Research Article

Exploration of Antimicrobial Potency and Mode of Action of Lintetralin and Mupirocin Derived from *Phyllanthus niruri* against *Enterococcus faecalis*

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ABSTRACT

Multidrug resistance dupped as 'silent pandemic' poses critical threat to public healthcare by increasing mortality rate and rendering antibiotic ineffective that necessitates efficacious development of effective antimicrobial agents. Use of medicinal plants as hidden resources of robust antimicrobial compounds is propelled to counteract antimicrobial resistance exhibited by bacterial pathogens to current antibiotics. The growing concern about the rapid emergence of antibiotic resistance in bacterial pathogens substantiates the modification in existing antibiotics and parallel development of novel antibiotics with higher therapeutic efficacies. Besides, the screening of bioactive phytochemicals in the leaf extract of *Phyllanthus niruri* have attracted considerable attention due to structural diversity, chemical stability and novelty to explore their therapeutic potentials as the alternatives to antibiotics with higher therapeutic efficacy to counter mechanism of antimicrobial resistance in MDR bacterial pathogens. The study elucidated the binding affinity of bioactive phytochemicals (Lintetralin and Mupirocin) from methanolic leaf extract of *Phyllathus niruri* with targeted genes identified in *Enterococcus faecalis* was predicted through *in silico* molecular docking analysis. The transcriptomics study revealed that lintetralin showed lowest minimum docking score of -5.347 Kcal/mol with esp protein (biofilm regulatory surface protein), -5.023 Kcal/mol with gelE protein (gelatinase) and -5.647 Kcal/mol with prgB protein (fibrinogen binding) exhibited by the down-regulated genes of *Enterococcus faecalis*. Besides, mupirocin also showed lowest minimum docking score of -9.209 Kcal/mol with esp (biofilm regulatory protein), -9.499 Kcal/mol with gelE (metalloprotease, hydrolase activity) and -8.382 Kcal/mol with prgB (biofilm regulatory pilus protein) of *Enterococcus faecalis.* The study clearly indicated that the phytochemicals (Lintetralin and Mupirocin) isolated from methanolic leaf extract of *Phyllanthus niruri* can be used as the potent lead molecule to be exploited towards the development of novel antimicrobials to combat against antibiotic resistance exhibited by MDR bacterial pathogens.

Keywords: Biofilm, *Enterococcus faecalis*, esp, gelE, lintetralin, mupirocin, prgB, Phyllathus.

INTRODUCTION

Multidrug resistance acquired by bacterial pathogens duped as 'silent pandemic' that pose critical global healthcare threat by increasing the mortality rate and rendering antibiotic ineffective thereby necessitates immediate and efficacious development of potent antimicrobial agents[1]. Utilizing medicinal plants as hidden resources of robust antimicrobial compounds is propelled to counteract the mechanism of antimicrobial resistance exhibited by bacterial pathogens against conventional antibiotics[2]. Plant extracts and phytochemicals derived from *Phyllanthus niruri* have been tested against MDR bacterial pathogens[3], which supplements the development of innovative strategies against the microbial infections that resist conventional antibiotics. The antimicrobial attributes of plant extracts and derivatives have proven beneficial in the exploration of potential therapies. However, the mechanism of action exhibited by bioactive compounds (Lintetralin and Mupirocin) derived from *Phyllanthus niruri* against *Enterococcus faecalis* at the molecular level remain unclear.

Moreover, the high-throughput transcriptome profiling has immense potential, which pave the way of greater understanding the phenomenon of antibiotic resistance in pathogenic MDR bacterial strains at molecular and functional levels[4]. RNA-Seq involves the sequencing of millions of paired or unpaired short-reads, making its highly effective for the detection of the gene expression patterns even in low abundance transcripts, which is crucial for characterization of MDR pathogens[5]. Researchers have revealed the significance of transcriptome analysis not only to identify the resistance genes but also elucidate the expression profiles in MDR pathogens including *Enterococcus faecalis*[6]. *Enterococcus faecalis* is a zoonotic enteric pathogen found in various regions especially hospital settings, which is the major causative agents for urinary tract infections, bacteremia, endocarditis and wound infections[7]. *Enterococcus faecalis* is mostly involved in nosocomial and community acquired enterococcal infections[8]. The genomic analysis revealed 34 antibiotics resistance genes in *Enterococcus faecalis*[9] with multitude mechanisms that renders therapies worthless[10]. Further, the studies revealed that biofilm formation is significantly associated with the expression of enterococcal surface protein (*esp* gene)[11,12,13], virulence gene *gelE* encoding gelatinase[14,15,16], prgB gene[17,18,19], endocarditis and biofilm associated pili ebpA gene[20] and additional biofilm related genes like asa1, ahrC, efaA, cylB, epaL[21,22] in enhancing its persistency across the varieties of settings.

