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Myristic acid methyl ester: A potential quorum quencher from *Melia dubia* against uropathogenic *E. coli*

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ABSTRACT

SdiA, transcriptional quorum sensing (QS) regulator controls the behavioral changes of uropathogenic *E. coli* in establishing biofilm and virulence. SdiA selective inhibitions characterized from the leaf ethanolic extract of *Melia dubia* showed > 50% inhibition of UPEC on biofilm in a time dependent manner without affecting cell growth. The compound C39 (Myristic Acid Methyl Ester, obtained from GC-MS) was found to have G-Score of 9.6 as it interacts with TYR67, SER138, SER 47 and TRP 71 residues via hydrogen bonds. The *in vitro* validation of C39 mode of action showed SdiA selective inhibition with mutants and wild types. The data was invariably higher than the existing SdiA selective inhibitor, furanone and this was confirmed by the fluorescence microscopy studies.
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KEYWORDS

Quorum sensing;
Quorum quenching;
Melia dubia;
Uropathogenic *E. coli*;
SdiA.

INTRODUCTION

Urinary tract infections (UTI), the most frequent infectious diseases encountered around the world that causes massive morbidity and mortality with an escalating threat to human community[1]. Uropathogenic *Escherichia coli* (UPEC) are the major cause of urinary tract infection and it is most frequently isolated from 50-90% UTI patients[2]. The clinical management of UTI is complicated by the increasing incidence of infection caused by *E. coli* that are resistant against commonly used antibiotics. In *E. coli*, there are two quorum sensing systems AI-2 / LsrR and HSL / SdiA and the AI-2 / LsrR has the ability to influence both gene regulation and bacterial fitness which are meant for interspecies communication[3]. SdiA is a transcriptional regulator protein (240 aminoacid) that belongs to the LuxR family of transcriptional regulators[4]. The N-terminus region of SdiA interacts with an unknown extracellular factor of *E. coli* to control the expression of virulence factors and biofilm formation[5,6]. Since *E. coli* does not produce a native AHL due to lack of *luxS* gene it is unlikely that, *E. coli* uses SdiA for detection of its own population density[7].

The rapid emergence of microbes that acquired resistant over most commonly used and even newly developed antibiotics has emphasized the demand for the development of new strategy against infectious diseases. Hence it is realized to have an attempt to attenuate bacterial pathogenesis by masking the activating domain of

its transcriptional regulator,

SdiA. Our approach is based on natural SdiA inhibitors isolated *Melia dubia* Cav., a plant from *meliacea* family used against urinary tract infections in the southern region of Tamil Nadu and also reported to have many biological activities[8-12]. The present study is highly specific and effective in negatively regulating the transcriptional regulator, SdiA to control the biofilm and virulence factors of uropathogenic *E. coli.*

MATERIALS AND METHODS

Bacteria and culture conditions

Uropathogenic *Escherichia coli* (UPEC) were isolated from the hospitalized patients with urinary tract infection from K. A. P Vishwanatham Government Medical College,

Trichy from September to December, 2009 and screened for multi drug resistance (MDR) against the antibiotics, Ampicillin, Ciprofloxacin, Levofloxin, Nitrofurantoin and Trimethoprim. The strain that showed maximum resistance (UPEC/QSPL/S4) was used as test strain. The test strain was cultured in LB (Luria Bertani) broth at 37°C for 24 hours and used throughout the study. The AsdiA and sdiA+ strains were employed to assess the efficacy of the drug and to find out the mode of action.

Plant material and extraction

Fresh leaves of *Melia dubia* were collected from the nearby town Kumbakonam, Tamil Nadu, India from August to November, 2009. The plant materials were identified and authenticated by Dr. M. Jegadeesan and the voucher herbarium specimens (TUH 285) were deposited in the Department of Environment and Herbal Science, Tamil University, Thanjavur, Tamil Nadu, India. The powdered leaf material was used for further extraction by cold percolation method[8, 13]. The leaf powder was soaked in five different solvents, water, ethanol (70%), methanol (70%), petroleum ether (70%) and hexane (70%) (1:10 W/V) at room temperature (25 ± 1 °C) to obtain the extract. The samples were subjected to frequent agitation and after 72 hours the supernatants were filtered through muslin cloth. Filtrates were dried, lyophilized and stored in amber-colored bottles in freezer (-80 °C) for further analysis.

In vitro *assays*

The LB media was supplemented with five different concentrations (10, 20, 30, 40, 50 mg/ml) of various extracts of *Melia dubia* leaf as test and with antibiotics Ciprofloxacin (2mg/ml), Trimethoprim (2mg/ml) were considered as reference to differentiates quorum quenching activity from antibiotic activity. LB without any supplementation was considered as control. C_8HSL were added (10µg/ml) for the entire assays unless mentioned. Extracts were reconstituted in phosphate buffer to evaluate the efficacy in different time intervals 12, 24, 48, 72 hours. The following assays were performed, cell density[14], swarming motility[15], protein[16], protease[17], hemolysis[18], hemagglutination[19], hydrophobicity[20], biofilm inhibition[21]. Studies have also been done for cell wet weight, cell dry weight and pH. All the tests were carried out in triplicates for the purpose of statistical analysis.

GC-MS analysis

GC-MS analysis was carried out to find out various active principle(s) present in the *M. dubia* leaf ethanolic extract. GC-MS analysis was carried out using PerkinElmer Clarus 500 with mass spectroscopy detector. The samples were dissolved in ethanol and one microliter of the sample was injected into the system. The identification of the compounds was done based on the comparison of their mass spectrum with NIST (National Institute of Standard and Technology) mass spectral library.

