

Original Research Article

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## Bioprospecting of antibiofilm potential of *Piper nigrum*, *Piper betle* and *Coscinium fenestratum* on *Escherichia coli*

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### ABSTRACT

#### Keywords

MDR, Biofilm, Quorum quenching, Piperidine, Eugenol, Berberine, *E. coli*

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Genetic language for social behavior in bacteria is mediated through Quorum sensing. This collective group behavior poses obvious advantages for the bacteria to adapt to suitable growth modes by migrating to amicable environmental conditions which makes them highly virulent and detrimental to the environment. 80% of the uncomplicated UTIs that occur in healthy individuals are due to uropathogenic *E. coli* which are the leading cause of morbidity. Current study investigates antibiofilm activity of methanol extracts from *Coscinium fenestratum*, *Piper betle* and *Piper nigrum* against clinically isolated, MDR *E. coli*. LC / MS-MS screened the herbal bioactive compounds. The extracts exhibited a MIC in a range of 0.1mg/mL- 0.15mg/mL. Reduction of EPS and rhamnolipids was found to be 79%, 68% and 62.0% respectively by *C. fenestratum*, *P. betle* and *P. nigrum*. Berberine and Eugenol can be used as quorum inhibitors for attenuation of MDR *E. coli* at sub inhibitory concentration of 50µg/mL.

### Introduction

Globally every year on an average 150 million cases of Urinary tract infections are reported with notable infectivity and high medical expenses. Furthermore, 80% of the uncomplicated UTIs that occur in healthy individuals are due to uropathogenic *E. coli* which are the leading cause of morbidity (Mary E. Davey *et al.*, 2003).

Large majority of nosocomial infections are

directly linked to multidrug resistant bacteria, capable of forming biofilms. Bacteria have developed high incidences of resistance to antibiotics due to its extensive use and abuse for almost seven decades giving rise to superbugs that are untreatable with antibiotics. These physiologically altered superbugs have shockingly altered their patterns of interaction with host and environment. Thus to minimize the resistance, rational strategies need to be implemented by gaining thorough insight about the mechanism of their emergence into

superbugs (Anderl, *et al.*, 2000).

The discovery and implementation of more valuable and improved alternatives is the need of the hour. This void is fulfilled by the process of Quorum Quenching (QQ) through QS inhibitors possibly by either blocking the AHL signal generation or blocking the AHL signal dissemination or by blocking the AHL signal reception depending on the stage of QS in the biofilm as explained by (Hentzer *et al.*, 2003).

*E. coli* is a facultative gram negative anaerobic bacteria present in the gastrointestinal tract that can form biofilm. The biofilm production is essentially regulated by LuxO gene. It also follows Autoinducer-2 based signaling. A variant of LuxO gene, LsrR affects the uptake of Autoinducer 2 and hence, it can be inferred that Lsr operon plays an important role in Quorum sensing related cellular functions (Blair R. G. Gordon *et al.*, 2010). The second signal is by CsgD cascade which are active in the stationary phase of cell growth together govern the flagellar production, biofilm matrix component activation such as curli fimbria and cellulose biosynthesis, that are inversely reciprocal leading to biofilm initiation and formation. Phytoligands are proven to have huge significance and inhibitory properties against virulent MDR pathogens and are potential drug targets due to variable alkaloid, flavonoids, terpenoid and phenolics contents. Phytoligands like reversitol, baicelein, luteolin showed promising docking properties against *S typhi*, *S aureus*, *E. coli* (Chan, 2002; Sinosh *et al.*, 2014).

The purpose of the study gives us insights about the various small molecules, new targets and their effectiveness on herbal drug formulations, thereby formulating a cost effective and zero side effect drugs against MDR bacteria.

## Materials and Methods

### Isolation and screening of clinical isolates

Microbial samples were collected from various hospitals across Bangalore. Isolation protocol such as swab test, air sampling, used catheters, disposal areas using ready-made LB and Nutrient agar plates. Collected samples were immediately transferred to lab and incubated at 37°C for 24- 48 hours. Also for comparing the study, positive control *E. coli* MG 1655 (MTCC No.1586) sample was procured from Institute of Microbial Technology, Chandigarh, India for the study. Glycerol stock cultures were prepared and kept at -20°C prior to use. The lyophilized cultures were revived by plating onto sterile tryptone soya agar (TSA) and incubated at 37°C for 18 h. This was followed by the inoculation of the bacteria into the sterile tryptone soy broth (TSB) which was placed in a shaking incubator at 37°C overnight. These cultures were then standardized to yield a concentration of 1x10<sup>6</sup> CFU/ mL before the biofilm formation. This was achieved by diluting the overnight cultures with TSB to obtain an absorbance of 0.1 (A 590 nm) using a spectrophotometer.