Keeping in view, the transcriptomic analysis of resistant *Enterococcus faecalis* based on RNA sequencing approach was performed to elucidate accurate gene expression and pathway prediction through the identification of potential genes involved in conferring the mechanism of resistance. Although, the application of transcriptomic based surveillance on the determinants of antibiotic resistance is technically feasible but the implementation of bioinformatics tools including molecular docking and molecular dynamics stimulation enhances the efficacy to many folds. Nevertheless, the possible binding mechanism of targeted bioactive phytochemicals (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* with targeted genes (esp gene, gelE protein and prgB gene) in *Proteus mirabilis* was predicted through molecular docking. The study provides a holistic approach to uncover novel gene(s) emphasizing the foundation for predicting mechanism of resistance, which not only substantiate the discovery of potent plant derived antimicrobial agents against bacterial infections, but also serve to combat the acquisition of antimicrobial resistance in MDR pathogens as well as their transmission.

MATERIALS AND METHODS

Whole transcriptome analysis

Whole transcriptome sequencing was performed between the treated and untreated MDR strain of *Enterococcus faecalis* with bioactive compounds (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* to understand its mode of action. Total RNA was isolated from the bacterial sample by Trizol method (Invitrogen) followed by DNAse treatment. Besides, the purity of RNA was assessed using Nanodrop and 1% agarose gel electrophoresis. Integrity of RNA was checked by Agilent Bioanalyzer, and samples with RNA integrity number (≥ 6.5) was prepared for sequencing. Purified cDNA library was prepared and evaluated by Agilent bioanalyzer and then followed by cluster generation using Illumina HiSeq 2000. Generated paired end data was submitted to NCBI Short Read Archive. Sequence reads were pre-processed by Trimmomatic and Printseq and aligned with available reference genome sequence using TopHat integrated with Bowtie software. Gene expression was analyzed using Cufflinks, in terms of 'fragments/kb of exon per million fragments mapped' (FPKM). Differential gene expression between treated and untreated *Enterococcus faecalis*, differential expression was determined using DESeq and edgeR using R Bioconductor package. To identify the novel targets, Cufflinks was used without the reference genome for constructing minimum number of transcripts. Differentially expressed genes with fold change >2 was further analyzed using Cytoscape plugins, DAVID, StringDB, and Ingenuity pathway analysis (IPA) to obtain canonical pathway, 16 GO ontologies, gene and protein networks, gene set analysis, gene clustering and target protein identification.

In silico Interactions of Lintetralin and Mupirocin with Targeted Proteins

Protein Preparation

Bioactive phytochemicals (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* were found to be involved in virulence, biofilm formation and multidrug resistance of *Enterococcus faecalis* through their interactions with different genes such as esp (PDB ID: 6ORI), gelE (PDB ID: 4GER) and prgB (PDB ID: 6GED), which were used in the study. PDB structures of these proteins were preprocessed through multistep procedures of protein preparation wizard (Schrödinger). Besides, the missing hydrogen atoms were added using protein preparation wizard. Besides, the missing side chain atoms of the amino acids were subsequently identified using Prime side-chain prediction tool and repaired using Prime (Schrödinger). Further, the structures of bioactive proteins were refined through energy minimization using Macromodel (Schrodinger) and OPLS 2005 force field. In the present study, the Polak-Ribiere Conjugate Gradient (PRCG) algorithm with energy gradient of 0.01 kcal/mol was used for energy minimization.

Preparation of Molecular Structure of Lintetralin and Mupirocin

Molecular structure of plant derived bioactive compounds (Lintetralin and Mupirocin) were built using ChemDraw (Figure 1) and imported into Maestro (Schrödinger package). Molecular structure was energy minimized using Macromodel (Schrödinger) and OPLS 2005 force field with PRCG algorithm (energy gradient of 0.001). The DFT (hybrid density functional theory) with Becke's three-parameter exchange potential and Lee-Yang-Parr correlation functional (B3LYP) with basis set 6-31G** by Jaguar (Schrödinger) for geometric optimization of structure[23]. Various conformations of plant derived bioactive compounds (Lintetralin and Mupirocin) derived from methanolic extract of *Phyllanthus niruri* were generated using Ligprep (Schrödinger).

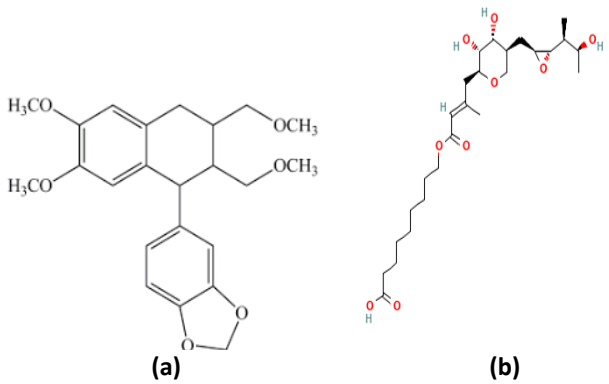


Figure 1: Molecular structure of bioactive molecules (A) Lintetralin and (B) Mupirocin derived from methanolic leaf extract of *Phyllanthus niruri*.