In silico *studies*

Homology modeling of uropathogenic E. coli SdiA

The amino acid sequence of UPEC SdiA (Swisprot accession number: Q8FGM5) and the NMR solution structure coordinates of *E. coli* SdiA (PDB Code: 2AVX) were loaded into the Modeller 9v8. The primary sequence of *E. coli* SdiA and UPEC SdiA were aligned carefully further checked to avoid deletions or insertions in the conserved regions. A series of the UPEC SdiA model (100 models) was constructed independently.

Evaluation of the stereochemical qualities of UPEC SdiA

The stereochemical qualities of the UPEC SdiA were

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accessed by Ramachandran plot. To access the quality of the model further, the Z-score was calculated using PROSA web server in order to check the overall model quality and to measure the deviation of the total energy of the structure with respect to an energy distribution derived from random confirmations.

Ligand preparation

The 53 compounds reported by the GC-MS were drawn using the SymxDraw. The Ligands files were prepared for docking using Schrodinger Ligprep software. In addition to the generation of energy minimized 3D structure, Schrodinger Ligprep was also used for adding hydrogens. For the computational studies, Ligprep was used to obtain low energy 3D structure for the set of ligands. OPLS_2005 force field was utilized to optimize the geometry and minimize the energy.

Docking studies

All the docking experiments were performed using the program GLIDE (Grid Based Ligand Docking with Energetics) module from Schrodinger suite. Coordinate of the modeled UPEC SdiA structure was prepared for Glide calculations by running the protein preparation wizard. Energy Minimization were run until the average root mean square deviation (RMSD) of the non hydrogrn atom reached 0.290 Â. Glide uses two boxes that share a common centre to organize its calculations: A larger enclosing box and a smaller binding box. The grids themselves are calculated within the space defined by the enclosing box. The binding box defines the space through which the centre of the defined ligand will be allowed to move during docking calculations. It provides a measure of the effective size of the search space. The only obligation on the enclosing box is that it should be large enough to contain all ligand atoms, even when the ligand centre is place at the edge or vertex of the binding box. Grid files were generated using the C_8HSL to the centre of the two boxes. The size of the binding box was set at 20 Â in order to explore a large region of the protein. The compounds were subjected to flexible docking using the pre computed grid files. For each compound the 100 top score poses were saved and only the best scoring pose was analyzed.

MBEC Determination and adherence assay

The MBEC (Minimum Biofilm Eradication Concentration) of the compound, QSI-MD^{MAME} was determined as described by Subhankari Prasad Chakraborty *et al.*, 2012[22]. For the adherence assay the test strains (AsdiA, sdiA+, UPEC, MTCC 729) individually cultured in the LB medium were supplemented with QSI-MD^{MAME} in three different doses (Low 5 µg /ml, Medium 10µg/ml, high 15µg/ml) in relation with the calculated MBEC concentration[21]. The dose response effect of QSI- MD was assessed in triplicates as compared with the negative (C8HSL) and positive control (indole, furanone).

Fluorescence microscopy

The sample preparation for fluorescence microscopic studies was similar to *in vitro* invasion assay as described previously Krut *et al.*, 2003[23]. Bacterial pellets of 10 CFU/ml were re-suspended in carbonate buffer (pH 9.0) containing 100µg of fluorescein isothiocyanate (FITC) / ml for 1 hour at room temperature. Again, washed twice with PBSE and further resuspended in PBS. The suspension was overlaid with a No.1 cover slip embedded on a 12 well microtitre plates and the reactions were preincubated for 1 hour at 37°C. Prior to the visualization in an Olympus BX-60 upright fluorescent microscope (100 x oil immersion phase-contrast lens; total magnification, x 1000) the cells were washed with PBS.

Cytotoxicity assay

The kidney carcinoma cell line (A498) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 370 C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

The potential influence of QSI-MD on cell viability was by using the MTT assay[24]. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is a yellow water soluble tetrazolium salt Succinate-dehydrogenase, a mitochondrial enzyme in living cells, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Once the cell density reached $1x10^7$ cells/ml, 100µl per well of cell suspension were seeded into 96- well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at

37ºC, 5% CO_2 and 100% relative humidity. After 24 hours the cells were treated with different concentrations (5, 10, 15, 100 µg/ml respectively designated as Low dose, medium dose, high dose and very high dose) of QSI-MD^{MAME}. The plates were incubated for an additional 24 hour at 37º C, 5% $C0_2$, 95% air and 100% relative humidity. 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 3 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The medium containing without QSI-MD^{MAME} served as control and the cell viability was estimated against control. All assays were performed in triplicate and mean ± SD values were used to estimate cell viability.

Statistical analysis

The experimental results were given as mean ± SE. Differences were considered statistically significant as the value of probability less than 5% ($P < 0.05$)[25].

RESULTS AND DISCUSSION

In this study we have analyzed the quorum sensing interfering efficiency of *M.dubia* various parameters like cell density, swarming motility, protein, protease, hemolysis, hemagglutination, hydrophobicity and biofilm inhibition. The overall results showed that the ethanolic extract of *M.dubia* leaves has significant quorum quenching activity, hence discussed in detail. The biofilm inhibitory potential of the drug lead and the mode of action were elucidated through confocal and fluorescent microscopy studies.

