ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2024; 12(4): 53-62

Received: 15-04-2024
Accepted: 20-05-2024

Karishma Patel
Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Odisha, India

Ankita Agrawal
Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Odisha, India

Amisha Mohanty
Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Odisha, India

Dr. Amiya Kumar Patel
Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Odisha, India

Corresponding Author:
Dr. Amiya Kumar Patel
Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar, Burla, Odisha, India

Elucidation of antimicrobial efficacy and mode of action of lintetralin and mupirocin derived from *Phyllanthus niruri* against *Proteus mirabilis* through transcriptome analysis

Karishma Patel, Ankita Agrawal, Amisha Mohanty and Dr. Amiya Kumar Patel

DOI: https://doi.org/10.22271/plants.2024.v12.i4a.1691

Abstract
Prevalence of bacterial multidrug resistance is a multifactorial problem raising healthcare threats globally rendering commercial antibiotic ineffective. Medicinal plants act as unexplored resources for the development of potent drugs. *Phyllanthus niruri* is reported to exhibit diverse biological properties including antimicrobial, antifungal, anticancer properties. Possible binding affinity of targeted bioactive phytochemicals (Lintetralin and Mupirocin) from methanolic leaf extract of *Phyllanthus niruri* against the resistance genes in *Proteus mirabilis* was predicted through *in-silico* analysis. This study suggested that lintetralin showed lowest minimum docking score (-3.830 Kcal/mol) against atfE protein, -3.248 Kcal/mol against lpxA protein and -3.103 Kcal/mol against mrpH protein from *Proteus mirabilis*. Similarly, mupirocin showed lowest minimum docking score of –7.552 Kcal/mol with atfE protein, -6.976 Kcal/mol with lpxA protein and -6.851 Kcal/mol with mrpH protein from *Proteus mirabilis.* The study revealed that lintetralin and mupirocin are potent antimicrobials, which can be used as potential alternative to antibiotics to combat MDR.

Keywords: Biofilm, atfE, lpxA, mrpH, *Phyllanthus niruri*, *Proteus mirabilis*

Introduction
Development of multidrug resistance among bacterial pathogens is a multifactorial problem that raises critical healthcare threats globally rendering commercial antibiotics ineffective. Thus, innovation of antibiotics with new mode of action is urgently needed [1]. Medicinal plants offer unexplored resources for the development of potent drugs against microbial infections to combat the phenomenon of resistance [2]. *Phyllanthus niruri* is reported to exhibit diverse biological properties including antimicrobial, antifungal, anticancer properties. The inhibiting effect of methanolic leaf extract of *Phyllanthus niruri* and their bioactive compounds have been reported against *Proteus mirabilis*. However, mode of action and binding affinity of bioactive compounds (Lintetralin and Mupirocin) against *Proteus mirabilis* at the molecular level remain unclear.

Molecular approaches aim to uncover the interactions between antibacterial agents and bacterial pathogens [3]. Transcriptome analysis is a powerful tool for monitoring antibiotic resistance at the transcriptional level and analyzes molecular mechanism of bacteriostatic agents including natural plant ingredients and antibiotics [4]. Moreover, whole transcriptomic analysis has great potential to identify specific genes involved in antibiotic resistance [5]. RNA-Seq is used to elucidate gene expression providing both qualitative/quantitative output [6] and involves the high-throughput sequencing driving millions of nucleotide sequences for transcriptome profiling with high resolution [7]. Besides, the RNA-Seq is used for precise quantitative analysis with complete annotation of all the transcripts with high accuracy [8].

Researchers have identified unique transcripts to elucidate the mechanism of antibiotic resistance in MDR bacterial pathogens including *Proteus mirabilis* [9]. *Proteus mirabilis* is an opportunistic pathogen prevalent in hospital settings poses major healthcare challenges [10]. Moreover, *Proteus mirabilis* exhibits both inherent and acquired resistance [11] and have diverse virulence factors that contribute to form intricate biofilm with multiple layers of polysaccharide matrix, exacerbating the severity of infections [12]. Studies revealed that biofilm formation is strongly associated with the expression of fimbrial adhesive genes atfE and ucaD

[11,13], lipopolysaccharide synthesis genes lpxA and eptB [14,15], mrpH gene [16,17], hmpA, zapA, mrpA, ureC, ureG and pnfA genes [18,19]. The pathogenicity, persistence and transmission of *Proteus mirabilis* infections are closely linked to its multidrug resistance and ability to form complex biofilms [20]. Keeping in view, transcriptomic analysis was performed to elucidate the gene expression in *Proteus mirabilis*. Besides, the *in-silico* molecular docking and molecular dynamic simulation was conducted to determine possible binding mechanism of targeted bioactive compounds (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* against resistance genes of *Proteus mirabilis*. Overall, the study provides a holistic approach to uncover novel gene(s) emphasizing the foundation for predicting the mechanism of resistance, which not only substantiate the discovery of potent antimicrobial phytochemicals, but also serve to combat the antimicrobial resistance in MDR pathogens.

Materials and Methods

Whole transcriptome analysis

Whole transcriptome sequencing was conducted to compare the treated and untreated MDR strain of *Proteus mirabilis* with the bioactive compounds (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* to understand its mode of action. Total RNA was isolated from bacterial sample using TRIzol method and DNA contamination was eliminated. RNA purity was tested by Nanodrop and 1% agarose gel electrophoresis, accepting sample with A260:A280 ratio greater than 2.2. RNA integrity was evaluated by Agilent Bioanalyzer having RNA integrity number (≥6.5) prepared for sequencing. Total RNA was then amplified and converted to ds cDNA for Illumina paired end sequencing library preparation, followed by high throughput sequencing and comparative analysis. Purified cDNA library was analyzed using an Agilent bioanalyzer, next to cluster generation on a HiSeq paired-end flow cell and massively-parallel sequencing on Illumina HiSeq 2000. Paired end data generated was uploaded to NCBI Short Read Archive. Trimmomatic and Printseq pre-processed sequence reads to filter out low quality reads. TopHat integrated with Bowtie software was used to align sequence with available reference genome sequence. TopHat removes reads from FASTQ files based on quality scoring associated with each read and maps them to the reference genome. Aligned reads from Tophat-Bowtie pipeline were analyzed by Cufflinks, which reported transcripts expression in 'fragments/kb of exon per million fragments mapped' (FPKM). To assess differential gene expression in treated and untreated *Proteus mirabilis*, Cuffdiff and Cuffcompare were used as the reference genome for comparison. Alternatively, differential expression was calculated through DESeq and edgeR using R Bioconductor package, which corrects biases by data normalization, limited number of highly expressed genes and other factors using relevant statistical and FDR tests. Cufflinks identifies novel targets, through construction of a minimum number of transcripts without bias towards known transcripts. Cytoscape plugins, DAVID, StringDB and Ingenuity pathway analysis (IPA) were used for canonical pathway, 16 GO ontologies, gene/protein networks, gene set analysis, gene clustering and target protein identification.

