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In vitro and in Silico Guided Identification of Anti-Biofilm Agents from Syzygium palghatense Against Pseudomonas aeruginosa

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Abstract

Background: Pseudomonas aeruginosa is a major opportunistic pathogen in cystic fibrosis, wound, and nosocomial infections, posing a serious burden to public health, due to its antibiotic resistance. Antibiofilm agents serve as an essential tool in the fight against antibiotic resistance.

Objective: This study investigates the in vitro and in Silico Guided Identification of Anti-Biofilm Agents from Syzygium palghatense Against Pseudomonas aeruginosa

Methods: Leaves and bark of Syzygium palghatense were collected. Reference strains which were used to evaluate the antimicrobial activity of the methanol extract of leaves and bark of Syzygium palghatense were Aspergillus niger (ATCC 16404) and Candida albicans (ATCC 10231) for fungi and Staphylococcus aureus (ATCC 25923), Streptococcus mutans (MTCC 890) and Enterococcus fecalis (ATCC 2912) for Gram Gram-positive bacteria and E. coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853) for Gram Negative Bacteria. MIC values for filamentous fungi were determined using broth microdilution with methanol leaf and bark extracts serially diluted in DMSO (10–300 μg/ml). Antibiofilm activity against Pseudomonas aeruginosa was assessed, and molecular docking of bioactive compounds was performed against the target proteins of P. aeruginosa.

Results: The antimicrobial activity of methanol extracts from the leaves and bark of Syzygium palghatense varied against different pathogenic microorganisms. Pseudomonas aeruginosa shows higher activity. Syzygium palghatense extract has the ability to suppress the biofilm formation by Pseudomonas aeruginosa. Molecular docking shows a good docking score.

Conclusion: Methanol extracts of Syzygium palghatense demonstrated significant antimicrobial and antibiofilm activity against Pseudomonas aeruginosa, supported by favourable molecular docking results. These findings suggest that S. palghatense could serve as a potential natural source of anti-biofilm agents for managing P. aeruginosa-associated infections.

Keywords: Syzygium Palghatense, Pseudomonas Aeruginosa, Antimicrobial, Antibiofilm, Molecular Docking

Introduction

Pseudomonas aeruginosa is a Gram-negative bacilli that is capable of surviving in adverse conditions. Its large genome (5.5–7 Mb) is composed of a conserved core and an accessory section with high plasticity. Thus this bacteria is highly versatile and can very much adapt to the harsh environmental conditions [1,2]. It is one of the major causes of hospital-acquired infections and of chronic pulmonary infections in patients suffering from cystic fibrosis (CF) or non-CF bronchiectasis, increasing mortality [3,4]. Pathogenicity of this bacterium is exaggerated by its innate virulence.

External components of Pseudomonas aeruginosa cells enable the establishment and persistence of infections. Outer membrane lipopolysaccharides protect the pathogen against phagocytosis and oxidative stress, but also promote as covering lectins the adhesion to host tissues. The type IV pili and flagellum allow planktonic cells to move from one infection site to another [5,6]. In addition, the bacterium also secretes virulence factors. Their production is tightly regulated by quorum sensing (QS), the bacterial cell–cell communication network. QS is based on the production, release, and perception of small molecules called autoinducers (AIs) that coordinate the expression of specific genes involved in virulence behaviours.

The development of anti-biofilm agents (ABAs) can alleviate the burden caused by P. aeruginosa infections. ABAs are specific anti virulence agents (AVAs) that reduce virulence by targeting the biofilm. ABAs and, more generally, AVAs differ from antibiotics by their lack of effect on bacterial cell growth. ABAs can hinder the formation or the dispersion of biofilm without eradicating sessile microcolonies. They can act as (i) c-di-GMP pathway inhibitors (slowing down the production of biofilm or activating its dispersion), (ii) QS inhibitors (QSIs) (diminishing the production of matrix components and AIs as well as toxin secretion) or (iii) lectin inhibitors (impairing the biofilm structure). Some conventional ATBs or antimicrobial peptides have also been studied for their anti-biofilm properties at concentrations that do not affect bacterial growth, as has the nanovectorization biotechnological approach allowing an extracellular matrix permeabilization. We aim to highlight the critical features that enable molecular key interactions with targets to pave the way for the rational development of new ABAs against P. aeruginosa.