### Screening of MDR pathogens using antibiotic assay (Boyan Bonev *et al.*, 2008)

As per clinical and laboratory standards institute (MS100 S23, 2013) some of the common antibiotics such as ampicillin 10µg, gentamicin 10µg, cefoperazone 30µg, ciprofloxacin 5µg, piperacillin 30µg, nalidixic acid 30µg and ceftazidime 30µg. The above antibiotics were used to screen out Multidrug-Resistant Bacteria (MDRB). Samples obtained from various hospitals were identified using basic biochemical test (Bergey, D. H., *et al.*, 1974).

## **Biofilm Establishment Assay**

### **Population growth analysis**

The population growth of microbes was monitored under *in vitro* culture using LB as culture medium. Population density was estimated by measuring the OD of 24h cultures at 600 nm in UV spectrophotometer. Generation time was calculated.

### **Tissue culture plate (TCP) method** (Christensen, *et al.*, 1982)

In TCP method, the adherence ability of the organisms to the hydrophobic surface was tested. Biofilm formation was initiated by addition of tryptone soya broth and the surface treated polystyrene microtitre plates were inoculated with the organisms. The plates were incubated at 37°C for 24h, 48h and 72 hours. Biofilm-coated wells of microtitre plate were vigorously shaken in order to remove all non-adherent bacteria. The remaining attached bacteria were washed twice with phosphate buffer saline (PBS) and then air-dried. Then, each of the washed wells was stained with aqueous 1% crystal violet solution.

### **Test tube method**

(Christensen, *et al.*, 1982)

The bacterial cultures were grown in tryptone soya broth and incubated at 37°C for 24h, 48h and 72 hours. The glass tubes were then decanted and washed with phosphate buffer saline (PBS) (pH 7.3) to remove loosely associated cells and dried. The dried test tubes were stained with 100 µL of 1% crystal violet and incubated at a room temperature for 15 min after which the tubes were washed 5 times with sterile distilled water to remove unabsorbed stain.

Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid

interface was not considered indicative of biofilm formation. Tubes were examined and the degree of biofilm formation was scored qualitatively.

### **Exopolysaccharides extraction**

(Smitinont, *et al.*, 1999)

The biofilm was estimated quantitatively in terms of the production of EPS by the microbe. The 10mL overnight cultures of all the bacteria were subjected to centrifugation at 10,000 rpm for 20 min at 4°C. To precipitate the EPS, two equal volumes of chilled ethanol was added to the sample CFE and incubating the mixture at 4°C for overnight. The precipitated EPS was collected by centrifugation at 10,000rpm for 20 min at 4°C and the supernatant was decanted. The pellet containing EPS was dried at room temperature. The total carbohydrate content in the EPS was estimated by phenol-sulphuric acid method.

### **Rhamnolipid analysis using FTIR**

(Diederich, *et al.*, 2003)

The CFE was subjected to liquid - liquid extraction using diethyl ether. The aqueous fraction was mixed with 2 ml of 1N H<sub>2</sub>SO<sub>4</sub> and the mixture was heated in water at 80°C for 20 minutes. Absorbance was recorded at 490 nm. L-rhamnose was used as a standard for calculating the concentration of rhamnolipids present in the CFE.

Fourier transform infrared spectroscopy was carried out to characterize rhamnolipids formed during the stationery stage of the bacterial growth curve. Infrared spectroscopy was carried out to know the structure of the rhamnolipid and compare it that of compound in hand. The IR spectra were recorded on a (Perkin-Elmer 31725 X, Germany) FTIR spectrophotometer using KBr discs (Reháková, *et al.*, 2014).

## Screening of Medicinal Plants

### Collection of plant material

For the current investigation, plants samples namely *Piper nigrum*, *Piper betle* and *Coscinium fenestratum*, were procured, identified and authenticated from Foundation for Rehabilitation of local Health tradition (FRLHT), Bengaluru. The deposited voucher numbers: 120014, 120015 and 120013 (FRLH Herbarium) respectively.

The leaves of parts of the *Coscinium fenestratum*, *Piper betle*, and dried unripe fruits (corns) of *Piper nigrum* plants used were washed in running tap water followed by distilled water. They were shade dried, weighed, powdered and stored for further use at 4°C. 50 g of each air dried plant materials were extracted with 200 mL methanol solvent using soxhlet apparatus.

