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Evaluation of Antimicrobial and Antibiofilm Potential of Syzygium diospyrifolium(Wall. Ex Duthie) S.N. Mitra via in Vitro Assays and Molecular Docking Against Bacterial Pathogens

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Abstract
The progressive insensitivity of pathogenic bacteria to antimicrobial agents to conventional treatments it is demands the search for unconventional antimicrobial agents from natural source. In this investigation the leaf and fruit of Syzygiumdiospyrifolium(Wall.ExDuthie) S.N.Mitra were evaluated for their antimicrobial and antibiofilm activities against pathogenic bacteria, S.aureus, B.subtilis, B.pumilus,M.luteus, E.coli and P.aeruginosa. Methanolic extract exhibited significant zone of inhibition and high biofilm percentage indicates potential antimicrobial agent. GC-MS report identified several bioactive palnt-derived compounds, which were carried for further studied to molecular docking against bacterial target protein. Among the identified compounds such as 5-(3-hydroxypropyl)-2,3- and D-allose displayed strong interactions with multiple bacterial proteins, in some cases approaching the binding energies of clinically used antibiotics,These investigation suggest that Syzygiumdiospyrifolium(Wall.ExDuthie) S.N.Mitra indicating positive outcome and innovative antimicrobial and antibiofilm agents. To the best of our review, this is the first documented on the medicinal value of these specific compounds from Syzygium diospyrifolium(Wall.ExDuthie) S.N.Mitra.
Keywords:*Syzygiumdiospyrifolium(Wall.ExDuthie) S.N.Mitra, Meghalaya, Antimicrobial activity, Antibiofilm activity, GC-MS, Molecular docking*

INTRODUCTION

Microbial infections continue to be a major global public health concern earthwide (Guo et al 2020). Over a past decade, the botanical and non-natural compounds with promising antimicrobial potential has become a primary concern of biomedical research. An essential aspects worening bacterial infection is biofilm formation. Biofilms are structured communities of microorganisms envelope in a self-produced extracellular matrix that adheres to complex interplay between biotic and abiotic surfaces (Vaseudevan 2014). Approximately 75% of bacterial infections involve biofilms, which act as barriers to many conventional therapies (Musk et al. 2005). The need for new strategies to prevent or disrupt biofilms is increasingly urgent (Simoes et al. 2007).Surprisingly, despite the advancements of modern medicine the growth of efficient antibacterial drugs pipeline is dwindling , the rising tide of antibiotic resistance enduring challenge for healthcare systems (Loolaie et al. 2017) .Plant-derived compounds have long served as critical resources for development of new therapies due to their historical usage in traditional medicine and perceived safety (Karuppiah and Mustaffa 2013,Rasamiravaka et al. 2015). Improvement in computational methodologies have enhanced the discovery of drug candidates through in silico analyses of intermolecular interactions. Among these computational tools, molecular docking is commonly used to predict the binding affinity and conformation of ligands with target proteins, thereby elucidating potential mode of action (Rasouli et al. 2017 & 2020). **Syzygiumdiospyrifolium(Wall.exDuthie) S.N. Mitra**which is found in Shillong Meghalaya, specifically in Jarain West Jaintia Hills, were consumed by the local people as a curry for their meal and commonly used by traditional healers and local people staying in the area for treating several human diseases and infections such as diarrhea, food poisoning, wounds, skin infections, ulcers, inflammatory disorders, gastric problems .In spite of the well-documented benefits of various Syzygium species, the antibacterial and antibiofilmpotential of **Syzygiumdiospyrifolium (Wall. Ex Duthie) S.N. Mitra**of leaf and fruits prepared in methanol remains

univestigated. Although various Syzygium species have recognized health benefits,no published research exist on the GCMS analysis and evaluation of antimicrobial as well as antibiofilm potential of Syzygiumdiospyrifolium (Wall. Ex Duthie) S.N. Mitra via in vitro assays and molecular docking against antibiotic resistance bacterial pathogens

MATERIAL AND METHOD

Plant Source and Analytical extraction process

The leaves and fruits ofSyzygiumdiospyrifolium(Wall.exDuthie) S.N Mitra were collected from the humid tropical zone of Shillong, Meghalaya,specifically in Jarain, close to the pitcher plant Park in Amlarem Block of West Jaintia Hills District in the month of April and November 2019. The Eastern Regional centre of Botanical Survey of India (BIS) Shillong, authenticated the collected botanical specimens (Ref: BSI/ERC//6/6/2024-2025-tech/619).The plant specimen were cut into smaller fragment and dried in shade (Azwanida 2015,Pandey et al. 2014). Then, they were crushed into fined powder and preserve in airtight containers in a cool and dry location for analytical purpose.Methanol were utilized, typically 1:2 ratio (plant material to solvent) for the isolation of the compounds. The powdered material is transferred into a flask containing methanol and subjected to vigorous shaking for 24 hours or more at ambient temperature. The resulting extracts is then utilized for antimicrobial and antibiofilm activity analysis(Alvarado Gomez et al.2016, Choi et al. 2017).