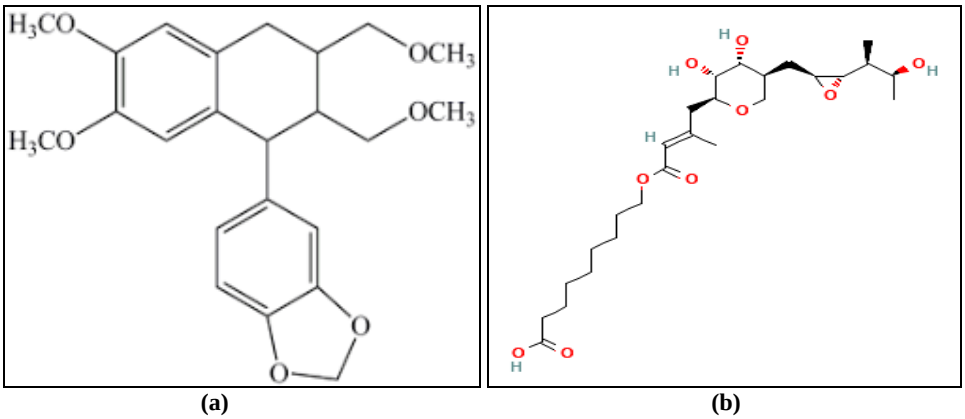
In silico interaction of lintetralin and mupirocin with targeted proteins

Protein preparation

Bioactive phytochemicals (Lintetralin and Mupirocin) derived from leaf extract of *Phyllanthus niruri* were found to be involved in pathogenesis, biofilm regulatory proteins and multidrug resistance of *P. mirabilis* through interactions with different genes such as atfE (PDB ID: 6H1Q), lpxA (PDB ID: 6OSS) and mrpH (PDB ID: 6Y4E), used in the study. PDB structures and addition of missing hydrogen atoms were processed by multistep procedures of protein preparation wizard (Schrödinger Inc., NY). Missing side chain atoms of the amino acids were subsequently identified using Prime side-chain prediction tool and repaired using Prime. Further, structures of bioactive proteins were refined through energy minimization using Macromodel (Schrodinger) and OPLS 2005 force field. In the present study, 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 Inc., NY) 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 Inc., NY) for geometric optimization of structure [21]. Various conformations of plant derived bioactive compounds (Lintetralin and Mupirocin) derived from methanolic extract of *Phyllanthus niruri* were generated using Ligprep (Schrödinger Inc., NY).



(a) **(b)**

Fig 1: Molecular structures of bioactive molecule (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 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 Inc., NY) 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 Inc., NY) and their binding poses were evaluated using Glide XP_{Score} function [22,23]. Single best conformation of lintetralin and mupirocin with lowest minimum docking score with protein was used for further analysis.

Results and Discussion

Transcriptome analysis

Data related to *Proteus mirabilis* (G-Control_1 & G-Control_2) treated with bioactive compounds 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 *Proteus mirabilis* was used as reference to align the 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 was mapped with reference genome. The study revealed that 99.98% and 100% reads from G-Control_1 and G-Control_2 were mapped with reference genome, whereas 100% reads from G-MUP_1 and G-MUP_2 were mapped with reference genome. Mapping results along with statistical summary were presented (ST 2). Further, the cufflinks package was used to calculate relative abundance of transcripts and find out database of essential genes (DEGs). 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 differential expression. Calculated number of DEGs was depicted (ST 3).

PPI network analysis

Protein-protein interaction network analysis was performed taking the down-regulated genes (n = 10), 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

The up-regulated genes exhibited by *Proteus mirabilis* after treated with methanolic leaf extract of *Phyllanthus niruri* include AID39897.1 (nucleic acid and catalytic activity), metG (ATP binding and tRNA binding,), rpmC (signal transduction regulatory protein), fsaA (aldolase activity). Functional annotations of up-regulated genes were collated (Table 1). Down-regulated genes of *P. mirabilis* identified after treated with leaf extract of *Phyllanthus niruri* were SAXN108_0407 (transferase activity), atfE (biofilm formation, virulence gene, fimbrial receptor binding), lpxA (transferase activity, lipopolysaccharide biosynthesis), metE/SAXN108_0406 (cellular metabolic activity, catalytic activity, transferase activity), nrpS (metabolic activity, amino acid adenylation), rplA/SAXN108_0592 (Organic cyclic binding, heterocyclic binding), rplK/SAXN108_0591 (nucleic acid binding, cellular component biogenesis), mrpH (metal binding adhesion activity, biofilm formation), bssS (biofilm regulatory protein) and zapD (biofilm regulatory protein). The study suggested that the down-regulated genes such as atfE, lpxA and mrpH were directly associated with biofilm formation in *Proteus mirabilis*. The down-regulated genes treated with the methanolic leaf extract of *Phyllanthus niruri* regulating biofilm formation were presented (Table 1).

Table 1: Functional annotation of the up-regulated genes of *Proteus mirabilis* 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.	AID39897.1	Pathogenesis	GO:0009405	Nucleic acid binding	GO:0003676	Integral component of membrane	GO:0016021
				Catalytic activity	GO:0003824		
		Interspecies interaction between organisms	GO:0044419	Endonuclease activity	GO:0004519	Cell periphery	GO:0071944
				Hydrolase activity	GO:0016787	Cellular anatomical entity	GO:0110165
2.	metG	Methionyl-tRNA aminoacylation	GO:0009987	ATP binding	GO:0044237	Cytoplasm	GO:0044237
				Metal ion binding	GO:0010467		
				tRNA binding	GO:0044237		
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	
		Intracellular signal transduction	GO:0035556				
4.	fsaA	Lyase activity	GO:0010467	Aldolase activity	GO:0060089	Cytoplasm	GO:0009927
		Metabolic process	GO:0016740				

Table 2: Functional annotation of the down-regulated genes of *Proteus mirabilis* identified after treated with the methanolic leaf extract of *Phyllanthus niruri*.