### LC-MS analysis

Methanolic extracts of *P. nigrum*, *P. betle*, and *C. fenestratum* were subjected for LC/MS-MS analysis by Waters, acquity (UPLC)-H class coupled with TQD-MS/MS equipment, equipped with a degasser, quaternary pump, automatic injection system, with a diode array detector and a temperature control compartment for the analytical column. The 2.1x50 mm UPLC BEH C18 column (Waters, USA) with 1.7 µm particles, protected by Vanguard BEH C18 with 1.7 µm guard column, operated at 250°C was used as the analytical column. The mobile phase, 0.5% formic acid in water as Solvent A and 0.2% formic acid in 90% methanol as Solvent B was delivered at a flow rate of 0.3 mL/min under the gradient program. The 5µL sample injection volume, with flow ramp rate of 0.45 min, high-pressure limit of 15000 psi and seal wash period of 5.00 min was used. The eluted metabolites were monitored using the UPLC column which was pumped directly into the

TQD-MS/MS (Waters, USA) system with source temperature 350°C, desolation gas flow of 650 L/h and temperature of 350°C. The LCMS data were collected in ESI+/- mode using timed SIR functions to maximize the sensitivity of the analysis. The compounds with highest peak at a particular retention time were found out and listed (Darrin Smith, *et al.*, 2009).

### Quorum Quenching Assay

#### Antibiofilm formation assay

Antibiofilm assay was adapted from as referred, employing crystal violet as biofilm indicator. In that case the glass tubes, 100 µL of the bacterial suspension, 100µL of the aqueous extract (concentration of 4.0 mg/mL in the tubes) and 10 mL of TSB were added. In both experiments, following the incubation period (37°C for 24 h) the content of the wells/tubes was removed and the wells/tubes were washed three times with sterile saline or PBS pH7.2. After washing, the remaining bacteria adhered to the walls of the test tubes were heat-fixed at 60°C for 1 hour in hot air oven. The adherent biofilm layer formed was stained with 0.4% crystal violet for 15 minutes at room temperature. The stain bound to the cells was solubilized with 99.5% DMSO and absorbance was measured at 570 nm. The biofilm formation control was considered to represent 100% of biofilm formation, and the extracts were replaced by 100 µL and 1000 µL of water in 96-well microtitre plates and glass tubes, respectively. Values higher than 100% represented a stimulation of biofilm formation in comparison to the control. (Trentin, D. D. S.*et al.*, 2011)

#### Study of the effect of plant extracts on EPS formation

The effects of plant extracts on the quenching the biofilm were qualitatively estimated. 100 µL of cells at exponential phase were

dispensed in glass tubes. Plant extracts of different concentrations were added to the tubes and incubated for 24 h at 37°C. The medium without extracts was used as the non-treated control. After incubation, media and unattached cells were decanted and washed with Phosphate Buffer Saline (PBS) pH 7.2. Then the tubes were air dried at 60°C for 1 hour and stained with 0.4% (w/v) Crystal Violet. In order to estimate the biofilm quantitatively, the overnight grown cultures were inoculated into test tubes containing 10 mL of TS broth and incubated for 24 hours for biofilm development. 0.1 mL of this was then added and the tubes were incubated for another 24 hours. The biofilm formation was expressed in terms of EPS which was estimated by Phenol-Sulphuric acid method. (Xiao, *et al.*, 2010)

## Results and Discussion

### Isolation and screening of clinical isolates

In the present study microbial samples were isolated from various hospitals across Bangalore during the period 2012-14. The samples were collected from urinary catheters, swab sampling and through air sampling. The following 9 bacterial species namely *Micrococcus sp*, *E. coli*, *Streptococcus sp*, *Staphylococcus sp*, *K. pneumonia* and *Vibrio sp* were identified. 34% of the samples were predominantly infected by *E. coli*, followed by 27% of *K. pneumonia*, 14% of *Streptococcus*, 12% of *Micrococcus sp*, 8% *Staphylococcus sp* and 5% *V. cholerae*. The microbes were screened based on MDRB by antibiotic screening as described and tabulated in Table 1.

### Screening of MDR pathogens using antibiotic assay

The clinical isolate of *V. cholerae* was subjected to antibiotic sensitivity test for confirming the multi-drug resistant (MDR)

status of the strain.

### Population Growth Analysis

*E. coli* samples were grown in LB medium. The optical density was measured at 600nm for every two hour intervals as shown in Fig 1. It was found that the *E. coli* doubling time was found very rapid and reached stationary phase within 12 hours of inoculation and declination phase was found after 22 hours. We inferred the hypothesis that the organisms express biofilm ability during late log phase and stationary phase. Furthermore in the next section the exopolysaccharides production and the highest level of EPS production at different physical conditions have been discussed.