In vitro assays

Biofilm represents a structured population of bacterial cells embedded in a self-produced polymeric matrix adherent to a natural or artificial surface which is protected from antimicrobial agents and host immune defense[26]. The biofilm inhibition was found to be increased in every time intervals and the best activity (67.74%) was observed at 30 mg/ml concentration (data not shown). Recently it was reported that the conjugated exocyclic vinyl bromide on the furanone ring was found to show biofilm inhibitory activity[27]. Similar results were recorded by Ren *et al.*,[28] which shows the efficiency of furanone to inhibit biofilm. The most important virulence factor of uropathogenic *E. coli* is hemolysin. It is a pore-forming toxin of the 'repeat toxin' (RTX) family with a promiscuous target cell spectrum including erythrocytes. The percentage of hemolysis inhibition was recorded in an increasing pattern with varying concentration and when it was supplemented with 30 mg/ml concentration it displayed much effective inhibition of hemolysin production (39.1± 1.66) (data not shown). Similar results were reported by Balague *et al.*,[29] when supplemented with herbicide, 2, 4-dichlorophenoxyacetic acid against the uropathogenic *E. coli* confirms our result.

The hydrophobicity of the bacterial surface is important for the adhesion of bacteria to the water-insoluble substrates. Aqueous extract showed salutary effect when supplemented with 30mg/ml concentration (35.05 ± 0.76, 35.98 ± 0.11, 42.53 ± 0.11) (Data not shown). Probably, the changes in the cell surface hydrophobicity were linked with alteration of surface properties such as capsule formation, thickness of the capsular layer, biofilm structure, outer membrane proteins (OMPs) and lipolysaccharide[30]. The protease level is high at 24th hour and in consecutive hours it is decreasing gradually (data not shown). Concentration dependent decrease was seen at 48th hour is 60.07%, 81.65%, 87.41%, 93.53% and 98.90% respectively. Theodar *et al.*,[31] reported that aqueous extract of garlic and onion inhibited the protease activity that coheres with our data obtained.

In addition, it was recorded that hemagglutination and swarming motility (42.50 % at 12th hour) was reduced when the media was supplemented with ethanolic extracts (data not shown). The cell density, cell wet weight, dry weight and pH were not much altered in the growth media supplemented with *M. dubia* leaf ethanolic extract but if the media is supplemented with antibiotics, the above said parameters were found to be decreased significantly (data not shown). These results suggest that the *M.dubia* leaf ethanolic extracts were not antibacterial rather it showed quorum quenching activity which cohere our earlier report[8].

GC-MS Analysis

The results of GC-MS analysis of *M. dubia* leaf

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ethanolic extract showed its wealth in secondary metabolites. The mass spectrum of each compound was compared to that in the NIST Library. Totally 53 compounds were identified and most of them were found to be oxalic acid derivatives. The GC-MS results showed the active principle richness of *M. dubia* leaf (TABLE 1).

TABLE 1 : List of ligands identified from *M. Dubia* leaves using GC-MS analysis

S.No	Compound Name	Retention
1	lH-Pyrrole, 1-methyl-	3.46
2	1-Butanol, 3-methyl-	3.56
3	Ethanol, 2-butoxy-	4.23
4	Diacetamide	4.38
5	2-Vinylethyl acetate	4.51
6	Furfural	5.00
7	lH-Pyrazole, 3,5-dimethyl-	5.10
8	Sulfoxide, methyl phenethyl	5.95
9	Butanedioic acid, phenyl-	6.05
10	2-Furancarboxaldehyde, 5-methyl	7.20
11	Octanoic Acid	7.47
12	1-Hexanol, 2-ethyl-	7.60
13	2-Hexanoic acid	8.07
14	Hexane, 1-chloro-	8.61
15	Nonanal	9.10
16	3 -Acetylthymine	9.19
17	2-Propanamine, N-methyl-N-nitro-	10.10
18	4H-Pyran-4-one,2,3-dihydro-3,5-	10.26
19	Oxalic acid, isobutyl 2-phenylethyl	10.72
20	Oxalic acid, allyl nonyl ester	10.87
21	Nonanamide, N-(1-b enzyl -2-	11.38
22	2-Furancarboxaldehyde, 5-	11.49
23	Oxalic acid, isobutyl nonyl ester	12.45
24	1-Penatanol, 2-ethyl-4-methyl-	14.12
25	Hexadecane	14.24
26	1,6,10-Dodecatriene, 7,11-	14.98
27	2-Formyl-9-[a-d-	15.19
28	Oxirane, (3,3-dimethylbutyl)-	15.90
29	Hexanoic acid	16.20
30	Nonanoic acid	16.98
31	Oxalic acid, allyl octyl ester	17.40
32	1 -Iodo-2-methylundecane	17.49
33	Benzenamine, N-phenyl -	18.22
34	Isosorbide Dinitrate	18.48
35	Undecanoic acid	19.92
36	1-Octadecyne	20.89
37	Phthalic acid, isobutyl octyl ester	21.33
38	9,9-Dimethoxybicyclo[3.3.1]nona-	22.58
39	Tetradecanoic acid, ethyl ester	22.91
40	2(3H)-Naphthalenone, 3-	23.04
41	2,6-Octadiene, 4,5-dimethyl-	23.21
42	3,7,11,15-Tetramethyl-2-hexadecen-	24.37
43	Sulfurous acid, 2-propyl tetradecyl	26.58
44	Hexanedioic acid, bis(2-ethylhexyl)	27.51
45	1 -Iodo-2-methylundecene	27.56
46	Eicosane	28.41
47	1-2-Benzenedicarboxylic acid,	28.77
48	Pentadecane, 8-heptyl-	29.17
49	Heptacosane	29.95
50	Eicosane, 2-methyl-	30.82
51	Squalene	31.05
52	Eicosane	31.81
53	Sulfurous acid, butyl hexadecyl	32.97