Sl.	Gene	Biological process	GOTerm ID	Molecular function	GOTerm ID	Cellular component	GOTerm ID
1.	SAXN108_0407	Metabolic process	GO:0008152	Binding	GO:0005488	Intracellular	GO:0005622
		Cellular process	GO:0009987	Transferase activity	GO:0016740	Cytoplasm	GO:0005737
		Cellular metabolic process	GO:0044237	Catalytic activity	GO:0003824	Cellular anatomical entity	GO:0110165
2.	atfE	Biofilm formation	GO:0016787	Cell adhesion	GO:0010467	Membrane	GO:0089587
		Activation of virulence gene	GO:0016847	Fimbrial receptor binding	GO:0086787		
3.	lpxA	Metabolic activity	GO:0016787	Transferase activity	GO:0018787	Outer membrane	GO:0058787
		Biofilm formation	GO:0016923	Lipopolysaccharides biosynthesis	GO:0024864		
		Catalytic activity	GO:0026787	*In vitro* cytotoxicity	GO:0016587		
4.	metE (SAXN108_0406)	Metabolic process	GO:0008152	Catalytic activity	GO:0003824	Intracellular	GO:0005622
		Cellular process	GO:0009987	ATP binding	GO:0005488	Cytoplasm	GO:0005737
		Cellular metabolic process	GO:0044237	Transferase activity	GO:0016740	Cytoplasm	GO:0005829
		Small molecule biosynthetic process	GO:0044283	Metal ion binding	GO:0046872	Cellular anatomical entity	GO:0110165
5.	nrpS	Metabolic activity	GO:0008152	Biosynthesis	GO:0008152	Cytoplasm	GO:0008152
		Peptide biosynthesis	GO:0009987	Metabolic activity	GO:0009987		GO:0009987
		Siderophore production	GO:0044237	Amino acid adenylation	GO:0044237		GO:0044237
6.	rplA (SAXN108_0592)	Metabolic process	GO:0008152	Nucleic acid binding	GO:0003676	Intracellular	GO:0005622
		Cellular process	GO:0009987	Ribosomal constituent	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)	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.	mrpH	Biofilm formation	GO:0008152	Single species biofilm formation	GO:0003676	Intracellular	GO:0005622
		Cellular process	GO:0008152	ATP binding	GO:0008152	Membrane	GO:0008152
		Metal binding adhesion	GO:0009987	Adhesion activity	GO:0009987		GO:0009987
9.	bssS	Biofilm formation	GO:0042710	Single species biofilm formation	CL: 3096 Integral membrane GO:0005806		
		Cell aggregation	GO:0098743	Cell division			
		Cellular process	GO:0009987	Cell division			
10.	zapD	Regulation of gene expression	GO:0010468	Bacterial secretion system	CL: 6573 Outer membrane GO:0005806		
		Biofilm formation	GO:1900190	Membrane binding activity			

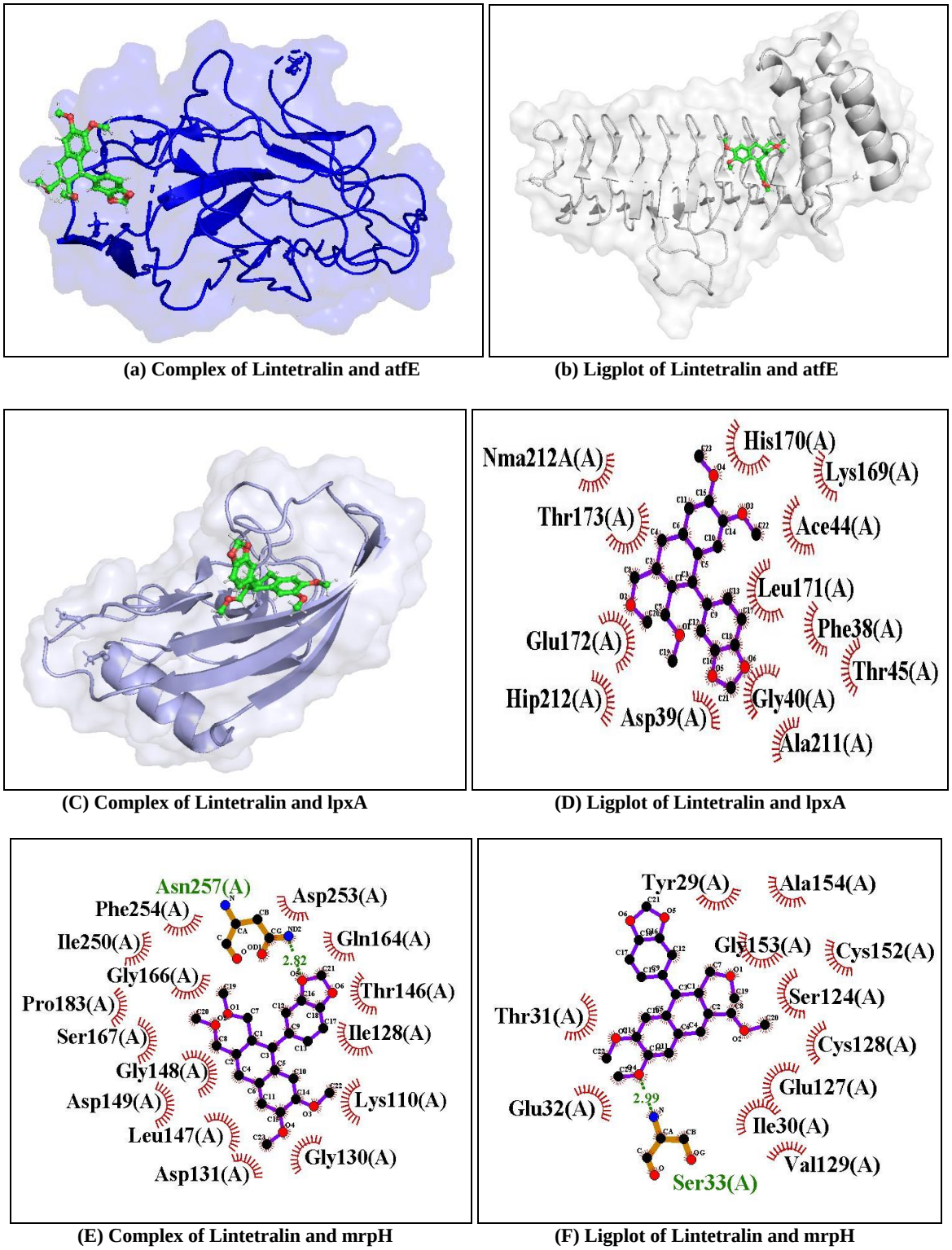
Molecular docking of lintetralin and mupirocin

The binding site of lintetralin and mupirocin onto proteins involve in pathogenesis, biofilm formation as well as drug resistance exhibited by *Proteus mirabilis* such as atfE protein (biofilm formation, fimbrial receptor binding protein), lpxA protein (transferase activity, lipopolysaccharide biosynthesis) and mrpH protein (metal binding adhesion activity, biofilm formation) 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. Binding site with lowest minimum docking score was considered as putative binding site for lintetralin and mupirocin individually.