Molecular Docking of Lintetralin and Mupirocin

Blind docking approach was used to investigate the molecular interactions of plant derived bioactive

compounds (Lintetralin and Mupirocin) with different proteins in absence of co-crystal structures. During blind docking, all the binding sites of protein were predicted using SiteMap (Schrödinger) and the receptor grid boxes were generated for each predicted binding site. An inner grid box of size (12Å x 12Å x 12Å) was defined at the centroid of binding site using Glide grid-receptor generation program. Within search space, the diameter midpoint of each docked ligand was required to be present. Besides, the outer grid box was also defined with an edge length of 20Å. All ligand atoms of a valid pose must be located. The various conformations of lintetralin and mupirocin were docked onto each predicted binding site using Glide XP (extra precision) algorithm (Schrödinger) and their binding poses were evaluated using Glide XP_{Score} function[24,25]. Single best conformation of lintetralin and mupirocin with lowest minimum docking score was used for further analysis.

RESULTS AND DISCUSSION

Transcriptome Analysis

The data related to *Enterococcus faecalis* (G-Control_1 & G-Control_2) treated with bioactive lintetralin (G-LIN_1 & G-LIN_2) and mupirocin (G-MUP_1 & G-MUP_2) were used for quality checking and trimming for accuracy. Statistical percentage passed quality checking and statistical summary of transcriptome sequencing was depicted (ST 1). Genome of *Enterococcus faecalis* was used as reference to align reads using HISAT2 software. About 99.98% and 100% reads obtained from G-Control_1 and G-Control_2 was mapped with reference genome, whereas 100% reads obtained from G-LIN_1 and G-LIN_2 were mapped with reference genome. The study revealed that 99.98% and 100% reads obtained from G-Control_1 and G-Control_2 were mapped with reference genome, whereas 100% reads obtained from G-MUP_1 and G-MUP_2 were mapped with reference genome. The mapping results along with the statistical summary were presented (Table 1).

Table 1: Statistical summary of the mapping results of genome of *Enterococcus faecalis* (Control and treated with Lintetralin/Mupirocin) using HISAT2 software.

Alignment details	***Enterococcus faecalis***		***Enterococcus faecalis*/Lintetralin**	
	G-Control_1	**G-Control_2**	**G-LIN_1**	**G- LIN_2**
Total reads	11485891 (100%)	11485129 (100%)	13153057 (100%)	13153826 (100%)
Aligned 0 times	11412609 (99.98%)	11412708 (100%)	14366721 (100%)	14367256 (100%)
Aligned exactly 1 time	84 (0%)	69 (0%)	229 (0%)	221 (0%)
Aligned >1 times	1734 (0.02%)	31 (0%)	461 (0%)	68 (0%)
Alignment details	***Enterococcus faecalis***		***Enterococcus faecalis*/Mupirocin**	
	G-Control_1	**G-Control_2**	**G-MUP_1**	**G-MUP_2**
Total reads	11376880 (100%)	11379253 (100%)	14670596 (100%)	14969021 (100%)
Aligned 0 times	11381850 (99.95%)	11381253 (100%)	12159096 (100%)	12646834 (100%)
Aligned exactly 1 time	79 (0%)	65 (0%)	223 (0%)	217 (0%)
Aligned >1 times	1698 (0.02%)	28 (0%)	452 (0%)	58 (0%)

Apart from that, cufflinks software was utilized to determine relative abundance of transcripts, which identifies the various database of essential genes (DEGs). Besides, *p* value was adjusted using *q* value. Additionally, the *q* value < 0.5 and log 2 (fold change) > 0.8 (up-regulated gene); < -0.8 (down-regulated gene) was set as the threshold for significant differential expression. The calculated number of DEGs found was depicted (ST 2).

PPI Network Analysis

Protein-protein interaction network analysis was performed taking the down-regulated genes (n = 12), which were used to construct PPI network. Genes involved in different signal pathway were integrated using STRING website to explore the association between DEGs, which showed close interaction between proteins involved in biofilm production (SF 1).

Functional Annotations of the Up-regulated and Down-regulated genes

Up-regulated genes exhibited by *Enterococcus faecalis* after treated with methanolic leaf extract of *Phyllanthus niruri* include murE (cell wall synthesis and cell cycle regulation), aspB (aminotransferase activity), rpmC (signal transduction regulatory protein), ftsL (cell growth), katA (catalase activity, metal ion binding). Functional annotations of up-regulated genes were collated (Table 2). Down-regulated genes of *Enterococcus faecalis* identified after treated with methanolic leaf extract of *Phyllanthus niruri* were phzM (transcription regulatory activity), SAXN108_1143 (catalytic activity), prgB (pilus biogenesis, biofilm formation, fibrinogen binding), fsrA (gelatinase activity), rplB (heterocyclic compound binding, nucleic acid binding, transferase activity), rplA and rplK (nucleic acid binding, organic cyclic and heterocyclic binding), rplJ (nucleic acid binding, rRNA binding), esp (biofilm formation, adhesion activity), gelE (biofilm formation, hydrolase activity), rcsD (transferase activity, transducer activity) and SAXN108_1534 (catalase activity). The study suggested that the down-regulated genes such as esp gene, gelE gene and prgB gene were found to be associated with pathogenesis and biofilm formation in *Enterococcus faecalis*. The down-regulated genes treated with methanolic leaf extract of *Phyllanthus niruri* regulating biofilm formation were presented (Table 3).

Table 2: Functional annotation of the up-regulated genes of *Enterococcus faecalis* identified after treated with methanolic leaf extract of *Phyllanthus niruri*.