Materials and Methods

Leaves and bark of Syzygium palghatense were collected from Nelliyampathy, Nemmara forest division, Palakkad, Kerala, India. Identification of sample was confirmed and a voucher specimen was deposited at the Calicut University Herbarium.

Then**,** Shade-dried, powdered leaves and bark of Syzygium palghatense (20 g each) were extracted with methanol in a Soxhlet apparatus[7]at 60°C for 24 hours. The solvent was removed using a rotary evaporator at 60°C and 100 rpm for 1 hour. All the prepared extracts were refrigerated at 4 °C for further analysis.

Reference strains which were used to evaluate the antimicrobial activity of the methanol extract of leaves and bark of Syzygium palghatense were Aspergillus niger (ATCC 16404) and Candida albicans (ATCC 10231) for fungi and Staphylococcus aureus (ATCC 25923), Streptococcus mutans (MTCC 890) and Enterococcus fecalis (ATCC 2912) for Gram Positive Bacteria and E. coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853) for Gram Negative Bacteria.

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Antifungal activity of methanol extracts of Syzygium palghatense leaves and bark against Aspergillus niger and Candida albicans was assessed by agar well diffusion[8,9] on Potato Dextrose Agar. Fungal cultures were swabbed onto plates, and wells (10 mm) were loaded with extract concentrations of 250, 500, and 1000 µg in DMSO. After overnight incubation at room temperature, inhibition zones were measured and compared with clotrimazole (10 mg/ml) as a standard. Each test was performed in triplicate.

Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

MIC values for filamentous fungi were determined using broth microdilution with methanol leaf and bark extracts serially diluted in DMSO (10–300 µg/ml). Conidial suspensions were standardized from 7-day-old PDA cultures. For Aspergillus niger and Candida albicans, MIC was defined as the lowest concentration with complete growth inhibition after 48 hours. To determine MFC, samples from optically clear tubes and the last growth-positive tube were subcultured on SDA and incubated at 35°C; MFC was the lowest concentration with no visible growth. All tests were performed in duplicate and repeated six time for consistency.

Antibacterial activity

The antibacterial activity of methanol extracts from leaves and bark of Syzygium palghatense was evaluated using the agar well diffusion method on Mueller Hinton Agar (MHA) plates. Bacterial cultures were adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/ml). Wells (10 mm) were filled with extract concentrations of 250, 500, and 1000 µg, and plates were incubated at 37°C for 24 hours. Inhibition zones were measured to assess activity, with streptomycin (10 mg/ml) as a positive control.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)[10,11]

MIC and MBC values were determined by standard broth microdilution in 96-well plates following CLSI guidelines (2012). Methanol extract of Syzygium palghatense (initially 10 mg/Ml) was twofold diluted to concentrations ranging from 300 to 10 µg/ml; appropriate positive and negative controls were included. MIC was recorded as the lowest concentration inhibiting visible growth, and MBC as the lowest killing concentration after subculturing onto MHA plates. All assays were performed in duplicate and repeated independently six times.

Effect of extracts on biofilm [12,13]

The studies were carried out on the microorganism with higher antimicrobial activities in the methanol extract of Syzygium palghatense leaves and bark. Pseudomonas aeruginosa was selected for the biofilm studies.

Congo red agar assay

Biofilm production by Pseudomonas aeruginosa was assessed using Congo red agar. Cultures were grown on Brain Heart Infusion Agar with Congo red and incubated at 37°C for 24 hours. A colour change of the medium from red to nearly black indicated biofilm formation, while no colour change signified non-biofilm producers.