The study suggested that lintetralin showed lowest minimum docking score of -3.830 Kcal/mol (atfE protein); -3.248 Kcal/mol (lpxA protein) and -3.103 Kcal/mol (mrpH protein) exhibited by down-regulated genes of *Proteus mirabilis* after treated with methanolic leaf extract of *Phyllanthus niruri* (Table 3). Besides, the study revealed that lintetralin was found to be well accommodated inside the binding cavity (Figure 2). Molecular docking studies revealed that the binding of lintetralin involved one hydrogen bond (represented in dashed line) with the binding site amino acid (Asn 257A) of lpxA protein (Figure 2e) and one hydrogen bond with amino acid (Ser 33A) of mrpH protein (Figure 2f). Besides, the study also revealed several hydrophobic interactions of lintetralin with the binding site amino acids of atfE, lpxA and mrpH proteins from *Proteus mirabilis* (Figure 2).

Table 3: Docking results of lintetralin against different binding sites onto the proteins involve in biofilm formation exhibited by *Proteus mirabilis*

Site ID	Site score	Volume (Å)^3	Glide XP score (Kcal/mol)
(a) atfE protein (PDB ID: 6H1Q)			
1	0.625	86.82	-3.732
2	0.626	75.20	-3.830
3	0.693	90.76	-3.410
4	0.558	56.29	-2.066
5	0.491	33.31	-3.608
(b) lpxA protein (PDB ID: 6OSS)			
1	0.514	69.71	-3.248
(c) mrpH protein (PDB ID: 6Y4E)			
1	0.620	84.72	-3.103



(a) Complex of Lintetralin and atfE **(b) Ligplot of Lintetralin and atfE**

(C) Complex of Lintetralin and lpxA **(D) Ligplot of Lintetralin and lpxA**

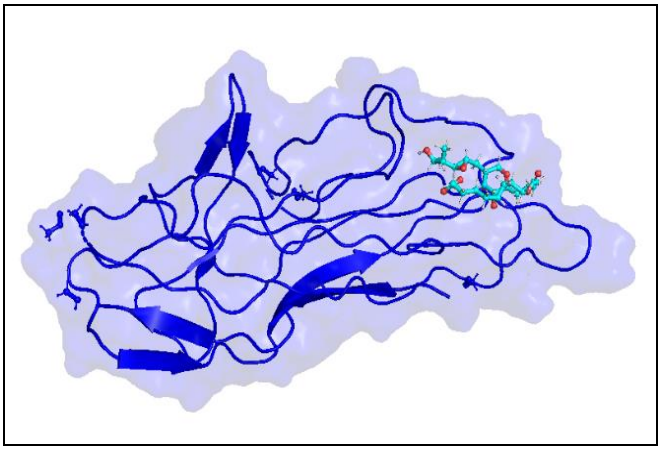
(E) Complex of Lintetralin and mrpH **(F) Ligplot of Lintetralin and mrpH**

Fig 2: Molecular docking of lintetralin onto different proteins such as (a) atfE, (b) lpxA and (c) mrpH involve in biofilm formation in *Proteus mirabilis*. Ligplot analysis of lintetralin showed interactions with binding site amino acids involving hydrogen bonds shown as dotted (green) lines whereas hydrophobic interactions with curved (red) lines

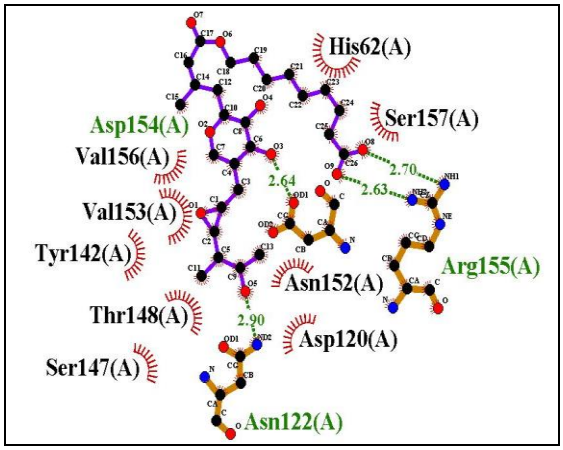
Further, the molecular docking studies of mupirocin with different binding sites of proteins involved in biofilm formation in *Proteus mirabilis* were presented (Table 4). The study suggested that mupirocin showed lowest minimum docking score of –7.552 Kcal/mol with atfE protein, -6.976 Kcal/mol with lpxA protein and -6.851 Kcal/mol with mrpH protein from *Proteus mirabilis* respectively (Table 4). Besides, the study revealed that the bioactive phytochemical mupirocin derived from the methanolic leaf extract of *Phyllanthus niruri* was found to be well accommodated inside the different binding cavity (Figure 3). The binding of mupirocin involved four hydrogen bonds (represented with dashed line) with three binding site amino acids (Asn 122A; Arg 155A and Asp 154A) of atfE protein (Figure 4.6d), three hydrogen bonds with the following amino acids (Thr 146A; Gln 164A and Arg 55A) of lpxA protein (Figure 4.5e) and one hydrogen bonding with binding amino acid (Ser 33A) of mrpH protein (Figure 4.5f). Moreover, the study suggested that the binding of mupirocin derived from methanolic leaf extract of *Phyllanthus niruri* involved several hydrophobic interactions with the binding site amino acids of atfE, lpxA and mrpH proteins (Figure 3).

Table 4: Docking results of mupirocin with respect to different binding sites onto the proteins involve in biofilm formation exhibited by *Proteus mirabilis*

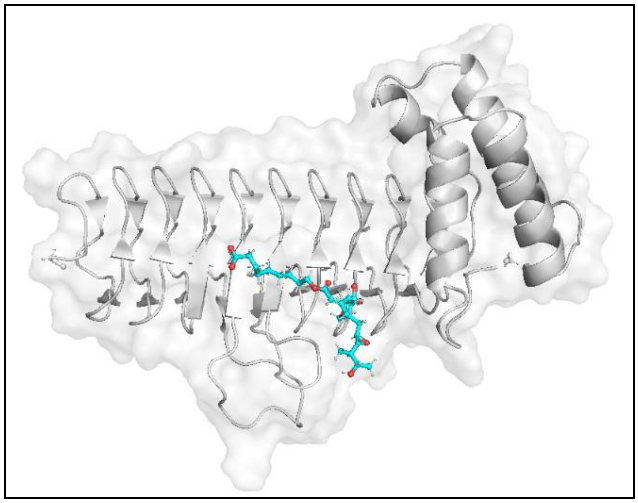
Site ID	Site score	Volume (Å)^3	Glide XP score (Kcal/mol)
(a) atfE protein (PDB ID: 6H1Q)			
1	0.625	86.82	-7.552
2	0.626	75.20	-6.246
3	0.693	90.76	-7.113
4	0.558	56.29	-6.953
5	0.491	33.31	-6.067
(b) lpxA protein (PDB ID: 6OSS)			
1	0.514	69.71	-6.976
2	0.498	68.29	-6.712
3	0.474	65.35	-6.375
4	0.385	59.21	-5.834
5	0.429	63.28	-6.167
(c) mrpH protein (PDB ID: 6Y4E)			
1	0.591	76.33	-6.749
2	0.620	84.72	-6.851
3	0.562	71.36	-6.438
4	0.543	65.25	-5.857
5	0.529	61.38	-5.624