### Biofilm Establishment Assay

Standard MTCC cultures and the chosen clinical isolates were investigated for their ability to form biofilm. Biofilm formation determines the capacity of an organism to survive in a particular environment. *E. coli* strains are known to secrete autoinducer-2, resultant of LuxS gene product which is a common bacterial cell-cell communication system found in *S. aureus* and *Vibrio* species. (Fitnat Yildiz, *et al.*, 2009; Bonnie L. Bassler *et al.*, 1998).

### Tissue Culture Plate Method

The selected *E. coli* species along with the standard MTCC cultures were monitored for their ability to establish biofilm on microtitre plates at 24 h, 48 h and 72 h (Fig.2). The growth of the organisms at different time duration can be visualized by the change in the color intensity.

### Test Tube Method

The biofilm formation was monitored for *E. coli* MG1655, clinical isolate *E. coli* at 24

hours, 48 hours and 72 hours fig(3). From both the above results, it is evident that the production of EPS was in the order *E. coli* MG1655 > *E. coli* sample. However, *E. coli* MG 1655 sample had only loosely aggregated cells adhered to the surface compared to the other samples.

### **EPS Estimation and Quantification at Different Temperature and pH Levels**

The EPS was estimated by extracting it from biofilm growing in Trypton soya broth and estimated in different time intervals. It was observed that the biofilm production by the microbe was increased with time till 72h and the increment in the yield of EPS was not significant after 72 h. So the quantification was measured until 72 hours. The quantification is also to find out the biofilm establishment and the growth parameters. For which standard glucose curve was spectrophotometrically determined to calculate the EPS produced at various temperature and pH (Fig.3a,b). The organisms produce considerable amount of EPS at pH 5 and 7 but there is a significant reduction at pH 9. EPS production was maximum at 48 h and there was significant change observed between 24 h and 72 h of EPS production.

### **Rhamnolipid Screening Using FTIR**

Rhamnolipids was for the first time described as secondary metabolite of *Pseudomonas aeruginosa* in 1949. It was noticed that for the growth of the bacterium on a hydrophobic carbon source, rhamnolipids were essential (Gong Peng Wang, 2015). These play a vital role in the formation of biofilms with pores (Mary E. Davey *et al.*, 2003). There have been reports that the rhamnolipids could be used for potential anti-bacterial and anti-biofilm activities (Parul Vatsa, *et al.*, 2010). The aim of the study was to identify a secondary metabolite that would have anti-biofilm

activity and which would not have any effect on the bacterial growth (Fig.4).

Rhamnolipids play a vital role in quorum sensing that is they help in cell dependent control of gene expression. The rhamnolipids of *E. coli* when subjected to Infra red spectroscopy, were found have absorption bands at 2920.11 cm<sup>-1</sup> and 2851.28 cm<sup>-1</sup> for C-H stretching of CH<sub>2</sub> and CH<sub>3</sub> groups. This was in accordance with the standard values of Infrared spectroscopy.

### **Liquid Chromatography Mass Spectrometry**

Three main phytoligands namely berberine from *C. fenestratum*, piperidine from *P. nigrum* and eugenol from *P. betle* have been used to evaluate their antibiofilm activity.

### **Analysis of herbal extracts**

The major bioactive compounds present in the extracts of *C. fenestratum*, *P. nigrum* and *P. betle* are presented in Fig 5a-5c Major alkaloids found in *C. fenestratum*, *P nigrum* and *P. betle* are shown in Table 2a-2c.

### **Anti-biofilm activity in the presence of herbal extracts**

Both the control and the clinical *E. coli* have established biofilm on the inner surface of the test tubes. However, biofilm establishment was disrupted in *E. coli* cultures exposed to the herbal extracts at sub inhibitory concentration of 0.1mg/mL(Fig 6a) and phytoligands at 0.05mg/mL(fig 6b). This result confirmed the quorum quenching activity of *C. fenestratum* is better than *P. betle* against *E. coli*. (Ankith *et al*, 2018) Eugenol inhibited biofilm formation in case of *Staphylococcus epidermidis* at MBIC 0.0312 mg/ml. Berberine displayed antibacterial effect against *E. coli* in a dose dependent manner at MIC 50µg/mL (Bandopadhyay, 2013).