Semi-quantitative adherence assay

Pseudomonas aeruginosa cultures were adjusted to 0.5 McFarland standard, and 20 µl inoculum was added to each well of a 96-well plate containing BHIB and sample concentrations ranging from 62.5 to 1000 µg. After 24 h incubation, biofilms were fixed with 95% ethanol, stained with 5% crystal violet for 5 min, air-dried overnight, and absorbance was measured at 570 nm to assess biofilm formation.

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Inhibition assay of the initial and pre-formed biofilm

A 96-well microtiter plate was inoculated with 100 µl Pseudomonas aeruginosa suspension and 100 µl methanol extract (62.5–1000 µg/ml), with growth control wells containing only inoculum and DMSO. Plates were incubated statically at 37°C for 24 hours. Wells were washed with PBS and fresh broth with extract was added for a second 24-hour incubation. Biofilm formation was quantified by measuring optical density at 570 nm.

Quantification of biofilm

Biofilms were allowed to form by incubating 20 µl of Pseudomonas aeruginosa inoculum per well at 37°C for 72 hours in a 96-well plate. After biofilm formation, sample concentrations (62.5–1000 µg) were added, and plates were incubated for 24 hours. Wells were washed with PBS, biofilms heat-fixed at 60°C for 1 hour, stained with 0.5% crystal violet for 15 minutes, and then washed and air-dried. Bound dye was solubilized with 30% acetic acid, and absorbance at 570 nm was measured to quantify biofilm inhibition

GC-MS analysis[14]

GC–MS analysis was performed using a Shimadzu QP2010S with an Elite-5MS capillary column (30 m × 0.25 mm, 0.25 µm). Conditions included an injector at 260°C; a column temperature program from 80°C (4 min) ramped to 280°C at 5°C/min (6 min hold); helium carrier gas at 1 ml/min; and a split ratio of 50:1. Electron ionization (70 Ev) was used, with ion source at 200°C and mass scan range 50–500 Da over 50 min. Compounds were identified by matching spectra and retention times to Wiley 8 and NIST 11 libraries, selecting peaks with similarity ≥70%.

Liquid Chromatography-Mass Spectrometry Quadrupole Time of Flight (LC-MS QTOF) analysis

LC-MS QTOF Analysis

- Agilent 1260 Infinity with QTOF 6545 protocol:
  - Agilent Technologies, 6200 Series and 6500 Series Q-TOF LC/MS Quick Start Guide, G3335-90268EN, 2021.
  - Agilent Technologies, Agilent 1260 Infinity Quaternary LC User Manual, G1311-90300, 2021.

Methanol extracts (100 mg) of leaves and bark were dissolved in methanol: water (1:1), diluted, and analyzed using an Agilent 1260 Infinity chromatograph. Chromatographic separation was performed on a C18 column (3.0 × 100 mm, 2.7 µm) with a mobile phase of 0.1% formic acid and 0.1% acetic acid in acetonitrile at 0.25 ml/min. The analysis was carried out with a QTOF 6545 (Agilent) under positive mode (gas temp. 325°C, capillary 2.0 Kv, nebulizer 60 psi, dry gas 6 L/min). Relative peak areas were compiled as a data matrix after analysis

Molecular docking[15,16]

Phytocompounds identified from GC-MS and LC-MS of Syzygium palghatense leaves (18 compounds) and bark (19 compounds) were docked against the quorum sensing regulators RhlR and LasR of Pseudomonas aeruginosa. Docking was carried out using AutoDock Vina. Interaction profiles, binding energies (ΔG), and key hydrogen bonds were analyzed for each extract.