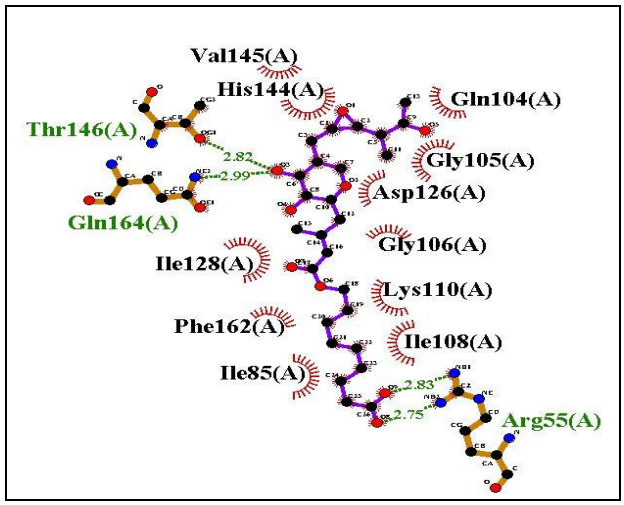
(a) Complex of Mupirocin and atfE



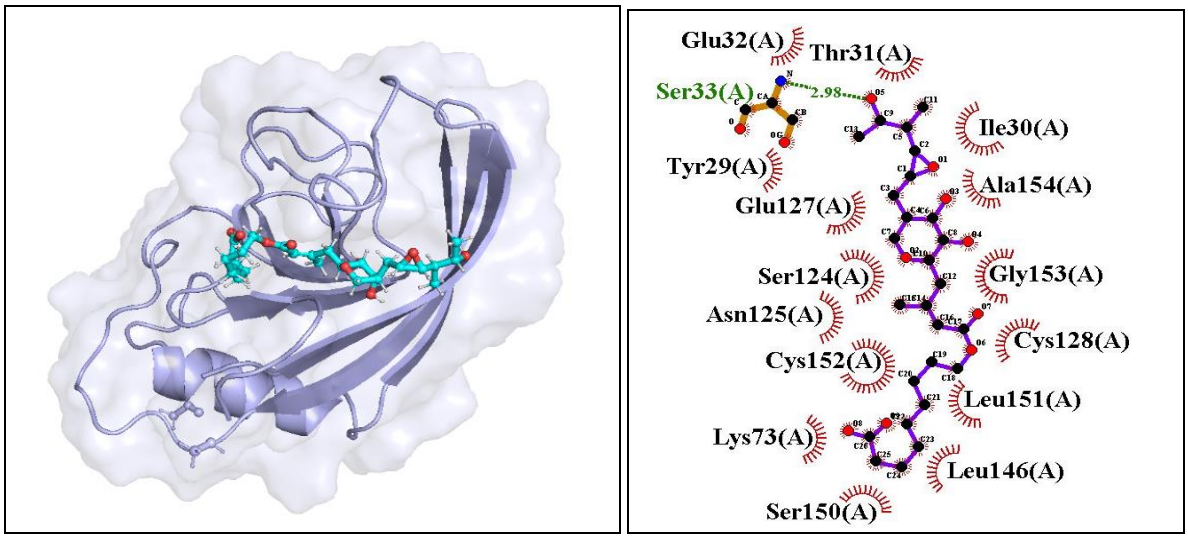
(b) Ligplot of Mupirocin and atfE



(b) Complex of Mupirocin and lpxA



(b) Ligplot of Mupirocin and lpxA



(c) Complex of Mupirocin and mrpH **(c)** Ligplot of Mupirocin and mrpH

Fig 3: Molecular docking of mupirocin onto different proteins such as (a) atfE, (b) lpxA and (c) mrpH involve in biofilm formation in *Proteus mirabilis*. Ligplot analysis of mupirocin showed interactions with binding site amino acids involving hydrogen bonds shown as dotted (green) lines whereas hydrophobic interactions with curved (red) lines

Discussion

The atfE gene identified in *Proteus mirabilis* through transcriptomic profiling encodes ambient temperature fimbriae adhesive proteins that strongly reveal its distinguishable role in biofilm formation of crystalline nature owing to urease activity [24, 25], which is one of the prime virulence factors influencing bacterial pathogenesis [13, 26]. The transcriptomics-based analysis of genes including lpxA involved in biofilm formation in *Proteus mirabilis* has been substantiated by several workers [15, 27]. The significance of lpxA protein involved in lipopolysaccharide biosynthesis was elucidated through rational drug design and suggested as attractive target for effective inhibitor design [28, 29]. The involvement of mrpH gene as the mannose-resistant/proteus like fimbriae adhesive protein in regulating biofilm formation in *Proteus mirabilis* was substantiated by several workers [17, 30, 31, 32].

Bioactive phytochemicals isolated from different extracts of *Phyllanthus niruri* show a wide spectrum of pharmacological activities such as antiplasmodial, antibacterial, antiviral, anti-inflammatory, antimalarial, anticancer, hypolipidemic, antidiabetic, nephroprotective, antioxidant, hepatoprotective, hypolipidaemic, anti-urolithiatic and diuretic properties [33,34]. The study suggested that lintetralin isolated from methanolic leaf extract of *Phyllanthus niruri* was found to be potent antibacterial compound against MDR strain *Proteus mirabilis* [35, 36, 37]. Antibacterial activity shown by lintetralin has been elucidated by several workers [34, 35, 38, 39]. Several studies reported the antimicrobial efficacy of mupirocin derived from *Phyllanthus niruri* that inhibit biofilm formation in MDR pathogens including *Proteus mirabilis* [40-44]. Existence of mupirocin in *Phyllanthus niruri* showed antibacterial activity against MDR pathogens including *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumoniae* [45-47]. The study indicated that the bioactive compounds (lintetralin and pupirocin) isolated from methanolic leaf extract of *Phyllanthus niruri* can be used as lead compounds, which can be contributed towards the development of novel antimicrobial agents to fight against antibiotic resistant MDR pathogens.

Increased multidrug resistant bacterial strains reflect ineffectiveness of commercially available antibiotics [48,49], which necessitates the discovery of novel chemotherapeutic phytochemicals from natural resources. Bioactive compounds derived from medicinal plants showed antimicrobial, antiviral, anti-inflammatory and antioxidant activities [48]. Some phytochemicals may not be effective when treated individually but revealed synergistic action in combination with commercially available antibiotics minimizing the acquisition of resistance [48]. Besides, the bioactive phytochemicals exhibit minimal side effects compared to commercial antibiotics and hence less prone in acquiring bacterial resistance [50].