Sl.	Gene	Biological process	GOTerm ID	Molecular function	GOTerm ID	Cellular component	GOTerm ID
1.	murE	Cell wall synthesis, cell cycle regulation	GO:0008052 GO:0009058	ATP binding Ligase activity	GO:0003735	Cytoplasm	GO:0005622
		Peptidoglycan biosynthesis	GO:0010467 GO:0008052	Magnesium binding	GO:0005198	Cytoplasm Cytoplasm	
2.	aspB	Biosynthetic process	GO:0009058	Aminotransferase activity Phosphate binding	GO:0015934	Cytoplasm	GO:0005737
3.	rpmC	Metabolic process	GO:0008152	Structural constituent of ribosome	GO:0003735	Intracellular	GO:0005622
		Biosynthetic process	GO:0009058			Cytoplasm	GO:0005737
		Gene expression	GO:0010467	Structural molecule activity	GO:0005198	Cellular anatomical entity	GO:0110165
		Translation	GO:0006412			Large ribosomal subunit	GO:0015934
		Signal transduction	GO:0035556				
4.	ftsL	Cell division	GO:0008152 GO:0009058	Cell growth and division	GO:0008152 GO:0009058	Plasma membrane Cytoplasm	GO:0110165
		Cytokinesis					
5.	katA	Hydrogen peroxide catabolic process	GO:0010467	Catalase activity	GO:0010467	Cytoplasm	GO:0110463
		Response to hydrogen peroxide	GO:0006412	Heme binding Metal ion binding	GO:0006412 GO:0008152		GO:0110438 GO:0110392

Table 3: Functional annotation of the down-regulated genes of *Enterococcus faecalis* identified after treated with methanolic leaf extract of *Phyllanthus niruri*.

Sl.	Gene	Biological process	GOTerm ID	Molecular function	GOTerm ID	Cellular component	GOTerm ID
1.	phzM	Phenazine biosynthesis	GO:0010467	Methyltransferase activity	GO:0046872	Cytoplasm	GO:0041869
		Pathogenesis	GO:0006412	Cell adhesion mediator activity	GO:0003676		GO:0003872
		Cell adhesion	GO:0010468	Transcription regulatory activity	GO:0003735		GO:0003643
2.	SAXN108_1143	Metabolic process	GO:0006412	Catalytic activity	GO:0003824	Plasma membrane	GO:0005886
		Cellular process	GO:0009987	ATP binding	GO:0005488	Membrane	GO:0016020
		Cellular metabolic process	GO:0044237	Electron transfer activity	GO:0009055	Cell periphery	GO:0071944
		Cellular respiration	GO:0045333	Organic cyclic compound binding	GO:0097159	Cellular anatomical entity	GO:0110165
3.	prgB	Intracellular signal transduction	GO:0003676	Pilus biogenesis	GO:1901363	Cell surface	GO:0005622
		Biofilm formation	GO:0003735	Adhesion activity	GO:0003676		GO:0005737
		Cellular process	GO:0045397	Fibrinogen binding	GO:0005488		GO:0005829

4.	fsrA	Metabolic process	GO:0008152	Catalytic activity	GO:0003824	Intracellular	GO:0005622
		Regulation of gene expression	GO:0009987	Gelatinase activity	GO:0005488	Cytoplasm	GO:0005737
			GO:0044237	Serum protease activity	GO:0016740		GO:0005829
		Biofilm formation	GO:0044283	Quorum sensing	GO:0046872	Cellular entity	GO:0110165
5.	rplB (SAXN108_2496)	Metabolic process	GO:0008152	Nucleic acid binding	GO:0003676	Intracellular	GO:0005622
		Cellular process	GO:0009987	Catalytic activity	GO:0003824	Cytoplasm	GO:0005737
		Metabolic process	GO:0044237	Transferase activity	GO:0016740	Cytoplasm	GO:0005829
		Gene expression	GO:0010467	Heterocyclic compound binding	GO:1901363	Cellular anatomical entity	GO:0110165
6.	rplA (SAXN108_0592)	Metabolic process	GO:0008152	Nucleic acid binding	GO:0003676	Intracellular	GO:0005622
		Cellular process	GO:0009987	Structural ribosome constituents	GO:0003735	Cytoplasm	GO:0005737
		Cellular metabolic process	GO:0044237	Organic cyclic compound binding	GO:0097159	Cytoplasm	GO:0005829
		Biological regulation	GO:0065007	Heterocyclic compound binding	GO:1901363	Cellular anatomical entity	GO:0110165
7.	rplK (SAXN108_0591)	Metabolic process	GO:0008152	Nucleic acid binding	GO:0003676	Intracellular	GO:0005622
		Cellular process	GO:0009987	ATP binding	GO:0005488	Cytoplasm	GO:0005737
		Cellular metabolic process	GO:0044237	Heterocyclic compound binding	GO:1901363	Cytoplasm	GO:0005829
		Cellular component biogenesis	GO:0044085	Organic cyclic compound binding	GO:0097159	Cellular anatomical entity	GO:0110165
8.	rplJ (SAXN108_0593)	Translation	GO:0006412	Nucleic acid binding	GO:0003676	Intracellular	GO:0005622
		Ribosome biogenesis	GO:0042254	Ribosomal subunit rRNA binding	GO:0070180	Large ribosomal subunit	GO:0015934
		Cellular component biogenesis	GO:0044085	Heterocyclic compound binding	GO:1901363	Protein-containing complex	GO:0032991
		Amide biosynthetic process	GO:0043604	rRNA binding	GO:0019843	Ribosomal subunit	GO:0044391
9.	esp	Biofilm formation	GO:0042710	Single species biofilm formation	CL: 3096 Cell surface extracellular GO:0005806		
		Cell aggregation	GO:0098743	Adhesion activity			
		Cellular process	GO:0009987	Catalytic activity			
10.	gelE	Regulation of gene expression	GO:0010468	Virulence gene expression	CL: 6573 Extracellular metalloprotease GO:0005806		
		Biofilm formation	GO:1900190	Adhesion activity			
		Metabolic process	GO:0021957	Hydrolase activity			
11.	rcsD	Cell communication	GO:0007154	Histidine phosphotransfer kinase activity	GO:0009927	Plasma membrane	GO:0005886
		Signal transduction	GO:0007165	Transferase activity	GO:0016740	Integral component of plasma membrane	GO:0005887
		Metabolic process	GO:0008152	Molecular transducer activity	GO:0060089	Cell periphery	GO:0071944
12.	SAXN108_1534	Metabolic process	GO:0007516	Catalytic activity	GO:0003824	Intracellular	GO:0005622
		Cellular process	GO:0009987	Binding	GO:0005488	Cytoplasm	GO:0005737
		Cellular metabolic process	GO:0044237	Organic cyclic compound binding	GO:0097159	Cytoplasm	GO:0005829
		Primary metabolic process	GO:0044238	Oxidoreductase activity	GO:0016491	Cellular anatomical entity	GO:0110165