In vitro Studies (Antimicrobial evaluation)

The antibacterial activities of leaf and fruits of Syzygiumdiospyrifolium(Wall.exDuthie) S.N Mitrawere examined against six bacterial strain which are known to be pathogenic against human four Gram positive bacteria, Bacilussubtilis(ATCC-6633), Baciluspumilus (ATCC-14884),Micrococcus luteus(ATCC-9341),Staphylococcus aureus(ATCC -6538);two Gran negative bacteria Escherichia coli(ATCC-1053),Pseudomonas aeruginosa (ATCC-9027) were provides by Regional Drug Testing Laboratory Guwahati, Assam.The methanolic leaf and fruit extracts of Syzygiumdiospyrifolium (Wall. ex Duthie) S.N. Mitra(10µg) were inoculated against six selected pathogenic bacteria strain using agar well diffusion method. The plates were then incubated at 37ºC for 24 hours. Following incubation, the diameters of the incubation zones were measured and compared with those produced by standard antibiotics (DamikiLaloo et al. 2020)

In vitro Studies (Antibiofilm evaluation)

Biofilm inhibition was carried out using crystal violet microtiterolate assay as illustrated by (Vasudevan 2014) with a slight modification.Bactrial structure were fixed to 1×10^6 CFU/mL and incubate with 100µg/mL of Syzygiumdiospyrifolium (Wall.exDuthie) S.N Mitra leaf and fruit methanol extract in 96-well plates at 37ºC for 24 hours. After incubation well were washed with PBS(phosphate-buffered saline),fixed with methanol stained with 0.1% Crystal violet and dye is solubilized in ethanol. Biofilm inhibition was determined by measuring absorbance 570nm, and inhibition was calculated compared to untreated control.

In silico analysis

(i) Compound Identification and Selection (GC-MS-Based Compound Identification)

The methanolic leaf and fruit extract of Syzygiumdiospyrifolium (Wall.exDuthie) S.N Mitra subjected to GCMS analysis. The GCMS analysis was performed in a Thermo Fisher Scientific GC system at the Central Instrumentation Facility (CIF), University of Science and Technology Meghalaya, India. The system was furnished with a split/splitless injection port with a consideration of 1/100 split ratio. It was accompanied by an electron impact mode set ISQ7000 mass spectrometer system. A column fused with TG-5MS silica capillary with the specification of 30 m × 0.25 mm i.d. and 0.25 µm film thickness was employed for the analysis. The initial temperature setting was for 3 min at 60 °C followed by elevation to 230°C at 5°C per minute. Both the transfer line and the injector temperature were maintained at 230°C and 290°C discretely. The ion source block was directly connected to the column outlet. Setting of the flow rate of ultrapure helium as a carrier gas was concluded at 1 ml per minute with 0.2.µL of injection volume. The Chromeleon™ Software which was provided along with the instrument was employed for the calculation of peak area percentages. The qualitative analysis was performed in dilute solutions of pre-determined concentrations. Confirmation of compound identifications was finalized by comparative analysis of the laboratory retention indices and NIST library-derived mass spectrum of undisputed standards as reference

(ii) **Selection of the protein target and preparation**

Selections of the protein targets for docking were selected on the basis research papers (Patel et al.2018,Tabassum et al., 2023, Balaji and Mahatingam 2025). The 3D structure of the proteins was downloaded from PDB (Berman et al. 2000) and modelled structure along with uniport IDs. For the protein whose crystal structures are not available, modelled structures were downloaded from Alphafold available in Uniprot database (Pettersen EF et al., 2004). The protein structures were optimized using the AutoDock Tools plug- in included in PyRx. The ligands corresponding to PUBMED IDs are processed in PyRx by minimizing all the ligands, ensuring no steric clashes and proper charge distribution. The prepared protein and ligand files were converted to pdbqt file format.

(iii) Selection of the ligands

Selection of the ligands was done using ADME and Toxicity prediction ADME and toxicity of all the compounds were predicted using SWISSADME and ADMETlab3.0 Lead likeliness of the compounds, pharmacokinetic properties and their violations to drug ability rules, such as Lipinski, Ghose and Veber were studied using SWISSADME (Michielin& amp; Zoete, V. (2017). The molecules that met the acceptance criteria from all the three rules will be presenting. The molecules which passed hERG Blockers, Ames mutagenesis, Rat oral acute toxicity, Eye corrosion, respiratory toxicity Genotoxic and immunotoxicity parameters obtained from ADMETlab3 web server will be presenting(Fu, L. & Cao, D. 2024).

ADME and Toxicity prediction

ADME and toxicity of all the compounds were predicted using SWISSADME (http://www.swissadme.ch/index.php)and ADMETlab3.0 (https://admetlab3.scbdd.com/) web servers.Leadlikeliness of the compounds, pharmacokinetic properties and their violations to drug ability rules, such as Lipinski, Ghose and Veber were studied using SWISSADME.