Homology modelling and docking analysis homology modelling

The model with the best PDF Total energy, PDF Physical energy and DOPE function was selected and chosen for the further stereochemical quality checks and docking studies (Figure 1). Analysis of Ramachandran plot revealed that 90.7 % of the residues were in the

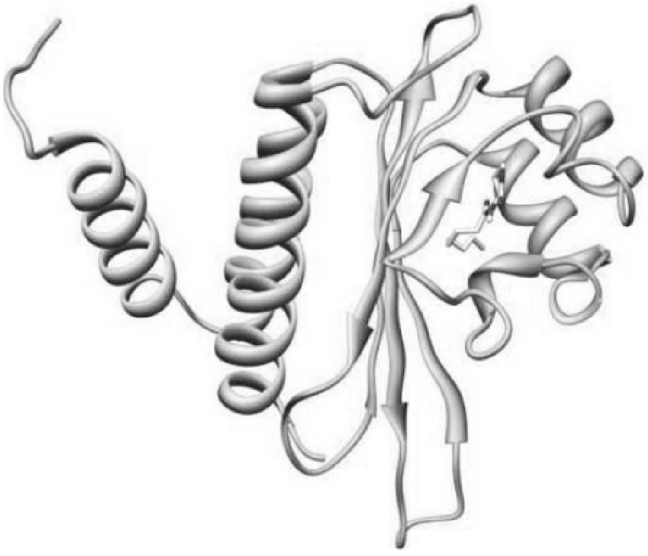

Figure 1 : A ribbon diagram showing the overall structure of the UPEC sdiA. The bound C_8HSL molecular at active site is shown as stick.

favoured region, 7.3 % in allowed region and only 1.9 % were in the disfavored region (Figure 2). The residues in the disallowed regions are located far away from the residue in the ligand binding site (LBS). These results indicate that the Phi and Psi backbone dihedral angles in the UPEC model are reasonably accurate. (Figure 3) shows the location of the Z- score for UPEC SdiA. The value -6.12 is in the range of native conformation. Hence the model was chosen for the further studies.

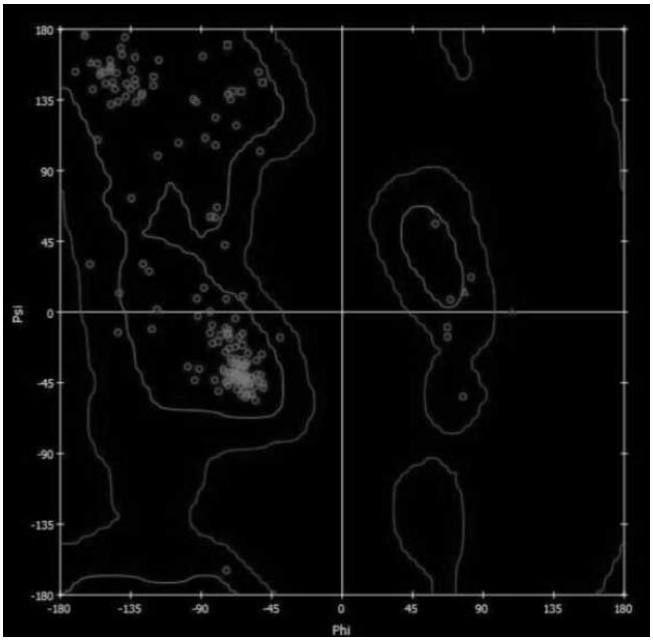

Figure 2 : The Ramachandran plot of the final model obtained by PROCKECK.

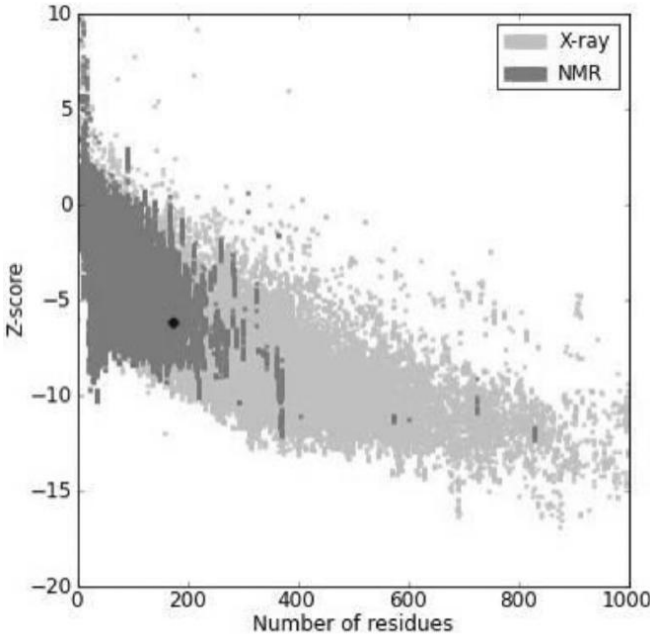

Figure 3 : Z-Plot of final model generated by ProSA-Web server

Identification and analysis of potential compounds

As control study, the C_8HSLwas docked to the protein, and this exercise which resulted in reproducing the NMR solution structure pose of the compound that yields -9.4 as the G score with 0.029A RMSD. G Score is nothing but the total GLIDE score: Sum of XP terms (Lipophilic EvdW, PhobEn, PhobEnHB, PhobEL, PairHB, HBond, Eleactro, SiteMap, Phi Stack, Cat, CLBR, LowM, Penalties, HBPenal, PhobicPenal, and RoatPNAL). The higher the contribution of XP term more will be the total GLIDE score. The score computed for this reference compound was used as reference value for identifying the possible leads.