Molecular docking of lintetralin and mupirocin

The binding site of lintetralin and mupirocin onto proteins crucial for virulence, biofilm formation as well as drug resistance exhibited by *Enterococcus faecalis* such as esp (biofilm regulatory surface protein), gelE (metalloprotease with hydrolase activity) and prgB (pilus biogenesis, biofilm regulatory protein) were subjected to docking with these proteins. Blind docking approach was followed to dock lintetralin and mupirocin to its suitable binding site on target proteins. All predicted protein binding sites were considered for docking of lintetralin and mupirocin followed by evaluation of docking score against the binding sites. The binding site against which docking score is found to be lowest minimum was considered as the putative binding site for lintetralin and mupirocin individually.

The study revealed that lintetralin showed lowest minimum docking score of -5.347 Kcal/mol (esp protein), -5.023 Kcal/mol (gelE protein) and -5.647 Kcal/mol (prgB protein) exhibited by the down-regulated genes of *Enterococcus faecalis* after treated with methanolic leaf extract of *Phyllanthus niruri* (Table 4). Besides, the study indicated that lintetralin was found to be well accommodated inside the binding cavity (Figure 5.4).

Table 4: Molecular docking results of lintetralin against different binding sites onto the proteins involved in biofilm formation exhibited by *Enterococcus faecalis*.

Site ID	Site score	Volume (Å)^3	Glide XP score (Kcal/mol)
(a) esp protein (PDB ID: 6ORI)			
1	1.009	894.33	-4.467
2	1.042	400.62	-5.347
3	0.900	177.97	-3.994
4	0.722	147.91	-2.472
5	0.689	139.25	-4.429
(b) gelE protein (PDB ID: 4GER)			
1	0.984	324.60	-5.023
2	0.588	93.46	-2.149
(c) prgB protein (PDB ID: 6GED)			
1	0.988	392.39	-4.495
2	0.975	575.98	-5.647
3	0.965	598.96	-3.310
4	0.629	92.39	-2.628
5	0.631	74.21	-3.453