Conclusion

Owing to therapeutic efficacies and antimicrobial activities exhibited by bioactive compounds (Lintetralin and Mupirocin) derived from methanolic leaf extract of *Phyllanthus niruri* prove them as potent antimicrobials against MDR bacterial pathogens based on their binding affinities with various proteins with diverse mode of action including biofilm formation. These bioactive compounds (Lintetralin and Mupirocin) derived from *Phyllanthus niruri* emerged as the potent chemotherapeutic antimicrobial agents that necessitate further research to elucidate their mode of action using network pharmacology followed by their efficacy evaluation through innovations in therapeutic strategies to combat MDR mediated infections resolving the issue of antibiotic resistance.

Acknowledgement

Authors are likely to acknowledge Department of Biotechnology and Bioinformatics, Sambalpur University for providing the scientific environment to carry out the research work.

Conflicts OF Interest

No conflict of interest.

References

1. Kumar V, Yasmeen N, Pandey A, Ahmad Chaudhary A, Alawam AS, Ahmad Rudayni H, *et al*. Antibiotic adjuvants: Synergistic tool to combat multi-drug resistant pathogens. Frontiers in Cellular and Infection Microbiology. 2023;13:1293633.

https://doi.org/10.3389/fcimb.2023.1293633
2. Nigussie D, Davey G, Tufa TB, Brewster M, Legesse B A, Fekadu A, *et al*. Antibacterial and antifungal activities of Ethiopian medicinal plants: A systematic review. Frontiers in Pharmacology. 2021;12:633921. https://doi.org/10.3389/fphar.2021.633921
3. Domínguez Á, Muñoz E, López MC, Cordero M, Martínez JP, Viñas M, *et al*. Transcriptomics as a tool to discover new antibacterial targets. Biotechnology Letters. 2017;39:819-828. https://doi.org/10.1007/s10529-017-2319-0
4. Wu J, Wang J, Li Z, Guo S, Li K, Xu P, *et al*. Antibiotics and antibiotic resistance genes in agricultural soils: A systematic analysis. Critical Reviews in Environmental Science and Technology. 2023;53(7):847-864. https://doi.org/10.1080/10643389.2022.2094693
5. Liu C, Sun S, Sun Y, Li X, Gu W, Luo W, *et al*. Antibiotic resistance of Escherichia coli isolated from food and clinical environment in China from 2001 to 2020. Science of the Total Environment; c2024. p. 173498. https://doi.org/10.1016/j.scitotenv.2024.173498
6. Deshpande D, Chhugani K, Chang Y, Karlsberg A, Loeffler C, Zhang J, *et al*. RNA-seq data science: From raw data to effective interpretation. Frontiers in Genetics. 2023;14:997383. https://doi.org/10.3389/fgene.2023.997383
7. Corchete LA, Rojas EA, Alonso-López D, De Las Rivas J, Gutiérrez NC, Burguillo FJ, *et al*. Systematic comparison and assessment of RNA-seq procedures for gene expression quantitative analysis. Scientific Reports. 2020;10(1):19737. https://doi.org/10.1038/s41598-020-76881-x
8. Sudhagar A, Kumar G, El-Matbouli M. Transcriptome analysis based on RNA-Seq in understanding pathogenic mechanisms of diseases and the immune system of fish: A comprehensive review. International Journal of Molecular Sciences. 2018;19(1):245. https://doi.org/10.3390/ijms19010245
9. Yang J, Shan G, Yu G, Wei J, Zhang Q, Su W, *et al*. Whole genome sequencing of multidrug-resistant Proteus mirabilis strain PM1162 recovered from a urinary tract infection in China. Journal of Global Antimicrobial Resistance. 2023;33:44-50. https://doi.org/10.1016/j.jgar.2023.02.014
10. Miryala SK, Anbarasu A, Ramaiah S. Gene interaction network approach to elucidate the multidrug resistance mechanisms in the pathogenic bacterial strain Proteus mirabilis. Journal of Cellular Physiology. 2021;236(1):468-479. https://doi.org/10.1002/jcp.29874
11. Elhoshi M, El-Sherbiny E, Elsheredy A, Aboulela AG. A correlation study between virulence factors and multidrug resistance among clinical isolates of Proteus mirabilis. Brazilian Journal of Microbiology. 2023;54(3):1387-1397. https://doi.org/10.1007/s42770-023-01080-5
12. Shaaban M, Abd El-Rahman OA, Al-Qaidi B, Ashour H M. Antimicrobial and antibiofilm activities of probiotic Lactobacilli on antibiotic-resistant Proteus mirabilis. Microorganisms. 2020;8(6):960. https://doi.org/10.3390/microorganisms8060960
13. Jiang W, Ubhayasekera W, Pearson MM, Knight SD. Structures of two fimbrial adhesins, AtfE and UcaD, from the uropathogen Proteus mirabilis. Acta Crystallographica Section D: Structural Biology. 2018;74(11):1053-1062. https://doi.org/10.1107/S2059798318012391
14. Gao C, Tian L, Lu J, Gong G. Transcriptomics- and metabolomics-based analysis of key biological pathways and core genes involved in the inhibition of Proteus mirabilis biofilm formation by protocatechuic acid. International Journal of Food Microbiology. 2024;412:110570. https://doi.org/10.1016/j.ijfoodmicro.2024.110570
15. Tian L, Gao C, Lu J, Liao S, Gong G. Key biological processes and essential genes for Proteus mirabilis biofilm development inhibition by protocatechuic acid. International Journal of Food Microbiology. 2024;412:110570. https://doi.org/10.1016/j.ijfoodmicro.2024.110570
16. Mirzaei B, Mousavi SF, Babaei R, Bahonar S, Siadat SD, Shafiee Ardestani M, *et al*. Synthesis of conjugated PIA–rSesC and immunological evaluation against biofilm-forming Staphylococcus epidermidis. Journal of Medical Microbiology. 2019;68(5):791-802. https://doi.org/10.1099/jmm.0.000910
17. Jiang Y, Geng M, Bai L. Targeting biofilms therapy: Current research strategies and development hurdles. Microorganisms. 2020;8(8):1222. https://doi.org/10.3390/microorganisms8081222
18. Sun X, Chen B, Xia B, Li Q, Zhu L, Zhao X, *et al*. Impact of mariculture-derived microplastics on bacterial biofilm formation and their potential threat to mariculture: A case in situ study on the Sungo Bay, China. Environmental Pollution. 2020;262:114336. https://doi.org/10.1016/j.envpol.2020.114336
19. Liu J, Wu S, Feng L, Wu Y, Zhu J. Extracellular matrix affects mature biofilm and stress resistance of psychrotrophic spoilage Pseudomonas at cold temperature. Food Microbiology. 2023;112:104214. https://doi.org/10.1016/j.fm.2023.104214
20. Shaaban M, Elshaer SL, Abd El-Rahman OA. Prevalence of extended-spectrum β-lactamases, AmpC, and carbapenemases in Proteus mirabilis clinical isolates. BMC Microbiology. 2022;22(1):247. https://doi.org/10.1186/s12866-022-02522-8
21. Santoshi S, Naik PK. Molecular insight of isotypes specific β-tubulin interaction of tubulin heterodimer with noscapinoids. Journal of Computational Aided Molecular Design. 2014;28:751-763. https://doi.org/10.1007/s10822-014-9756-9
22. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT *et al*. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. Journal of Medicinal Chemistry. 2004;47(7):1739-1749. https://doi.org/10.1021/jm0306430
23. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, *et al*. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. Journal of Medicinal Chemistry. 2004;47(7):1750-1759. https://doi.org/10.1021/jm030644s
24. Scavone P, Iribarnegaray V, Caetano AL, Schlapp G, Härtel S, Zunino P, *et al*. Fimbriae have distinguishable roles in Proteus mirabilis biofilm formation. FEMS Pathogens and Disease. 2016;74(5):033. https://doi.org/10.1093/femspd/ftw033
25. Yuan F, Huang Z, Yang T, Wang G, Li P, Yang B, *et al*. Pathogenesis of Proteus mirabilis in catheter-associated urinary tract infections. Urologia Internationalis. 2021;105(5-6):354-361.