All those compounds that exhibited weaker binding in comparison with the reference compound were shortlisted for further analysis. From the docking studies, it was observed that compound 39 (QSI-MD^{MAME})

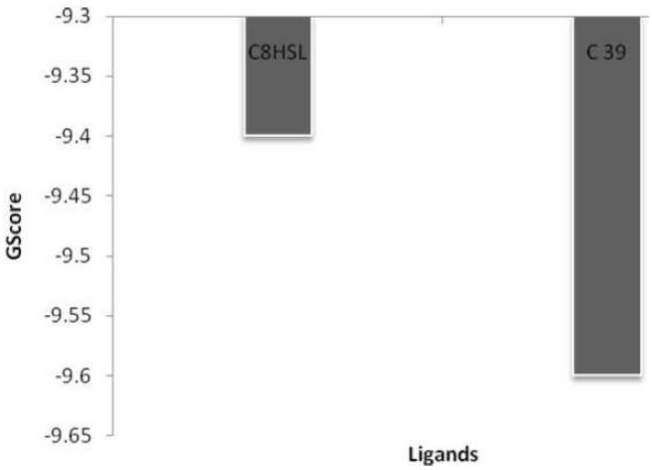

Figure 4a : The predicted GScore of C8HSL and QSI-MD^{MAME}. The QSI-MD^{MAME} shows the maximum binding ability to SdiA that the native ligand C_8HSL.

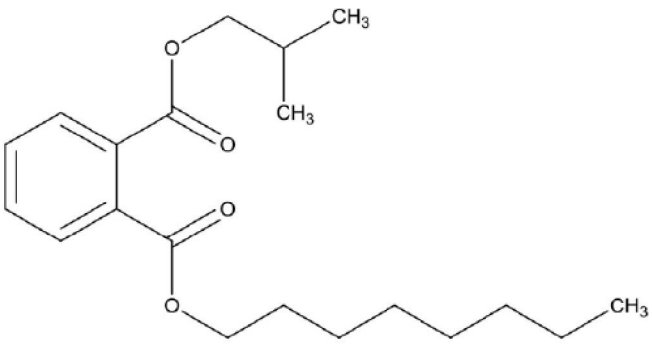

Figure 4b : Structure of Myristic Acid Methyl Ester

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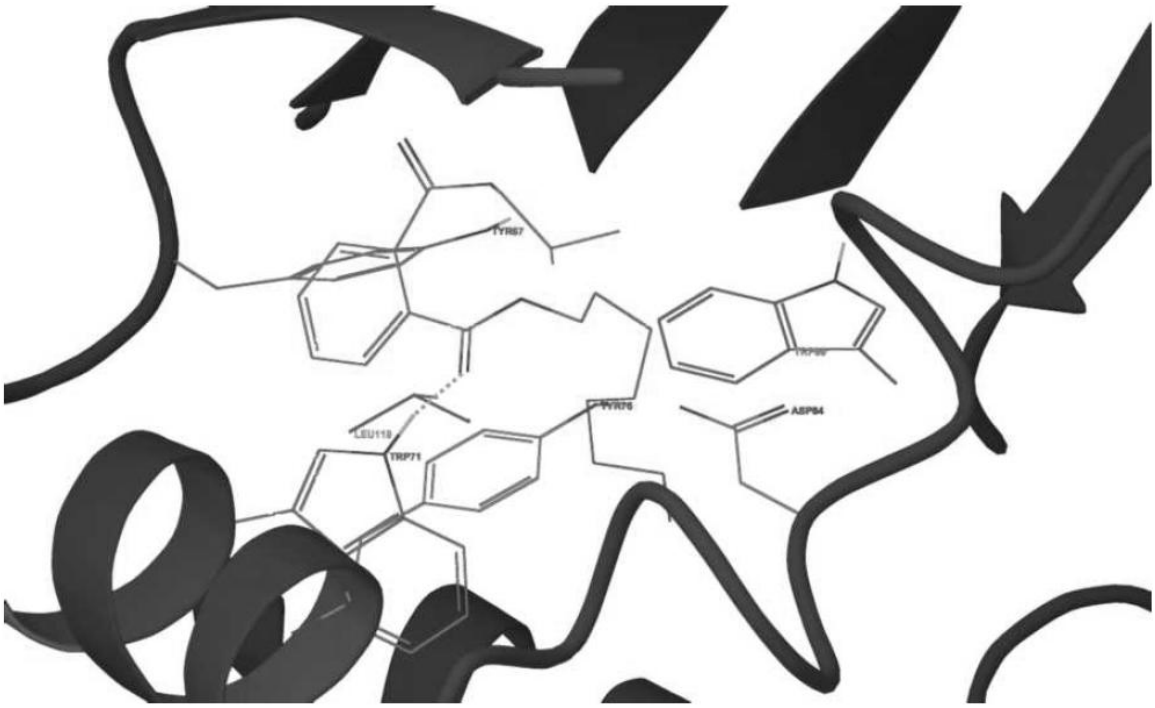

Figure 5 : Docking model of UPEC sdiA-QSI-MD^{MAME}. The hydrogen bond interactions with the key residues are show as dotted lines.

is having better GScore of 9.6 than the native ligand C_8HSL (Figure 4a and b). QSI-MD^{MAME} forms one strong hydrogen bond interactions with the amino acid TRP71 (Figure 5). From the previous experiment, it was proved that TRP67 and TYR 71 are highly conserved and the key residue for LuxR type proteins and SER 43 is a homologus residue of SdiA family. Since QSI-MD^{MAME} was able to make strong hydrogen bond interactions with those key residues, this compound could be a possible reason for the quorum quenching activity. Hence this compound can be further evaluated for their individual activities in *in vitro* and *in vivo* as well.