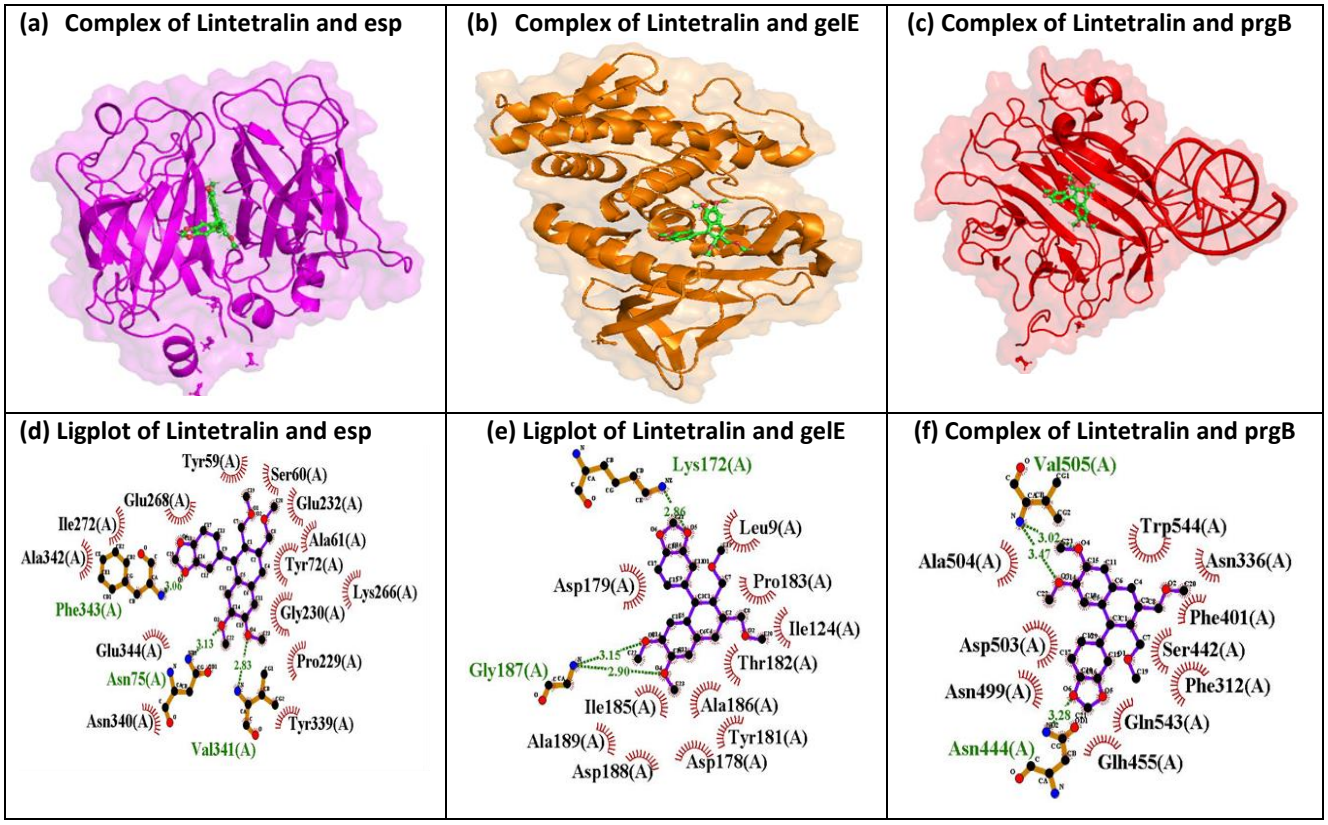


Figure 2: Molecular docking of lintetralin onto different proteins such as (a) esp protein, (b) gelE protein and (c) prgB protein involved in pathogenesis and biofilm formation in *Enterococcus faecalis*. Ligplot analysis of lintetralin revealed the interactions with binding site amino acids of (d) esp protein, (e) gelE protein and (f) prgB protein respectively. Binding involved hydrogen bonds were represented as dotted (green) lines whereas the hydrophobic interactions with curved (red) lines.

Binding of lintetralin involved three hydrogen bonds (represented in a dashed line) with binding site amino acids (Val 341A; Asn 75A; Phe 343A) of esp protein (Figure 2d), two hydrogen bonds with the following amino acids (Lys 172A and Gly 187A) of gelE protein (Figure 2e), and two hydrogen bonds with the following amino acids (Val 505A and Asn 444A) of prgB protein (Figure 2f). Besides, the study revealed several hydrophobic interactions of lintetralin with the binding site amino acids of these proteins (Figure 2).

Molecular docking of mupirocin with different protein binding sites in *Enterococcus faecalis* suggested that mupirocin showed lowest minimum docking score of -9.209 Kcal/mol with esp (biofilm regulatory protein), -9.499 Kcal/mol with gelE (metalloprotease, hydrolase activity) and -8.382 Kcal/mol with prgB (biofilm regulatory pilus protein) (Table 5). The study indicated that mupirocin was found to be well accommodated inside the binding cavity (Figure 5). Binding of mupirocin involved three hydrogen bonds (represented with dashed line) with three binding site amino acids (Phe 244A, Asp 243A and Tyr 214A) of esp protein (Figure 5.6d), four hydrogen bonds with amino acids (Arg191A, Trp108A. Glu 43A and Asn 188A) of gelE protein (Figure 3e), and two hydrogen bonds with amino acids (Lys 371A and Lys 290A) of prgB protein (Figure 3f). Besides, the binding of mupirocin involved several hydrophobic interactions with the binding site amino acids of proteins (Figure 3).

Table 5: Molecular docking results of mupirocin with respect to different binding sites onto the proteins involved in biofilm formation exhibited by *Enterococcus faecalis*.

Site ID	Site score	Volume (Å)^3	Glide XP score (Kcal/mol)
(a) esp protein (PDB ID: 6ORI)			
1	1.009	894.33	-9.209
2	1.042	400.62	-8.153
3	0.900	177.97	-6.362
4	0.722	147.91	-4.991
5	0.689	139.25	-5.504
(b) gelE protein (PDB ID: 4GER)			
1	0.984	324.60	-9.499
2	0.588	93.46	-5.365
(c) prgB protein (PDB ID: 6GED)			
1	0.988	392.39	-8.382
2	0.975	575.98	-7.800
3	0.965	598.96	-6.176
4	0.629	92.39	-6.434
5	0.631	74.21	-7.228