Molecular Docking

A total of 31 compounds and 6 reference molecules have been docked with 11 proteins of bacteria using PyRx software. A grid box was defined around the whole protein covering the entire possible binding pocket to ensure accurate docking of ligands. For all the proteins, grid box allocation dimensions will be present.The docking process was executed using the AutoDockVina algorithm integrated within PyRx. For each protein
ligand complex, 8 docking runs were performed. The scoring function outputted predicted binding affinities and docking pose (Trott& Olson, 2010).

RESULT AND DISCUSSION

Antibacterial activity

The metabolic extracts of leaves and fruits (1 mg/mL) exhibited varying degrees of antibacterial activity against six bacterial strains, as evaluated by the zone of inhibition (mm) as shown asin **Table 1**. Across all bacterial strains tested, the standard antibiotics generally showed higher or comparable efficacy than the plant extracts, except in the case of Staphylococcus aureus, where the leaf extract was more effective. Among the Gram-positive bacteria, the leaf extract showed the highest activity against Staphylococcus aureus (21.25 ± 1.09 mm), which surpassed the activity of the standard antibiotic tobramycin (20.25 ± 0.3 mm). Other notable zones of inhibition for the leaf extract included 18.75 ± 0.5 mm for Bacillus pumilus, 18.25 ± 0.5 mm for Micrococcus luteus, and 15.75 ± 0.5 mm for Bacillus subtilis. In the case of Gram-negative bacteria, the leaf extract exhibited moderate activity, with zones of inhibition measuring 13.5 ± 0.2 mm for Escherichia coli and 13.75 ± 0.3 mm for Pseudomonas aeruginosa. These values were lower compared to their respective standard antibiotics (streptomycin and neomycin). The fruit extract demonstrated comparatively lower antibacterial activity, with the highest zone observed against Staphylococcus aureus (18.75 ± 0.5 mm).

Table 1. Antibacterial activity of methanolic leaf and fruit extract of Syzygiumdiospyrifolium (Wall.exDuthie) S.N Mitra against selected human pathogens

Antibiofilm activity

The methanolic leaf extract exhibited significant biofilm inhibitory activity against all tested bacterial strains, with inhibition percentages ranging from 31% to 80.9% as shown in **Table 2**. The highest biofilm

Bacterial strain	MethanolicLeaf extract(1mg/ml)	MethanolicFruit extact(1mg/ml)	Standard antibiotic	Zone of standard antibiotic(1mg/ml)
Staphylococcus aureus	21.25±1.09	18.75±0.5	*Tobramycin*	20.25±0.3
Bacillus subtilis	15.75±0.5	12.75±0.95	*Amikacin*	16.75±0.95
Bailluspumilus	18.75±0.5	15.25±0.5	*Gentamicin*	20.5±0.4
Micrococcus luteus	18.25±0.5	16.25±0.5	*Erythromycin*	20..25±0.3
Escherechia coli	13.5±0.2	11.5±0.2	*Streptomycin*	15.75±0.5
Psudomonasaeruginosa	13.75±0.3	11.75±0.5	*Neomycin*	18.75±0.5

inhibition was recorded against Pseudomonas aeruginosa (80.9%), followed closely by Bacillus pumilus (80%), Escherichia coli (77.47%), and Bacillus subtilis (76%). Notably, the extract showed greater inhibition in all strains compared to their respective standard antibiotics.ForStaphylococcus aureus, the extract inhibited biofilm formation by 63.2%, which was higher than tobramycin (55%). Similarly, for E. coli, the extract showed 77.47% inhibition compared to 59.8% by streptomycin. The lowest inhibition was observed against Micrococcus luteus (31%), which was lower than the standard erythromycin (51.4%).

Table 2.Biofim inhibition (%) of leaves extract of Syzygiumdiospyrifolium (Wall.exDuthie) S.N. Mitra prepared in methanol compared with Reference Standard Antibiotic against six bacterial strains

The methanolic fruit extract exhibited strong antibiofilm activity against a range of Gram-positive and Gram-negative bacterial strains, with inhibition percentages ranging from 36% to 82.8% as shown in **Table 3**. The highest inhibition was observed against Bacillus pumilus (82.8%), followed closely by Pseudomonas aeruginosa (82.5%) and Escherichia coli (81.3%). These values were substantially higher than those obtained with their respective standard antibiotics, which generally showed inhibition around 55–60%.In the case of Staphylococcus aureus and Bacillus subtilis, the fruit extract inhibited biofilm formation by 67.8% and 80%, respectively, both exceeding the performance of the standard antibiotics (55% in both cases). For Micrococcus luteus, the extract showed the lowest antibiofilm activity (36%), which was less effective compared to erythromycin (51.4%).

Table 3.Biofilm inhibition (%) of fruit extract of Syzygiumdiospyrifolium (Wall.exDuthie) S.N. Mitra prepared in methanol compared with Reference Standard Antibiotic against six bacterial strains

Compound Identification and Selection (GC-MS-Based Compound Identification)

The GCMS chromatogram of both fruits and leaves extract of S. diospyrifolium (Wall.exDuthie) S.N. Mitra prepared in methanol confirms the eventuality of numerous compounds with different retention times. Comprehensive analysis of compounds present in fruits and leaves by GC-MS analysis is shown in **Table 4a,b**.Fruits are dominated by sugars and fatty acid amides whereas; leaves contain more sterols, terpenoids, and phenolic compounds.