**EPS production in presence of herbal extracts and specific phytoligands**

EPS modulates the initial cell attachment to various substrates and protects the bacterial aggregates against its habitat stress and dehydration (Freeman, Bassler,1999). Therefore, EPS has been selected as one of the testing tools for evaluating the efficacy of the phytoligands on biofilm formation. The percentage of EPS reduction in the clinical *E. coli* sample and *E. coli* MG 1655 was tested using the herbal extracts. 79%, 95% and 67% reduction was seen in *E. coli* control against *P. betle*, *C. fenestratum* and *P. nigrum* respectively at a concentration of 150µg/mL. However in clinical *E. coli* sample EPS reduction was highest in *C. fenestratum* with 89.5% followed by *P. nigrum* with 72% and only 57% in *P. betle* (Fig.7). Cranberry extract (500µg/ml) could reduce the formation of *S. epidermidis* biofilm on soft contact lenses (Leshem et al.,2014)

**Rhamnolipid production in presence of herbal extracts**

Rhamnolipids play a vital role in quorum sensing as they facilitate the cell dependent control of gene expression (Vu Chen, 2009). Therefore, it has been used to estimate

rhamnolipids as a tool for assessing the quorum quenching efficiency of the phytoligands. Quantitative estimation of rhamnolipids content in unit volume of the bacterial culture without treatment with the phytoligands and those with treatment showed a considerable level of reduction in the quantity of these compounds in the treated cultures (Table 3). *C. fenestratum* showed more than 80% reduction in rhamnolipids in both the strains of *E. coli* tested followed by *P. betle* and *P. nigrum*.

Selected bacteria were subjected for biofilm establishment and quenching studies using various techniques such as tube test, microtitre plate test, EPS extraction for carbohydrate analysis, lipid testing using FTIR were carried out both before and after quenching using plant metabolites. Biofilm quenching by *Coscinium fenestratum* extract is very prominent in *E. coli* in all the methods followed.

**Analysis of Herbal Extracts**

The major bioactive compounds present in the extracts of *C. fenestratum*, *P. nigrum* and *P. betle* are presented in Fig 5a-5c Major alkaloids found in *C. fenestratum*, *P nigrum* and *P. betle* are shown in Table 2a-2c.

**Table.1** Results of antibiotic sensitivity test of bacteria showing MDRs against commercial antibiotics

Sample	Ampicillin	Gentamicin	Cefoperazone	Ciprofloxacin	Piperacillin	cefalotin	nalidixic acid	ceftazidime
<i>E. coli</i> MG1655	+	+	+	+	-	-	-	-
Sample 24	+	+	+	-	+	+	+	+
Sample 47	+	-	+	-	-	+	+	+
Sample 49	+	+	+	+	+	-	+	+
Sample 50	+	+	+	-	+	+	+	+

+ Resistant, - Sensitive

**Table.2a** Major bioactive compounds found in the *C. fenestratum* leaf extract using LC MS (SIR)

Sl. No	Retention time	Name of the compound	Mol Mass	Formula
1	8.39	Berberine	336.36	C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup>
2	3.51	Proto-berberine	232.13	C <sub>17</sub> H <sub>14</sub> N <sup>+</sup>
3	7.83	Jatrorrhizine	338.21	C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub> <sup>+</sup>
4	7.85	Tetra hydro-berberine	339.16	C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub> <sup>+</sup>
5	4.81	Magnoflorine	342.41	C <sub>20</sub> H <sub>24</sub> NO <sub>4</sub> <sup>+</sup>
6	8.64	Palmatine	352.23	C <sub>21</sub> H <sub>22</sub> NO <sub>4</sub> <sup>+</sup>

**Table.2b** Major bioactive compounds found in the *P. nigrum* seed extract using LC MS (SIR)

Sl. No	Retention time	Name of the compound	Mol Mass	Formula
1	3.98	Piperidine	85.14	C <sub>5</sub> H <sub>11</sub> N
2	13.48	Piperlyne	271.31	C <sub>16</sub> H <sub>17</sub> NO <sub>3</sub>
3	14.56	Piperine	285.33	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>
4	14.59	Piperanine	287.3	C <sub>17</sub> H <sub>21</sub> NO <sub>3</sub>
5	16.04	Piperittine	311.37	C <sub>19</sub> H <sub>21</sub> NO <sub>3</sub>

**Table 2c** Major bioactive compounds found in the *P betle* leaf extract using LC MS (MRM/SIR)

Sl. No	Retention time	Name of the compound	Mol Mass	Formula
1	3.52	Hydroxy-chavicol	150.17	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>
2	16.06	Chavibetol	164.2	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
3	10.12(MRM) 15.97(SIR)	Eugenol	164.2	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
4	16.59	Pellitorine	223.35	C <sub>14</sub> H <sub>25</sub> NO
5	15.5	Ilepcimide	259.3	C <sub>15</sub> H <sub>17</sub> NO <sub>3</sub>

**Table.3** Effect of herbal extracts on rhamnolipids production by *E. coli*

Bacterial strains	Percentage reduction in rhamnolipid content		
	<i>P. nigrum</i>	<i>P. betle</i>	<i>C. fenestratum</i>
<i>E. coli</i> MG 1655	68%	74%	83%
<i>E. coli</i> sample	62%	68%	79%