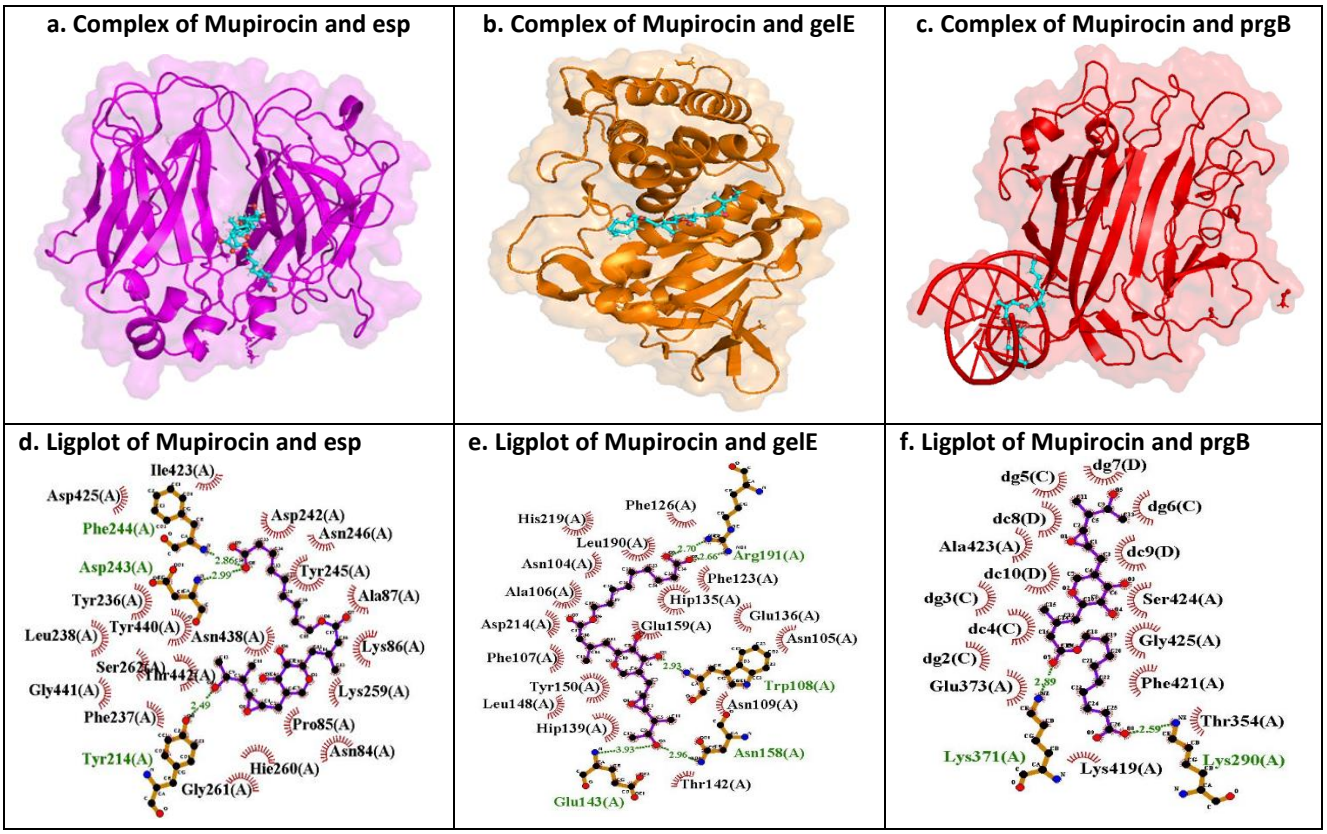


Figure 3: Molecular docking of mupirocin onto different proteins such as (a) esp protein, (b) gelE protein and (c) prgB protein involved in pathogenesis and biofilm formation in *Enterococcus faecalis*. Ligplot analysis of mupirocin showed interactions with binding site amino acids of (d) esp protein, (e) gelE protein and (f) prgB protein respectively. Binding involved hydrogen bonds were represented as dotted (green) lines whereas the hydrophobic interactions with curved (red) lines.

The esp gene has been identified by transcriptomic profiling of *Enterococcus faecalis* and suggested as key contributor in enhancing biofilm formation influencing pathogenesis and transmission[10,12,26] and revealed the role of esp gene in colonization and persistence of *Enterococcus faecalis* - during urinary tract infections[27,28]. Besides, the gelE gene encoding gelatinase is an extracellular zinc-endopeptidase/protease that facilitates transmission[29], colonization and persistence through biofilm formation influencing pathogenicity caused by *Enterococcus* faecalis[15,30,31]. Presence of gelE and esp genes was reported to enhance biofilm formation and confer resistance against antimicrobials promoting the virulence nature in *Enetrococcus faecalis*[16]. Involvement of prgB gene in promoting cellular aggregation, biofilm formation and efficiency of plasmid transfer in *Enetrococcus faecalis* was reported[19,32].