Bacterial strain	Fruit ($OD_{control}$)	Fruit ($OD_{treated}$)	Inhibition (%)	Std.Antibiotic	$OD_{control}$	$OD_{treated}$	Inhibition (%)
Staphylococcus aureus	1.00±0.02	0.322±0.002	67.8%	Tobramycin	1.00±0.02	0.450±0.002	55%
Bacillus subtilis	1.00±0.02	0.200±0.001	80%	Amikacin	1.00±0.02	0.450±0.002	55%
Bacillus pumilus	1.05±0.02	0.180±0.001	82.8%	Gentmicin	1.05±0.02	0.450±0.002	55%
Escherechia coli	1.02±0.001	0.190±0.001	81.3%	Streotomycin	1.02±0.001	0.410±0.001	59.8%
Pseudomonas aeruginosa	1.10±0.01	0.192±0.001	82.5%	Neomycin	1.10±0.01	0.460±0.001	58.1%
Micrococcusluteus	1.00±0.02	0.640±0.001	36%	Erythromycin	1.00±0.02	0.486±0.002	51.4%

Table 4a:GC-MS analysis of Syzygiumdiospyrifolium (Wall.exDuthie) S.N. Mitra leaves extract prepared in methanol

S.No	**RT**	**Compound name**	**% Peak area**	**Mol. Formula**	**Mol. weight (g/mol)**
1	3.113	Cyclohexene-3,5-diol, cis-	0.31%	$C_6H_{10}O_2$	114.14
2	3.779	Melibiose	0.40%	$C_{12}H_{22}O_{11}$	342.30
3	4.681	Cyclopentane, 1-acetyl-1,2-epoxy-	3.28%	$C_7H_{10}O_2$	126.15
4	5.317	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	3.85%	$C_6H_8O_4$	144.12
5	5.983	5Hydroxymethylfurfural	15.30%	$C_6H_6O_3$	126.11
6	6.623	6-Acetyl-ß-d-mannose	0.13%	$C_6H_{14}O_7$	222.19
7	7.293	1,2,4-Benzenetriol	4.53%	$C_6H_6O_3$	126.11
8	8.391	Melezitose	13.91	$C_{18}H_{32}O_{16}$	504.4
9	8.585	Maltose	1.52%	$C_{12}H_{22}O_{11}$	342.3
10	10.075	Lactose	4.66%	$C_{12}H_{22}O_{11}$	342.3
11	14.367	1-Heptatriacotanol	0.18%	$C_{37}H_{76}O$	537.0
12	14.904	n-Hexadecanoic acid	0.62%	$C_{16}H_{32}O$	256.43
13	17.758	Linoelaidic acid	0.47%	$C_{18}H_{32}O_2$	280.4
14	23.741	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3.18%		
15	26.230	(Z)-3-(Heptadec-10-en-1-yl)phenol	8.97%	$C_{22}H_{38}O$	330.6
16	26.768	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.91%	$C_{21}H_{42}O_4$	358.5
17	27.693	Palmitoleamide	24.71%	$C_{16}H_{31}NO$	
18	34.008	ß-Sitostero	0.89%	$C_{29}H_{50}O$	414.7
19	35.352	Methyl glycocholate, 3TMS derivative	0.53%	$C_{36}H_{69}NO_6Si_3$	696.2
20	38.410	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecam	0.39%	$C_{16}H_{50}O_7Si_8$	576.15

Table 4b:GC-MS analysis of Syzygiumdiospyrifolium(Wall.exDuthie) S.N. Mitra fruits extract prepared in methanol

S.No	RT	Compound name	% Peak area	Mol. Formula	Mol. weight (g/mol)
1	3.773	DL-Arabinose	0.64%	$C_5H_{10}O_5$	150
2	4.674	Cyclopentane, 1-acetyl-1,2-epoxy-	0.57%	$C_7H_{10}O_2$	126.15
3	5.317	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.96%	$C_6H_8O_4$	144.12
4	5.463	l-Gala-l-ido-octose	0.03%	$C_8H_{16}O_8$	240
5	5.977	5-Hydroxymethylfurfural	1.05%	$C_6H_6O_3$	126.11
6	7.269	1,2,3-Benzenetriol	12.23%	$C_6H_6O_3$	126.11
7	8.395	D-Allose	1.41%	$C_6H_{12}O_6$	
8	9.776	Melezitose	0.52%	$C_{18}H_{32}O_{16}$	504.4
9	12.986	Neophytadiene	2.21%	$C_{20}H_{38}$	278.5
10	13.922	5-(3-Hydroxypropyl)-2,3-dimethoxyphenol	1.55%	$C_{11}H_{16}O$	212.2
11	14.928	n-Hexadecanoic acid	1.92%	$C_{16}H_{32}O$	256.43
12	17.891	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	2.00%	$C_{21}H_{36}O_2$	320
13	23.523	Phenol, 3-pentadecyl-	1.57%	$C_{21}H_{36}O$	304
14	26.268	(Z)-3-(Heptadec-10-en-1-yl)phenol	29.21%	$C_{23}H_{38}O$	330.6
15	27.693	13-Docosenamide, (Z)-	%1.39	$C_{23}H_{43}NO$	337.6
16	28.352	Squalene	2.14%	$C_{30}H_{50}$	410.7
17	31.903	dl-a-Tocopherol	3.55%	$C_{29}H_{50}O_2$	430.7
18	34.026	ß-Sitosterol	1.96%	$C_{29}H_{50}O$	414.7
19	37.233	Friedelan-3-one	2.40%	$C_{30}H_{50}O$	426.7