https://doi.org/10.1159/000514097
26. Bouhrour N, Nibbering PH, Bendali F. Medical device-associated biofilm infections and multidrug-resistant pathogens. Pathogens. 2024;13(5):393. https://doi.org/10.3390/pathogens13050393
27. Raina D, Husain U, Kumar P, Pandita AK, Negi N, Kunar P, *et al*. Bacterial isolates and their antimicrobial susceptibility profile in osteomyelitis patients: An experience from a tertiary care center in a hilly area of Uttarakhand. Cureus. 2023;15(8):e44263. https://doi.org/10.7759/cureus.44263
28. Cole JN, Nizet V. Bacterial evasion of host antimicrobial peptide defenses. In: Virulence Mechanisms of Bacterial Pathogens; c2016. p. 413-443. https://doi.org/10.1128/9781555819286.ch15
29. Fereshteh S, Kalhor H, Sepehr A, Rahimi H, Zafari M, Cohan RA, *et al*. Rational design of inhibitors against LpxA protein of *Acinetobacter* baumannii using virtual screening method. Journal of the Indian Chemical Society. 2022;99(2):100319. https://doi.org/10.1016/j.jics.2021.100319
30. Bode NJ, Debnath I, Kuan L, Schulfer A, Ty M, Pearson MM, *et al*. Transcriptional analysis of the MrpJ network: Modulation of diverse virulence-associated genes and direct regulation of mrp fimbrial and flhDC flagellar operons in Proteus mirabilis. Infection and Immunity. 2015;83(6):2542-2556. https://doi.org/10.1128/iai.02978-14
31. Ghaima KK, Hamid HH, Hasan SF. Biofilm formation, antibiotic resistance and detection of mannose-resistant proteus-like (MR/P) fimbriae genes in Proteus mirabilis isolated from UTI. International Journal of Chem. Tech Research. 2017;10(5):964-971.
32. Majnooni MB, Ghanadian SM, Mojarrab M, Bahrami G, Mansouri K, Mirzaei A, *et al*. Antibacterial, antibiofilm, antiswarming, and antioxidant activities of flavonoids isolated from Allium colchicifolium leaves. Journal of Food Biochemistry; c2023. p. 1-10. https://doi.org/10.1155/2023/5521661
33. Lee NY, Khoo WK, Adnan MA, Mahalingam TP, Fernandez AR, Jeevaratnam K, *et al*. The pharmacological potential of *Phyllanthus niruri*. Journal of Pharmacy and Pharmacology. 2016;68(8):953-969. https://doi.org/10.1111/jphp.12565
34. Kaur N, Kaur B, Sirhindi G. Phytochemistry and pharmacology of *Phyllanthus niruri* L.: A review. Phytotherapy Research. 2017;31(7):980-1004. https://doi.org/10.1002/ptr.5825
35. Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J. Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: A review. Journal of Pharmacy and Pharmacology. 2006;58(12):1559-1570. https://doi.org/10.1211/jpp.58.12.0001
36. Kumar S. *Phyllanthus amarus* Schum. and Thonn. as herbal medicine: Ethnobotany, phytochemistry, and pharmacology aspects. Botanical Leads for Drug Discovery; c2022. p. 179-199. https://doi.org/10.1007/978-981-15-5917-4_9
37. Olayem BS, Olaitan OB, Akinola AB. Immunomodulatory plant-based foods, its chemical, biochemical and pharmacological approaches. In: Medicinal Plants-Chemical, Biochemical, and Pharmacological Approaches; c2024. https://doi.org/10.5772/intechopen.112406
38. Pereira RG, Garcia VL, Rodrigues MVN, Martínez J. Extraction of lignans from *Phyllanthus amarus* Schum. and Thonn using pressurized liquids and low-pressure methods. Separation and Purification Technology. 2016;158:204-211. https://doi.org/10.1016/j.seppur.2015.12.007
39. Salsabila S, Hartati R, Al Muqarrabun LM, Qomaladewi NP, Haniffadli A, Rosandy AR, *et al*. Isolation of bioactive compounds with tyrosinase inhibitory activity from the methanol extract of meniran herb (*Phyllanthus niruri* Linn.). Current Research on Biosciences and Biotechnology. 2022;3(2):196-201. https://doi.org/10.5614/crbb.2022.3.2/GOO6ZULV
40. Horii T, Morita M, Muramatsu H, Muranaka Y, Kanno T, Maekawa M, *et al*. Effects of mupirocin at subinhibitory concentrations on flagella formation in *Pseudomonas aeruginosa* and Proteus mirabilis. Journal of Antimicrobial Chemotherapy. 2003;51(5):1175-1179. https://doi.org/10.1093/jac/dkg226
41. Jones A, Morgan D, Walsh A, Turton J, Livermore D, Pitt T, *et al*. Importation of multidrug-resistant *Acinetobacter* spp. infections with casualties from Iraq. The Lancet Infectious Diseases. 2006;6(6):317-318.
42. Petersen FC, Ahmed NA, Naemi A, Scheie AA. LuxS-mediated signalling in *Streptococcus anginosus* and its role in biofilm formation. Antonie Van Leeuwenhoek. 2006;90:109-121. https://doi.org/10.1007/s10482-006-9065-y
43. Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone: A review. Journal of Ethnopharmacology. 2016;190:372-386. https://doi.org/10.1016/j.jep.2016.06.061
44. Ahmed ME, Hasan HM, Kttafah AJ. Characterization and antibacterial activity of biogenic iron nanoparticles using Proteus mirabilis. Medical Journal of Babylon. 2024;21(1):39-45. DOI: 10.4103/MJBL.MJBL_27_23
45. Titiladunayo SO, Oladunmoye MK. In silico and *in vitro* combinatorial study in the fight against the multidrug-resistant uropathogen, *Pseudomonas aeruginosa*. Microbes and Infectious Diseases; c2023. DOI: 10.21608/mid.2023.217574.1542
46. Sigalingging YEWKA, Tarigan AI, Nadapdap T. Comparison of Healing Rates Between Giving Meniran Leaf Extract (*Phyllanthus niruri*) and Topical Antibiotics for Cuts on the Backs of White Wistar Rats (*Rattus norvegicus*). Jurnal Edu. Health. 2023;14(04):118-128. https://ejournal.seaninstitute.or.id/index.php/healt/article/view/3064
47. Ramalingam S, Chandrasekar MJN, Krishnan GG, Nanjan MJ. Plant-based natural products as inhibitors for efflux pumps to reverse multidrug resistance in Staphylococcus aureus: A mini review. Mini Review in Medicinal Chemistry. 2024;24(3):272-288. https://doi.org/10.2174/1389557523666230406092128
48. Álvarez-Martínez FJ, Barrajón-Catalán E, Micol V. Tackling antibiotic resistance with compounds of natural origin: A comprehensive review. Biomedicines. 2020;8(10):405. https://doi.org/10.3390/biomedicines8100405
49. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 2021;9(10):2041. https://doi.org/10.3390/microorganisms9102041
50. Ruddaraju LK, Pammi SVN, Sankar Guntuku G,

