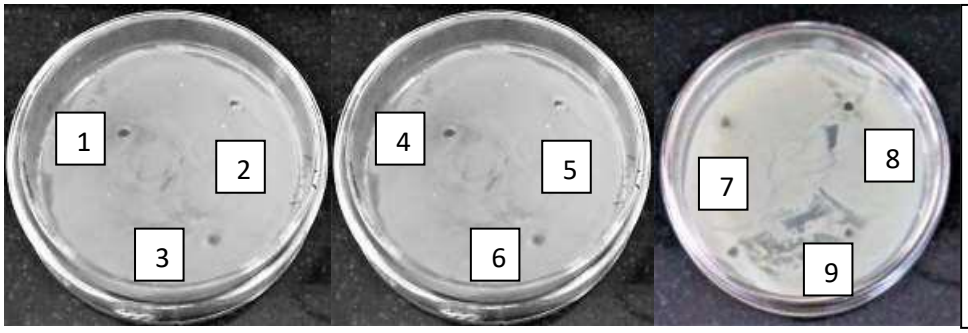

*Magnifera indica*fruit
2.*Magniferaindica*twig
3. Magniferaindica leaves
4.*Punica granatum*pericarp
5.*Catharanthus roseus*flower
6.*Hibiscus roseus*flower
7.*Manilkara zapota*leaves
8.*Manilkarazapota*fruit
9. *Manilkarazapota*twig

Figure 8: Well diffusion assay for sample 2

3.9 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 4: Absorbance of the sample 2 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

Table 5: Percentage inhibition showed by *Punica granatum*

	Percentage inhibition		
	1% Extract	**5% Extract**	**10% Extract**
Sample 2	88	89	90

Table 4 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 0.047, 0.044 and 0.039 OD respectively. Table 5 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%, 5% and 10% 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.

4. Conclusion

In the present investigation it has been observed that the UPEC isolates have a strong biofilm forming abilities. The key component that is responsible for biofilm formation is N-acylhomoserine lactone in gram negative bacteria. AHL was extracted by Liquid-Liquid Extraction. Qualitative estimation of AHL was carried out by FT-IR. 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. The plant extract was found to possess antibiofilm activity due to the presence of phytochemical group present. Further research has to be carried out to ascertain the specific component and 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," *Atlanta, Clin Infect Dis*; *vol 33 (8)*, pp. 1387-1392, 2001.
2. Rutter, J.M., "A study of the carbohydrate fermentation reactions of clostridium oedematiens (cl. Novyi)," *Med. Microbiol, vol 3*, pp. 283-289, 1971.
3. Hans Christian Gram., "The differential staining of Schizomycetes in tissue sections and in dried preparations, Schnitt-und Trockenpriparaten," *Fortschitte der Medicin, vol 2*, pp. 185-189, 1884.
4. 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 & Molecular Biology, vol* 3(2) pp. 56-61, 2015.
5. Stepanović, S., Vuković, D., Hola, V., Bonaventura, G.D., Djukić, S., Ćirković, I., Ruzicka, F., " Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm samples," *Institute of Microbiology and Immunology, School of Medicine, vol 115(8),* pp. 891-899, 2007.
6. Dietrich, J.A., Mckee, A..E, Keasling, J.D., "High-throughput metabolic engineering: advances in small-molecule screening and selection," *Annual review of biochemistry, vol* 79, pp. 563-590, 2010.
7. Yang, Y.H., Lee, T.H., Kim, J., Kim, E.J., Joo, H.S., Lee, C.S., Kim, B.G., " High-throughput detection method of quorum-sensing molecules by colorimetry and its applications," *Analytical Biochemistry, vol 356(2)* pp. 297–299, 2006.
8. Shikh-Bardsiri, H., Shakibaie, M.R., " Antibiotic resistance pattern among biofilm producing and nonproducing Proteus strains isolated from hospitalized patients; matter of hospital hygiene and antimicrobial stewardship," *Pak J Biol Sci, vol 16(22)* pp. 1496-502, 2013.
9. Vashist Hemaraj, D. Sharma, A. Gupta, "A Review of commonly used biochemical tests for bacteria," *Department of Pharmacy, L.R.Institute of Pharmacy, vol 1(1)* pp. 1-7, 2013.
10. Ponnusamy, P., Natarajan, V., Sevanan, M., " In vitro biofilm formation by uropathogenic Eschericia coli and their antimicrobial susceptibility pattern," *Asian Pac J Trop Med.vol 5(3)* pp. 210-23, 2012.