Selection of the protein target and preparation

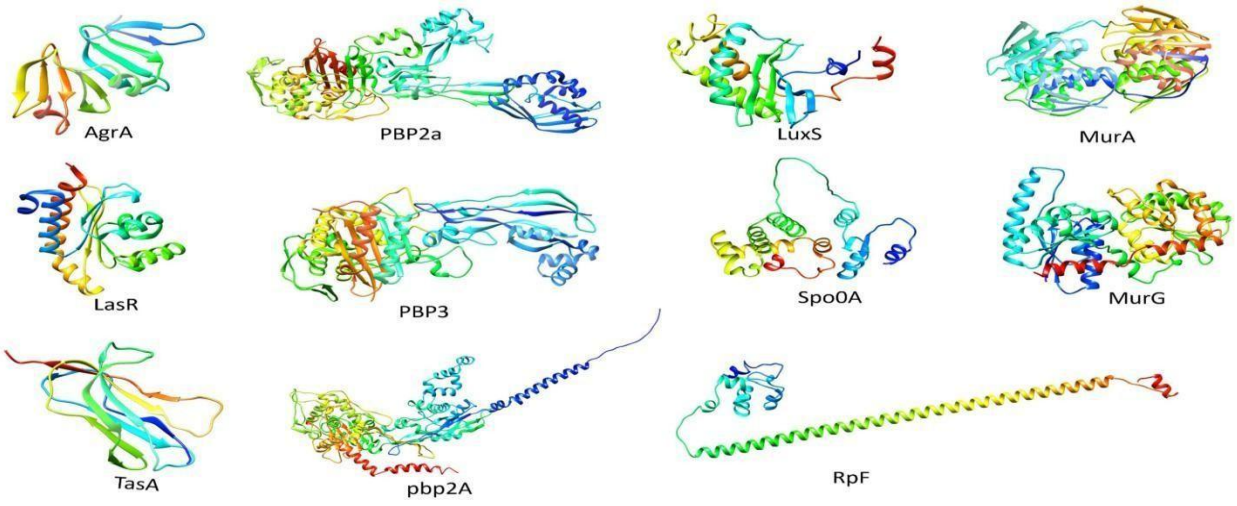
Protein targets responsible for antibiofilm and antibacterial were taken into consideration for the study. The 3D structure of the proteins was downloaded from PDB (Bermanet al. 2000) and modelled structure along with uniport IDs are listed in Table5 and Fig.1 . For the protein whose crystal structures are not available, for them modelled structures were downloaded from Alphafold available in Uniprot data base.The protein structures were optimized using the AutoDock Toolsplug-inincluded in PyRx.

Table5. List of target proteins displaying the PDBIDs and modelled structure's uniport IDs

Bacteria	Proteinsymbol	ProteinName	Function	Antibiofilm/ Antibacterial	Resolutionand Method	Sequencelength
Staphylococcus aureus	AgrA	4G4K	Accessory gene regulator A) – Regulates quorum sensing for biofilm control	Antibiofilm	1.52Å,X-ray	103
	PBP2a	1VQQ	Penicillin-binding protein 2a) – Key for β-lactam resistance.	Antibacterial	1.80 Å	646
Escherichia coli	LuxS	Modelled (Q8X902)	LuxS (Autoinducer synthase) – Regulates quorum sensing.	Antibiofilm	-	171

	MurA	3SWD	MurA (UDP-N-acetylglucosamineenolpyruvyltransferase) – Key for the first step of peptidoglycan biosynthesis	Antibacterial	2.5Å,X-ray	418
Pseudomonas aeruginosa	LasR	3IX3	LasR (Quorum sensing regulator) – Controls virulence and biofilm formation.	Antibiofilm	1.40Å,X-ray	173
	PBP3	4WEL	PBP3 (Penicillin-binding protein 3) – Important for cell wall integrity.	Antibacterial	1.99Å,X-ray	538
Bacilluspumilus	Spo0A	Modelled (P52933)	Spo0A (Sporulation master regulator) – Controls biofilm initiation.	Antibiofilm	-	191
	MurG	Modelled (A8FCY1)	MurG (UDP-N-acetylglucosaminetransferase) – Essential for peptidoglycan biosynthesis.	Antibacterial	-	364
Bacillus subtilis	TasA	6HQC	TasA (Amyloid-like protein for matrix stability) – Required for robust biofilm structure.	Antibiofilm	1.28Å,X-ray	116
	pbp2A	Modelled (P54488)	PBP2 (Penicillin-binding protein 2) – Key for cell wall biosynthesis.	Antibacterial	-	716
MicroccousLeteus	Rpf	Modelled (Q6UC89)	Rpf (Resuscitation-Promoting Factor) protein plays a crucial role in bacterial growth and resuscitation of dormant cells	Antibacterial	-	210