### Population Growth Curve

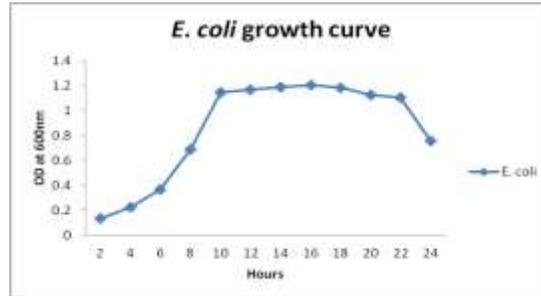


Fig.1 *E. coli* generation time estimated for 24 hours

### Tissue Culture Plate

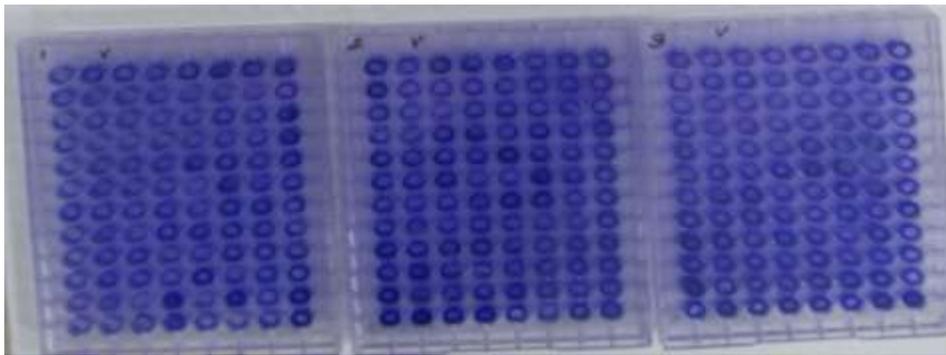


Fig.2 Microtiter plate assay of biofilm formation for *E. coli* sample 24 after 24h, 48h and 72h.

### Test Tube Method



Fig.3 Test tube assay of biofilm formation for *E. coli* sample 24 after 24h, 48h and 72h

### EPS Estimation and Quantification at Different Temperature and pH levels

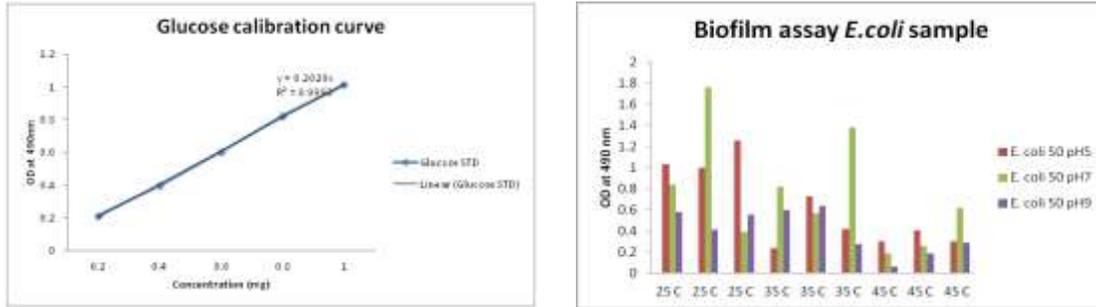


Fig.3a. Biofilm produced by *E. coli* at pH 5, pH 7, pH 9, vs 25°C 35°C 45°C  
Fig.3b Illustrates the standard glucose curve for the prediction of the EPS.