MBEC Determination and in vitro biofilm adherence assay

The MBEC of QSI-MD^{MAME} was found to be 10µg/ml and that taken into further studies. Biofilms are attached of microorganisms to a surface of polysaccharides, proteins, and nucleic acids to form a community. The intracellular biofilms are responsible for a dormant reservoir of pathogens inside the bladder cells, which outlast the strong host immune response. So, time dependent response of the lead compound C39, QSI-MD^{MAME} was studied to elicit its mode of SdiA selective biofilm inhibition on polystyrene plates at 12, 18 and 24th hours. The data showed consistent effect on sdiA null strain with no response over the behavioral change in adhering the plastics irrespective of treating with or without C39 as compared with the wild type strain (Figure 6).

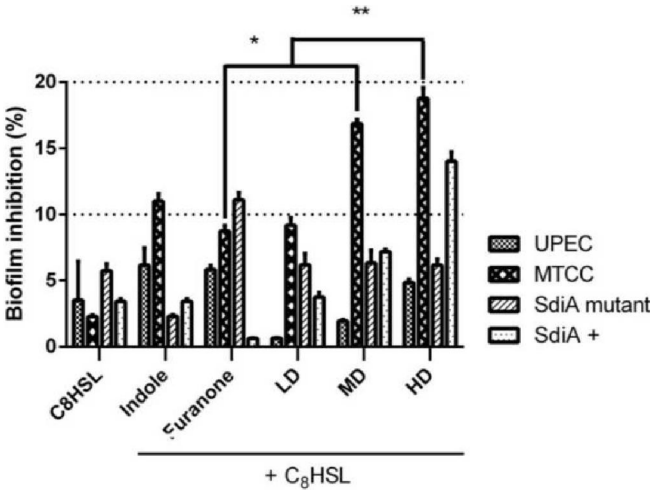

Figure 6 : The interference of MAME towards the biofilm formation on various *E. coli* strains at 24th hour. The biofilm inhibition was found high when MAME administered and a dose dependent increase was also found. There is no significant difference in the biofilm inhibition was found and this confirms that the MAME is functioning through SdiA. Bars show the mean value of the experiments (n = 3) and Error bars show standard deviation, * = p <0.0001.

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The adhesion pattern of the strains was reported as discussed above are in accordance with the earlier report by Stepanovic *et al.*,[32]. Based on the Optical density (OD) measured against bacterial films, strains were classified into the following categories: no biofilm producers, weak, moderate or strong biofilm producers, as previously described[32]. Briefly, the cut-off OD (OD_c) was defined as three standard deviations above the mean OD of the negative control. Strains were classified as follows: O.D < OD_c = no biofilm producer, OD_c < OD < (2 x OD_c) = weak biofilm producer, (2 x OD_c) < OD= (4 x OD_c) = moderate biofilm producer and (4 x OD_c) < OD = strong biofilm producer. It is found that all the tested strains except sdiA null mutant were under no biofilm producing category (data not shown) when administered with QSI-MD^{MAME}.

The overall result shows that the lead does not affect the AsdiA strains as it is acting through as sdiA. It is been reported by Ren *et al.*,[28] that the quorum-sensing disrupter (5Z)-4-bromo-5-(bromomethylene)-3-