Figure 1. 3Dimagesofallthe11bacterialproteins



ADME and Toxicity prediction

A total of 8 molecules got acceptance from all the three rules as shown in Table 6 . Most of the 8 molecules passed hERG Blockers, Ames mutagenesis, Rat oral acute toxicity, Eye corrosion, respiratory toxicity Genotoxic and immunotoxicity parameters obtained from ADMETlab3 web server as shown in Table 7(Supplementary file)

Table6. The lead likeliness and pharmacokinetic profiles of the selected compounds and reference molecule

Sl.No.	Compound CID	MW<500	HBA<10	nHD≤5	ThreeruleViolation			MLogP ≤4.15	TPSA(Å^2) <140	Bioavailability Score > 0.1
					Lipinski	Ghosh	Veber			
1	854 (DL-Arabinose)	150.05	5	4	0	0	0	-2.48	97.99	0.55
2	119838 (4H-Pyran-4-one, 2,3-dihydro-3,5-)	144.04	4	2	0	0	0	-1.77	66.76	0.85
3	219659 (l-Gala-l-ido-octose)	240.08	8	7	0	0	0	-3.81	158.68	0.55
4	237332 (5-Hydroxymethylfurfural) (HMF)	126.03	3	1	0	0	0	-1.06	50.44	0.55
5	439507 (D-Allose)	180.06	6	5	0	0	0	-2.75	110.38	0.55
6	537123 (Cyclopentane)	126.07	2	0	0	0	0	0.42	29.6	0.55
7	565903 (Cyclohexene-3,5-diol, cis-)	114.07	2	2	0	0	0	0.07	40.46	0.55
8	72942686 (5-(3-Hydroxypropyl)-2,3-)	212.1	4	2	0	0	0	0.93	58.92	0.55
9	36294 Tobramycin	467.26	14	15	2	1	1	-6.3	268.17	0.17
10	37768 Amikacin	585.29	18	17	3	3	2	-8.42	331.94	0.17
11	3467 Gentamicin	477.32	12	11	2	2	1	-3.33	199.73	0.17
12	12560 Erythromycin	733.46	14	5	2	3	1	1.79	193.91	0.17
13	19649 Streptomycin	581.27	19	16	3	4	1	-8.16	336.43	0.17
14	8378 Neomycin	614.31	19	19	3	4	1	-8.9	353.11	0.17

Table7. Toxicity parameters were calculated using ADMET lab3.0 for the selected compounds

Sl. No	Phytochemicals	hERG Blockers	Ames	Ratoralacute toxicity	Eyecorrosion	Respiratory Toxicity	Immunotoxicity	Genotoxicity
1.	854 (DL-Arabinose)	0.39 (Negative)	0.20 (Negative)	0.031 (Negative)	0.0009 (Negative)	0.003 (Negative)	0.02 (Negative)	0.001 (Negative)

2.	119838 (4H-Pyran-4-one, 2,3-dihydro-3,5-)	0.08 (Negative)	0.82 (Positive)	0.26 (Negative)	0.64 (Positive)	0.28 (Negative)	0.06 (Negative)	0.68 (Negative)
3.	219659 (l-Gala-l-ido-octose)	0.40 (Negative)	0.43 (Negative)	0.02 (Negative)	2.32 (Positive)	0.000103 (Negative)	0.01 (Negative)	0.0004 (Negative)
4.	237332 (5-Hydroxymethylfurfural) (HMF)	0.25 (Negative)	0.80 (Positive)	0.42 (Negative)	0.89 (Positive)	0.68 (Positive)	0.04 (Negative)	0.96 (Positive)
5.	439507 (D-Allose)	0.21 (Negative)	0.88 (Positive)	0.22 (Negative)	0.09 (Negative)	0.22 (Negative)	0.10 (Negative)	0.37 (Negative)
6.	537123 (Cyclopentane)	0.43 (Negative)	0.60 (Positive)	0.52 (Positive)	0.07 (Negative)	0.56 (Positive)	0.06 (Negative)	0.69 (Positive)
7.	565903 (Cyclohexene-3,5-diol, cis-)	0.26 (Negative)	0.33 (Negative)	0.27 (Negative)	0.99 (Positive)	0.53 (Positive)	0.02 (Negative)	0.008 (Negative)
8.	72942686 (5-(3-Hydroxypropyl)-2,3-)	0.51 (Positive)	0.26 (Negative)	0.12 (Negative)	0.88 (Positive)	0.62 (Positive)	0.05 (Negative)	0.009 (Negative)
9.	36294 Tobramycin	0.018 (Negative)	0.41 (Negative)	0.003 (Negative)	0.0001 (Negative)	0.015 (Negative)	0.27 (Negative)	0.02 (Negative)
10.	37768 Amikacin	0.02 (Negative)	0.66	0.002 (Negative)	1.77 (Positive)	0.004 (Negative)	0.31 (Negative)	0.64
11.	3467 Gentamicin	0.4 (Negative)	0.20 (Negative)	0.50 (Positive)	2.83 (Positive)	0.30 (Negative)	0.11 (Negative)	0.47 (Negative)
12.	12560 Erythromycin	0.03 (Negative)	0.32 (Negative)	0.03 (Negative)	0.0001 (Negative)	0.02 (Negative)	0.38 (Negative)	0.22 (Negative)
13.	19649 Streptomycin	0.004 (Negative)	0.98	0.01 (Negative)	1.45 (Positive)	0.0009 (Negative)	0.24 (Negative)	0.99
14	8378 Neomycin	0.01 (Negative)	0.79	0.001 (Negative)	1.68 (Positive)	0.006 (Negative)	0.30 (Negative)	0.04 (Negative)