### Rhamnolipid Screening Using FTIR

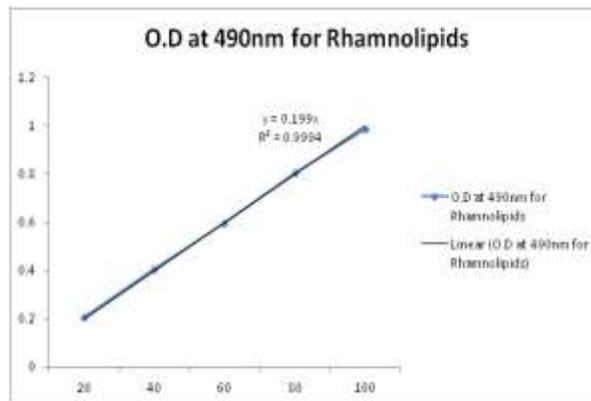


Fig.4a Calibration curve for Rhamnolipids

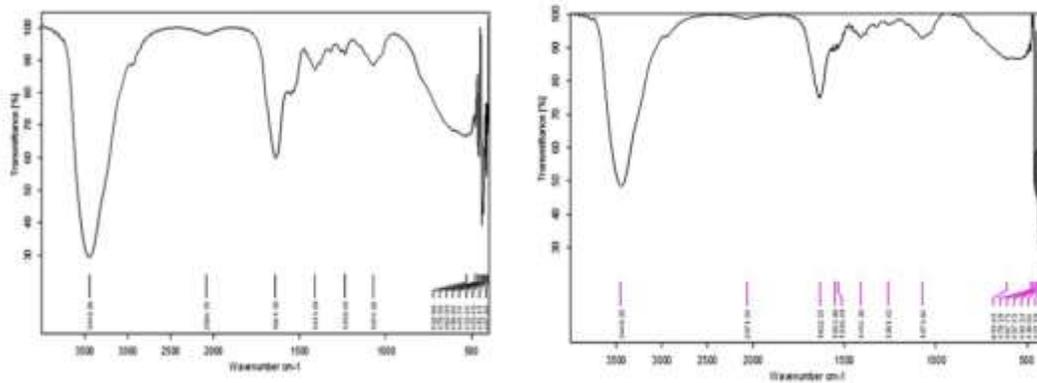


Fig.4b FTIR spectrum of the controls and the samples (1) *E. coli* MG 1655 (2) *E. coli*.

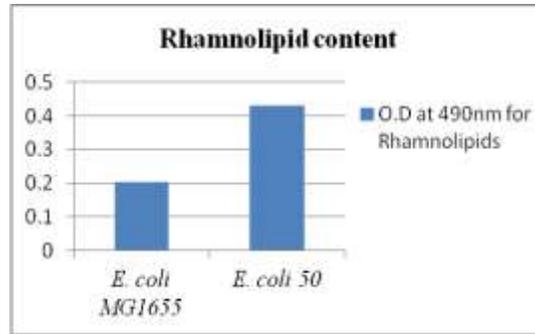


Fig.4c Rhamnolipid analysis spectrophototically for the standards vs the *E. coli* sample

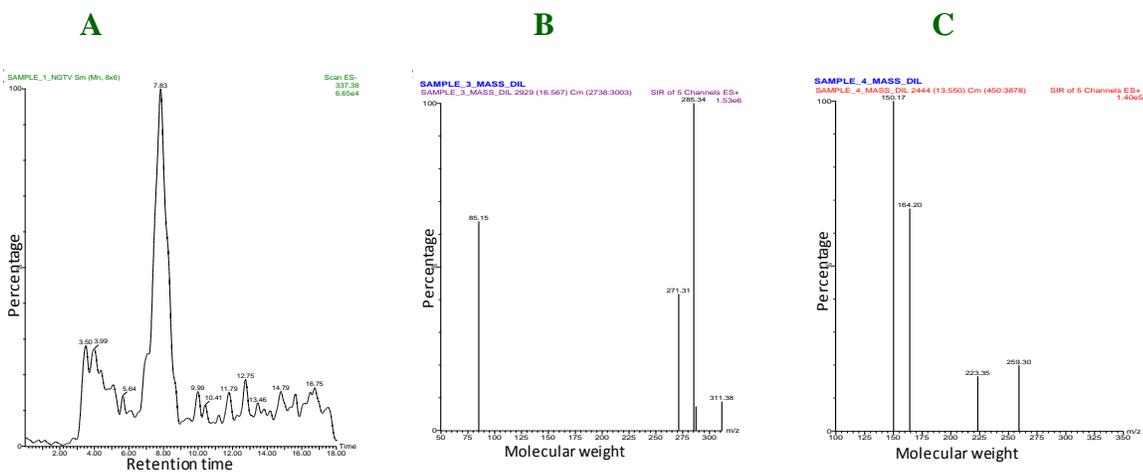


Fig.5a: Major bioactive peaks in *C. fenestratum*; Fig.5b. Major bioactive peaks in *P. nigrum*  
Fig.5c. Major bioactive peaks in *P. betle*

### EPS Production In Presence Of Herbal Extracts

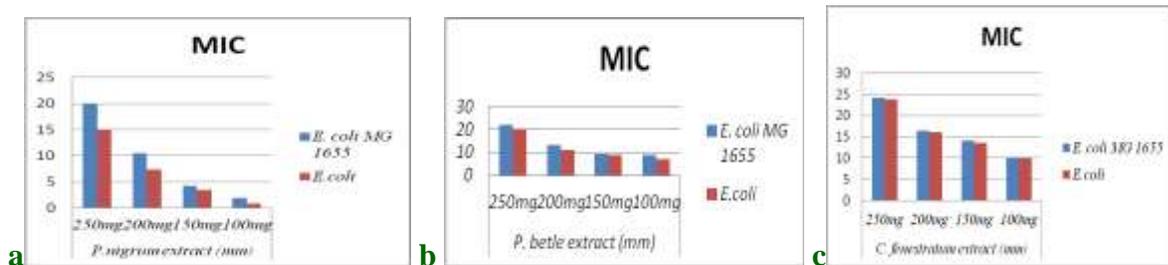


Fig 6.1 a-c: Shows the MIC of *P. nigrum*, *P. betle* and *C. fenestratum* extracts against *E. coli* respectively