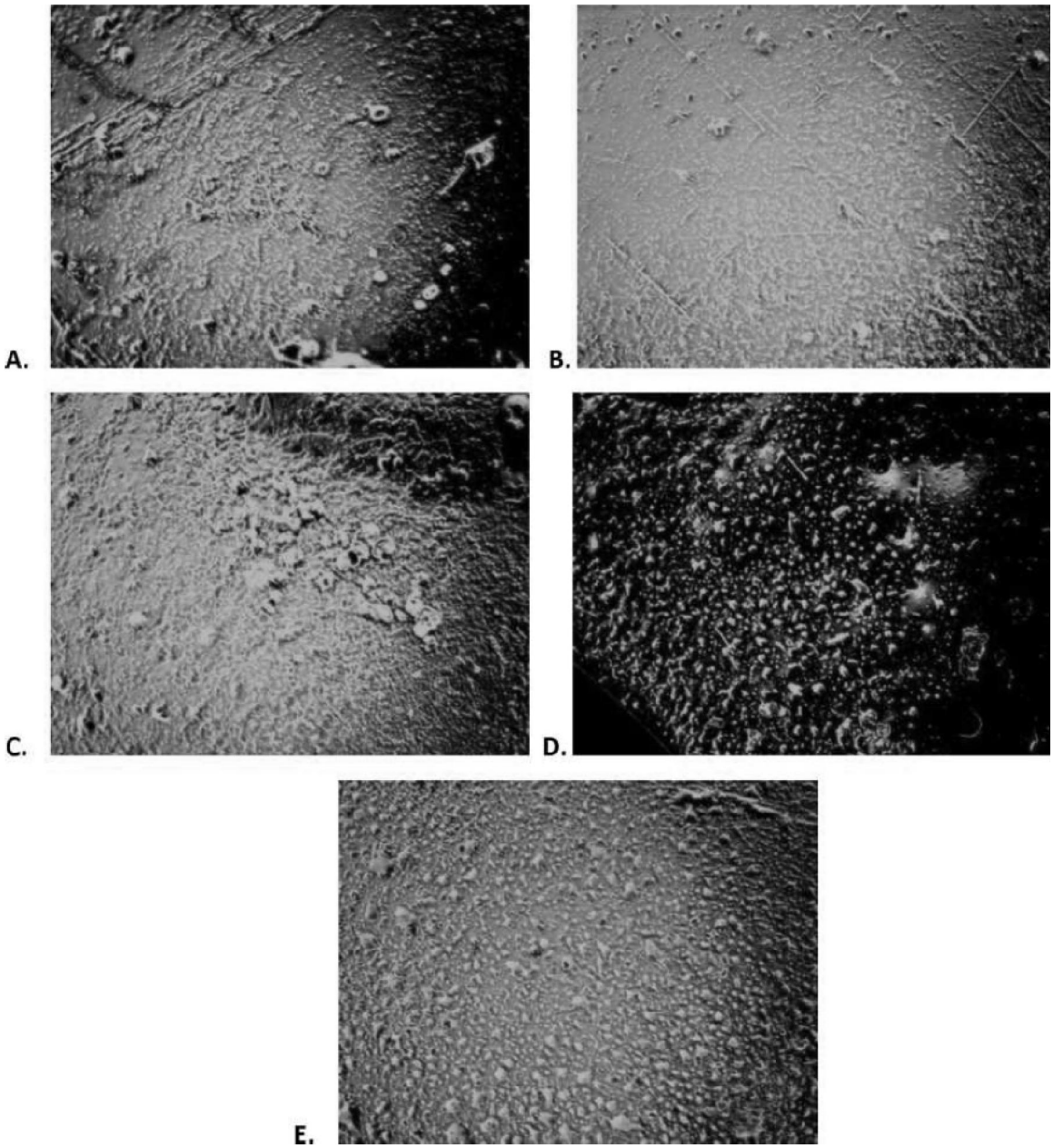

Figure 7 : The FITC labelled *E. coli* cells under fluorescent microscopy. A) Treated with C_8HSL, showed high biofilm colonies B) Treated with indole showed moderate decrease in biofilm C) Treated with Furanone showed moderate decrease in biofilm D) Treated with drug lead showed efficient biofilm inhibition as distracted individual colonies. E) Untreated.

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butyl-2(5H)-furanone (furanone) of the alga *Delisea pulchra* inhibited the biofim and swarming of *Escherichia coli.* It has been recently reported that natural products-inspired organosulfur compounds inhibits biofilm by inhibiting quorum sensing[33]. The drug lead QSI-MD^{MAME} showed a better efficacy profile than the known inhibitors like indole and furanone as well.

Fluorescence microscopic studies

The fluorescent microscopic studies revealed that the QSI-MD^{MAME} has a potentiality to curb UPEC/S4 biofilm formation through quorum sensing inhibition. The cells were highly visible when supplemented with C_8HSL whereas indole and furanone showed was moderately inhibited the biofilm and the lead showed better efficiency (Figure 7). These results were confirmed with the earlier studies of Shahrooei *et al.,* [34] against *S.aureus.*

From the studies, it is very clear that the biofilm formation was inhibited by quorum sensing inhibition through SdiA and the data obtained from this study has good correlation with biofilm inhibition studies.

Cytotoxicity

QSI-MD^{MAME} was tested for its toxic effects on human kidney carcinoma cell line (A498). It is found that it is not inhibited the cell growth at all the tested concentrations significantly. Even in very high dose of QSI-MD^{MAME} (100µg/ml), there is no significant change in the cell viability (Figure 8). This confirms that the compound QSI-MD^{MAME} is efficient drug against UPEC quorum sensing with no toxic effects.

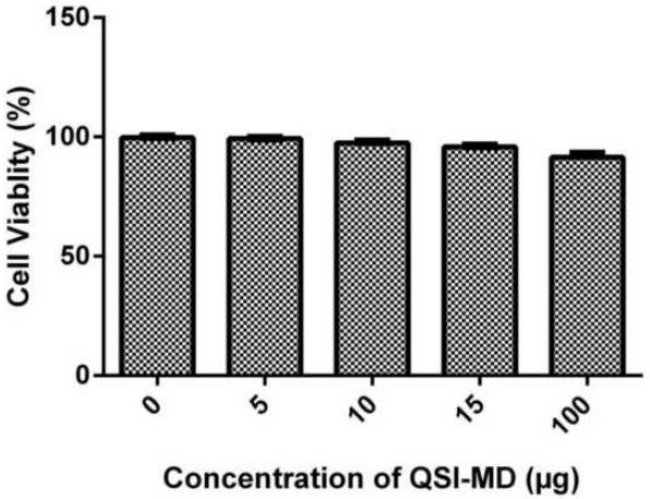

Figure 8 : Percentage of viable cells after 48 hours pretreatment of Kidney carcinoma cells (A498), against QSI-MD^{MAME} evaluated by MTT assay. Bars show the mean value of the experiments (n = 3) and Error bars show standard deviation.

CONCLUSION

The results suggested that the active principles derived from *Melia dubia* can be used as quorum quenching agents that would strongly inhibit the virulence character expression as well as biofilm formation. Based on the docking results it is exceedingly clear that the ligands from *M. dubia* can be used as a lead compound to develop an effective drug for urinary tract infections caused by uropathogenic *E. coli.* It is clear from the biofilm and fluorescent microscopic studies that the QSI-MD^{MAME} (Myristic Acid Methyl Ester) is having a potential biofilm inhibitory potentiality.