Selection of the ligands

Theligands correspondingtoPUBMEDIDs areprocessedinPyRxbyminimizingall the ligands, ensuring no steric clashes and proper charge distribution. The prepared protein and ligand fileswereconvertedtopdbqtfileformat. (Table for ligand)

Molecular Docking

The prepared protein and ligand files were converted to pdbqt file format. A grid box was defined around the whole protein covering the entire possible binding pocket to ensure accurate docking of ligands. For all the proteins, grid box allocation dimensions are presented in **Table 8 (Supplementary file)**. A total of 8 compounds and 6 reference molecules have been docked with all the 11 proteins of bacteria using PyRx software as shown in Table 9. The binding affinity analysis presented in Table 9 demonstrates that the selected eight ADMET-validated compounds exhibit variable interaction strengths with multiple bacterial protein targets when compared with standard antibiotics. Overall, the reference molecules such as Streptomycin (–7.9 kcal/mol), Gentamicin (–7.4 kcal/mol), and Erythromycin (–6.7 kcal/mol) showed stronger binding affinities, which was expected given their established antimicrobial efficacy. Among the phytocompounds, PubChem ID 72942686 (5-(3-Hydroxypropyl)-2,3-) emerged as the most promising candidate, with docking scores reaching –6.0 kcal/mol against LasR and –5.8 kcal/mol against MurG, values that approach those of conventional antibiotics. Similarly, D-Allose (PubChem ID 439507) and l-Gala-l-ido-octose (PubChem ID 219659) demonstrated consistent binding affinities across multiple targets, notably against pbp2A, LasR, and MurG, suggesting potential broad-spectrum activity. In contrast, compounds such as Cyclopentane

(PubChem ID 537123) and Cyclohexene-3,5-diol (PubChem ID 565903) showed weaker interactions, indicating limited antimicrobial promise. Target-specific trends were also evident, with LasR and MurG being the most sensitive proteins, receiving moderate to strong affinities from several natural compounds, whereas AgrA and Spo0A showed weaker interactions overall. Although none of the tested natural molecules surpassed the binding efficiencies of antibiotics, their overlapping affinities with certain bacterial targets highlight their potential as lead scaffolds for antimicrobial drug development.The docking process was executed using the AutoDockVina algorithm integrated within PyRx. For each protein-ligand complex, 8 docking runs were performed. The scoring function outputted predicted binding affinities and docking pose.Those 8 molecules passed most of the toxicity parameters displayed less binding affinity than the reference molecules.

Table8. Grid box allocation for the proteins

Sl No	**Bacteria**	**Proteins**	**Center**	**Size**
1.	S.Aureus	AgrA(4G4K)	X=17.57,Y=15.32,Z=42.03	X=38.23,Y=46.30,Z=43.90
2.		PBP2a(1VQQ)	X=23.40,Y=26.68,Z=41.65	X=76.76,Y=70.60,Z=134.32
3.	E.Coli	LuxS(Modelled)	X=4.66,Y=1.58,Z=3.60	X=61.93,Y=44.60,Z=49.98
4.		MurA(3SWD)	X=1.10,Y=-0.95,Z=0.49	X=55.36,Y=67.59,Z=57.80
5.	P. Aeruginosa	LasR(3IX3)	X=11.45,Y=1.60,Z=17.76	X=44.49,Y=54.92,Z=60.52
6.		PBP3(4WEL)	X=4.93,Y=22.47,Z=7.09	X=66.72,Y=73.17,Z=102.74
7.	B.Pumilus	Spo0A(Modelled)	X=-10.40,Y=-6.86,Z=8.65	X=62.49,Y=66.56,Z=68.16
8.		MurG(Modelled)	X=-4.10,Y=1.70,Z=-1.55	X=69,Y=55.97,Z=66.52
9.	B.Subtilis	TasA(6HQC)	X=8.42,Y=-0.71,Z=-15.29	X=48.26,Y=48.03,Z=40.04
10.		pbp2A(Modelled)	X=-1.01,Y=5.51,Z=-13.29	X=93.03,Y=80.52,Z=111.40
11.	Micrococcus luteus	Rpf(Modelled)	X=-21.69,Y=2.76,Z=32.59	X=72.25,Y=53.55,Z=58.64