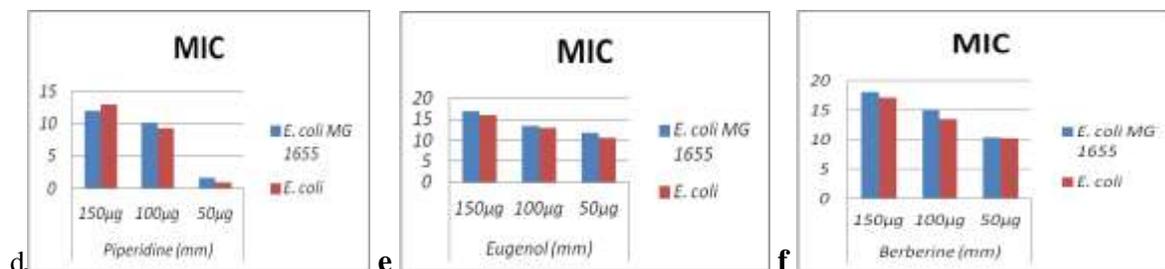


Fig 6.2 d-f: Shows the MIC of Piperidine, Eugenol and Berberine extracts against *E. coli* respectively.

### Percentage Reduction of EPS By Herbal Extracts

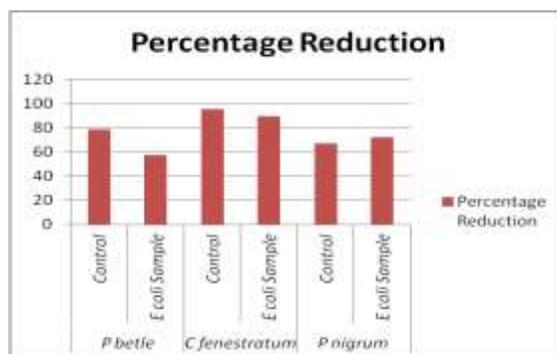


Fig.7 Shows percentage reduction of EPS by Herbal extracts

The social behavior of microbe at genetic level is studied. These quorum sensing inhibitors breakdown cell-cell communication and bacterial cells remain in planktonic stage, retaining their susceptibility to antimicrobials. The more potent approach with the help of plant extracts and secondary metabolites acting as QS inhibitors is surfacing as an anti-biofilm forming remedy. Extensive investigation into individual molecules, mechanisms and genes at the grass-root level is the future scope of this area of research. Current study was carried out with an objective of isolating and identifying multidrug resistant bacteria from clinical samples and finding the effectiveness of ethnobotanical extracts. The study could isolate 6 strains of MDRs' and narrowed it down to *E. coli* from clinical samples collected from hospitals. The conditions for biofilm formation for the bacteria were evaluated and optimised under in vitro

conditions. Biofilm formation was confirmed qualitatively using crystal violet staining assay on test tubes and titre plates and quantitatively by EPS and rhamnolipid analysis.

The next part of the study was focused on evaluating the effectiveness of quorum quenching of the selected bacteria along with the selected plant extracts. *In vitro* studies on quorum quenching was carried out by crystal violet staining assay on test tubes and titre plates, and EPS estimation of bacteria treated with individual extracts from selected plants (*Piper nigrum*, *Piper betel*, *Coscinium fenestratum*) along with controls. These sets of experiments have shown the extract of *Coscinium fenestratum* as the most effective agent for quorum quenching. The results have indicated that, berberine has a potential to inhibit the clinical strains by inhibiting vital proteins involved in QS and inturn biofilm production. Similarly Eugenol also has a

potential to inhibit the clinical strains by binding to it strongly as indicated by the study. Piperidine however wasn't as effective against *E. coli*. As this compound acts directly against the QS pathway, the virulence factor, viability and developing resistance against this herbal prodrug would be challenging for these pathogens. The utilization of quorum quenching as a promising strategy of anti-virulence therapy is demonstrated. The bioactive ingredient provides an opportunity for the development of next generation clinical therapeutics to more effectively treat refractory and deleterious bacterial-human infections. Bacterial ability to evolve resistance against multiple drugs shall be minimized as the pathogen is not killed and is allowed to dwell in planktonic state. The need for the bacteria to progress into a resistant microbe is thus nullified. Extended study on functional consequences of biofilm using *in vivo* models and role of mixed species system shall be explored.

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