Comprehensive studies have been demonstrated the pharmacological potentials of multiple bioactive compounds derived from leaf plant extracts of *Phyllanthus niruri* with wide translational therapeutic significance such as antioxidant, antinociceptive, analgesic activity, hepatoprotective, antidiabetic-hypoglycemic, anti-inflammatory, hypolipidaemic, antiplatelet, vasorelaxant, antibacterial, antiviral, antiulcer, antiurolithiatic, antineoplastic, spasmolytic, immunomodulatory activity[33,34]. Lintetralin is one of potent bioactive phytochemicals isolated from methanolic leaf extract of *Phyllanthus niruri* was found to exhibit antibacterial compound against MDR pathogens including *Enterobacter faecalis*, which have been elucidated by several workers[35,36]. Besides, mupirocin derived from methanolic leaf extract of *Phyllanthus niruri* has been reported to exhibit antimicrobial activity through the inhibition in biofilm formation in MDR pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E. coli* and *Enterococcus faecalis*[37,38]. The study clearly indicated that the bioactive phytochemicals (Lintetralin and Mupirocin) derived from *Phyllanthus niruri* can be used as the potent lead molecule to be exploited towards the development of novel antimicrobial agents to fight against antibiotic resistance exhibited by MDR bacterial pathogens.

CONCLUSION

MDR bacterial pathogen responsible for nosocomial infections is unprecedented and pacey, which is the major concern for global healthcare and necessitate the development of alternative and effective treatment. Phytochemicals with wide therapeutic properties are used to overcome antibiotic resistance developed in MDR pathogens. The study revealed the therapeutic efficacies exhibited by bioactive compounds (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* as potent antimicrobials against *Enterococcus feacalis* based on their binding affinities with proteins with diverse mode of action including biofilm formation. These phytochemicals have been proved not only as the promising alternatives in response to the shrinking pool of conventional antibiotics, but also proved to inhibit the major resistance-gaining determinants vital for sustenance and resistance of bacterial pathogens. Besides, the bioactive phytochemicals exhibit minimal side effects compared to commercial antibiotics and are less prone in acquiring bacterial resistance. Though the phytochemicals (Lintetralin and Mupirocin) derived from *Phyllanthus niruri* have displayed success as potent antimicrobial agents, their translational application is quite low, which necessitates the fast-track research and clinical approval to elucidate their mode of action using network pharmacology followed by their efficacy evaluation with innovations in therapeutic strategies resolving the issues of antibiotic resistance along with their effectiveness against MDR associated clinical complications.

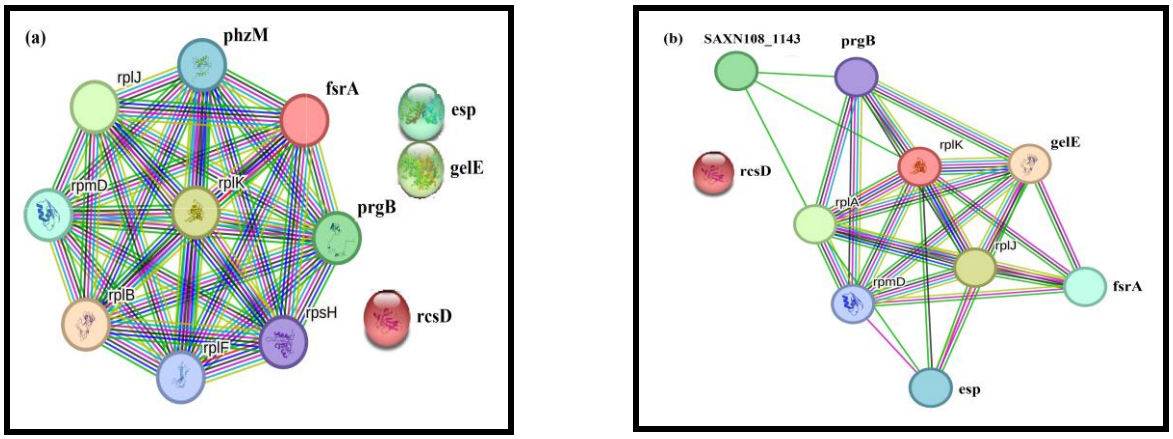
Supplementary Data

Supplementary Table 1: Statistical summary of the quality control of transcript sequencing.

File name	G-Control_1.fastq	G-Control_2.fastq	G-LIN_1.fastq	G-LIN_2.fastq
Total sequences	11485891	11485129	13153057	13153826
Encoding	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9
Sequence length	153	153	153	153
GC %	48	48	48	48
Basic statistics	pass	pass	pass	pass
File name	**G-Control_1.fastq**	**G-Control_2.fastq**	**G-MUP_1.fastq**	**G-MUP_2.fastq**
Total sequences	11376880	11379253	14670596	14969021
Encoding	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9
Sequence length	153	153	153	153
GC %	48	48	48	48
Basic statistics	pass	pass	pass	pass

Supplementary Table 2: Statistical summary of the predicted genes with database of essential genes.

Details of genes	*p* value	Fold change (log 2)	DEGs
Total significant genes	*p* value < 0.5		1041
Total up-regulated genes	*p* value < 0.5	≥ 0.8	5
Total down-regulated genes	*p* value < 0.5	≤ -0.8	12



Supplementary Figure 1: Protein-protein interaction network analyses of down-regulated genes from *Enterococcus faecalis* treated with bioactive phytochemicals such as (a) lintetralin and (b) mupirocin revealed the closer interactions between different proteins.

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