Table 9. Binding affinity of the selected eight compounds which obeys ADMET along with reference molecules

Sl.No.	**PubChemIDS**	**S.aureus**		**E.coli**		**P.aeruginosa**		**B.pumilus**		**B.subtilis**		**M. luteus**
		AgrA (4G4K)	PBP2a (1VQQ)	LuxS (Modelled)	MurA (3SWD)	LasR (3IX3)	PBP3 (4WEL)	Spo0A (Modelled)	MurG (Modelled)	TasA (6HQC)	pbp2A (Modelled)	Rpf(Modelled)
1.	854 (DL-Arabinose)	-4.1	-4.1	-3.7	-4.3	-5.1	-4.8	-3.5	-4.7	-4.2	-4.4	-3.9
2.	119838 (4H-Pyran-4-one, 2,3-dihydro-3,5-)	-4.1	-4.9	-4.1	-5.1	-6.0	-5.2	-4.2	-5.0	-4.6	-5.4	-4.2
3.	219659 (l-Gala-l-ido-octose)	-4.4	-5.2	-4.0	-5.2	-5.6	-5.5	-4.7	-5.5	-4.7	-5.5	-4.5
4.	237332 (5-Hydroxymethylfurfural) (HMF)	-4.0	-4.8	-4.0	-4.7	-5.4	-4.7	-4.1	-4.9	-4.1	-5.1	-4.2

5.	439507 (D-Allose)	-4.5	-5.1	-4.7	-5.4	-5.6	-5.3	-4.3	-5.4	-5.0	-5.9	-4.4
6.	537123 (Cyclopentane)	-3.7	-4.6	-4.4	-4.7	-5.5	-4.8	-4.3	-5.3	-3.9	-4.5	-4.5
7.	565903 (Cyclohexene-3,5-diol, cis-)	-3.8	-4.2	-3.9	-4.6	-4.9	-4.5	-4.1	-5.1	-3.9	-4.3	-4.1
8.	72942686 (5-(3-Hydroxypropyl)-2,3-)	-4.6	-5.5	-4.9	-5.6	-6.0	-5.5	-4.6	-5.8	-5.1	-5.7	-5.0
9.	36294 Tobramycin	-5.5	-6.3									
10.	19649 Streptomycin			-6.3	-7.9							
11.	Neomycin8378					-5.9	-6.4					
12.	3467 Gentamicin							-5.7	-7.4			
13.	37768 Amikacin									-5.4	-6.5	
14	12560 Erythromycin											-6.7

CONCLUSION

The present study highlights the promising antibacterial and antibiofilm potential of methanolic leaf and fruit extracts of Syzygiumdiospyrifolium (Wall. ex Duthie) S.N. Mitra against a broad spectrum of pathogenic bacteria. The methanolic leaf extract exhibited notably higher antibacterial activity than the fruit extract, with zones of inhibition in several cases comparable to or exceeding those of standard antibiotics—particularly against Staphylococcus aureus, Bacillus pumilus, and Micrococcus luteus. Antibiofilm assays further confirmed the efficacy of the leaf extract, demonstrating substantial inhibition rates—up to 80.9% against Pseudomonas aeruginosa—often surpassing the inhibitory effects of the tested standard antibiotics. In silico molecular docking supported these experimental observations by revealing favorable binding affinities of selected bioactive compounds to key bacterial targets involved in cell wall synthesis, quorum sensing, and biofilm formation. Notably, compounds such as 5-(3-hydroxypropyl)-2,3- and D-allose displayed strong interactions with multiple bacterial proteins, in some cases approaching the binding energies of clinically used antibiotics. The methanolic leaf extract showed very good growth inhibition against Gram-positive bacteria like S. aureus (21.25 mm) and B. pumilus (18.75 mm), which were on par with their standard antibiotics; however, Gram-negative bacteria (E. coli, P. aeruginosa) showed smaller zones (13–14 mm), which reflect less activity. The leaf extract always showed larger antibiofilm inhibition than its antibacterial inhibition zones. In silico studies showed moderate to excellent binding affinities, although less than standard antibiotics. Binding scores show likely antibiofilm-related target inhibition versus antibacterial protein targets. In summary, the complementary in vitro and in silico studies put forward that the methanolic leaf extract, which is rich with efficacious phytoconstituents, holds much promise as a future source of novel antibacterial and antibiofilm moieties. However, more studies are required to uncover the therapeutic possibility of these plant origin bioactives in the control of multidrug-resistant bacterial infections.

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