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REFERENCES

[1] M.Abhilash; Int.J.Pharma.Bio.Sci., **1**, 1-7 **(2010)**.
[2] M.Starcic Erjavec, M.Rijavec, V.Krizan-Hergouth, A.Fruth, D.Zgur-Bertok; Int.J.Antimicrob.Agents, **30,** 436-442 **(2007)**.
[3] T.Xue, L.Zhao, H.Sun, X.Zhou, B.Sun; Cell Res., **19**, 1258-1268 **(2009)**.
[4] J.Lee, T.Maeda, S.H.Hong, T.K.Wood; Appl.Environ.Microbiol., **75**, 1703-1716 **(2009)**.
[5] M.Aldea, T.Garrido, J.Pla, M.Vicente; EMBO J., **9**, 3787-3794 **(1990)**.
[6] X.D.Wang, P.A.J.de Boer, L.I.Rothfield; EMBO J., **10**, 3363-3372 **(1991)**.
[7] V.Sperandio, A.G.Torres, J.A.Giron, J.B.Kaper; J.Bacteriol., **183**, 5187-5197 **(2001)**.
[8] V.Ravichandiran, K.Shanmugam, K.Anupama, S.Thomas, A.Princy; Eur.J.Med.Chem., **48**, 200-205 **(2012)**.

[9] S.Malarvannan, R.Giridharan, S.Sekar, V.R.Prabavathy, S.Nair; J.Biopestic., **2**, 64-71 **(2009)**.
[10] M.A.H.Nagalakshmi, D.Thangadurai, T.Pullaiah; Phytother.Res., **17**, 414-416 **(2003)**.
[11] T.Susheela, P.Balaravi, J.Theophius, R.Narendar Reddy, P.U.M.Reddy; Curr.Sci., **94**, 9 **(2008)**.
[12] N.Rama Rao, L.N.Henry; The Ethnobotany of Eastern Ghats of Andhra Pradesh, India, Botanical Survey of India, Calcutta, India **(1996)**.
[13] V.Gupta, S.Saggu, R.K.Tulsawani, R.C.Sawhney, R.Kumar; Food Chem.Toxicol., **46**, 1645-1652 **(2008)**.
[14] D.M.Carlberg; Determining the effects of antibiotics on bacterial growth by optical and electrical methods, In: Antibiotics in Laboratory Medicine, Williams & Wilkins, Baltimore, 64-92 **(1986)**.
[15] J.Lee, T.Bansal, A.Jayaraman, W.E.Bentley, T.K.Wood; Appl.Environ.Microbiol., **73**, 4100-4109 **(2007)**.
[16] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; J.Biol.Chem., **193**, 265 **(1951)**.
[17] M.Kunitz; J.Gen.Physiol., **30**, 291--310 **(1947)**.
[18] J.F.Van den Bosch, P.Postma P, J.De Graaff, D.M.MacLaren; FEMS Microbiol.Lett., **8**, 75-77 **(2006)**.
[19] D.G.Evans, D.J.Evans, W.Joa; Infect.Immun., **18**, 330-337 **(1977)**.
[20] J.S.Chapman, N.H.Georgopapadakou; Antimicrob.Agents Chemother., **32**, 438-442 **(1988)**.
[21] O.M.Vandeputte, M.Kiendrebeogo, S.Rajaonson, B.Diallo, A.Mol, M.El Jaziri, M.Baucher; Appl.Environ.Microbiol., **76**, 243-253 **(2010)**.
[22] S.P.Chakraborty, S.K.Sahu, P.Pramanik, S.Roy; Asian Pac.J.Trop.Biomed., **2**, 215-219 **(2012)**.
[23] O.Krut, O.Utermohlen, X.Schlossherr, M.Kronke; Infect.Immun., **71**, 2716-2723 **(2003)**.
[24] T.Mosmann; J.Immunol.Methods, **65**, 55-63 **(1983)**.
[25] J.H.Zar, 'Biostatistical analysis', 4th Edition, Prentice Hall International Inc.Press, London **(1998)**.
[26] A.Marchese, M.Bozzolasco, L.Gualco, E.A.Debbia, G.C.Schito, A.M.Schito; Int.J.Antimicrob.Agents, **2**, 95-100 **(2003)**.
[27] Y.Han, S.Hou, K.A.Simon, D.Ren, Y.Y.Luk; Bioorg.Med.Chem.Lett., **18**, 1006-1010 **(2008)**.
[28] D.Ren, J.J.Sims, T.K.Wood; Environ.Microbiol., **3**, 731-736 **(2001)**.
[29] C.E.Balague, C.S.de Ruiz, R.Rey, A.M.Evangelista de Duffard, M.E.Nader- Macias; Toxicology, **177**, 143-155 **(2002)**.
[30] W.Dorota, J.Stanislaw; Int.J.Antimicrob.Agents, **29**, 700-704 **(2007)**.
[31] E.Teodor, W.Buzgariu, A.Ruginsa, M.Diaconu, G.L.Radu; Innov.Chem.Biol., **41**, 353-360 **(2009)**.
[32] S.Stepanovic, I.Cirkovic, L.Ranin, M.Svabic-Vlahovic; Lett.App.Microbiol., **38**, 428-432 **(2004)**.
[33] N.C.Cady, K.A.McKean, J.Behnke, R.Kubec, A.P.Mosier, S.H.Kasper, D.S.Burz, R.A.Musah; PLoS One; 7, e38492 **(2012)**.
[34] M.Shahrooei, V.Hira, B.Stijlemans, R.Merckx, P.W.Hermans, J.Van Eldere; Infect.Immun., **77**, 3670-3678 **(2009)**.