Padavala VS, Kolapalli VRM. A review on antibacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nanotechnological combinations. Asian Journal of Pharmaceutical Sciences. 2020;15(1):42-59. https://doi.org/10.1016/j.ajps.2019.03.002

Supplementary DATA

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

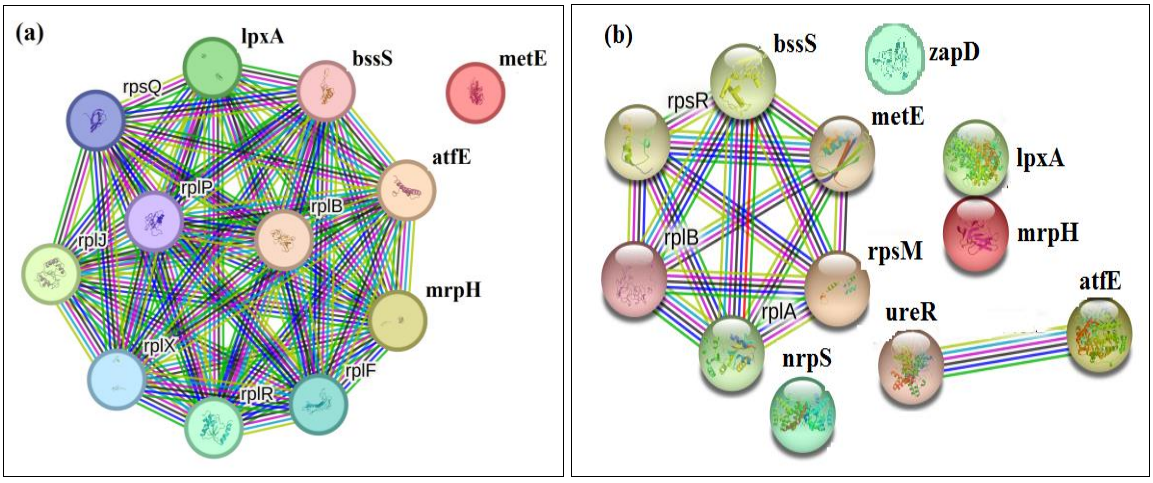
File name	G-Control_1.fastq	G-Control_2.fastq	G-LIN_1.fastq	G-LIN_2.fastq
Total sequences	10385880	10385223	11104596	11942921
Encoding	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9	Sanger/ Illumina 1.9
Sequence length	151	151	151	151
GC %	51	51	51	51
Basic statistics	pass	pass	pass	pass
File name	**G-Control_1. fastq**	**G-Control_2.fastq**	**G-MUP_1.fastq**	**G-MUP_2.fastq**
Total sequences	11380880	11380223	14104596	14942921
Encoding	Sanger / Illumina 1.9	Sanger / Illumina 1.9	Sanger / Illumina 1.9	Sanger / Illumina 1.9
Sequence length	151	151	151	151
GC %	51	51	51	51
Basic statistics	pass	pass	pass	pass

Supplementary Table 2: Statistical summary of the mapping results of the genome of *Proteus mirabilis* (Control and treated with plant derived phytochemicals such as lintetralin/mupirocin) using HISAT2 software.

Alignment details	*Proteus mirabilis*		*Proteus mirabilis* /Lintetralin	
	G-Control_1	G-Control_2	G-LIN_1	G- LIN_2
Total reads	10385880 (100%)	10385223 (100%)	11104596 (100%)	11942921 (100%)
Aligned 0 times	11388800 (99.98%)	11388223 (100%)	14114596 (100%)	14942921 (100%)
Aligned exactly 1 time	88 (0%)	72 (0%)	232 (0%)	224 (0%)
Aligned >1 times	1751 (0.02%)	35 (0%)	469 (0%)	70 (0%)
Alignment details	***Proteus mirabilis***		***Proteus mirabilis* /Mupirocin**	
	G-Control_1	**G-Control_2**	**G-MUP_1**	**G-MUP_2**
Total reads	11380880 (100%)	11380223 (100%)	14104596 (100%)	14942921 (100%)
Aligned 0 times	10380850 (99.98%)	10381223 (100%)	12104596 (100%)	12942921 (100%)
Aligned exactly 1 time	85 (0%)	68 (0%)	228 (0%)	220 (0%)
Aligned >1 times	1748 (0.02%)	31 (0%)	463 (0%)	64 (0%)

Supplementary Table 3: 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		1024
Total up-regulated genes	*p* value < 0.5	≥ 0.8	4
Total down-regulated genes	*p* value < 0.5	≤ -0.8	10



Supplementary Fig 1: Protein-protein interaction network analyses of the down-regulated genes from *Proteus mirabilis* treated with the bioactive phytochemicals such as (a) lintetralin and (b) mupirocin showed closer interactions among different proteins