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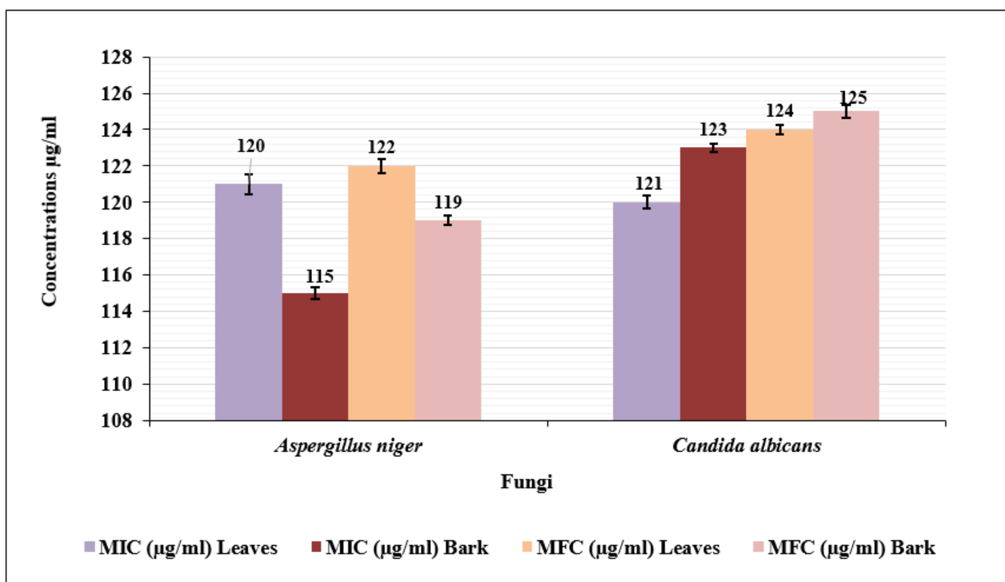
Results

Table 1: Antimicrobial activity of methanol extracts of the leaves and bark of Syzygium palghatense against the pathogenic microorganisms.

Fungus	Concentrations (Zone of inhibition in mm)							
	Clotrimazole (100 µg/ml)		250 (µg/ml)		500 (µg/ml)		1000 (µg/ml)	
	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
Aspergillus niger	23 ± 0.45	23 ± 0.61	13 ± 0.45	13 ± 0.31	15 ± 0.25	15 ± 0.22	16 ± 0.33	16 ± 0.19
Candida albicans	20 ± 0.57	20 ± 0.13	13 ± 0.21	12 ± 0.61	14 ± 0.16	14 ± 0.18	16 ± 0.28	15 ± 0.17
Bacteria	Streptomycin (100 µg)		250 (µg/ml)		500 (µg/ml)		1000(µg/ml)	
	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
Staphylococcus aureus	27 ± 0.24	27 ± 0.47	11 ± 055	Nil	12 ± 0.44	Nil	13 ± 0.27	11 ± 0.24
Streptococcus mutans	27 ± 0.14	27 ± 014	Nil	Nil	11 ± 0.25	11 ± 0.27	12 ± 0.24	11 ± 0.28
Enterococcus fecalis	25 ± 0.26	25 ± 0.31	12 ± 0.22	13 ± 0.21	13 ± 0.19	14 ± 0.31	14 ± 0.16	17 ± 0.14
Escherichia coli	28 ± 0.16	28 ± 0.21	12 ± 0.14	11 ± 0.21	13 ± 0.21	13 ± 0.17	14 ± 0.15	14 ± 0.12
Pseudomonas aeruginosa	28 ± 0.13	28 ± 0.14	12 ± 0.13	13 ± 0.16	13 ± 0.15	15 ± 0.14	15 ± 0.89	18 ± 0.12

Table 1 shows Antimicrobial activity of methanol extracts of the leaves and bark of Syzygium palghatense against various pathogenic microorganisms including Staphylococcus aureus, Streptococcus mutans, Enterococcus fecalis, Escherichia coli and Pseudomonas aeruginosa.



Minimum inhibitory and fungicidal concentrations of leaves and bark of methanol extracts of Syzygium palghatense (Mean ± SE, n=6)

Figure 1: Shows the Minimum inhibitory and fungicidal concentrations of leaves and bark of methanol extracts of Syzygium palghatense against Aspergillus niger and Candida albicans.

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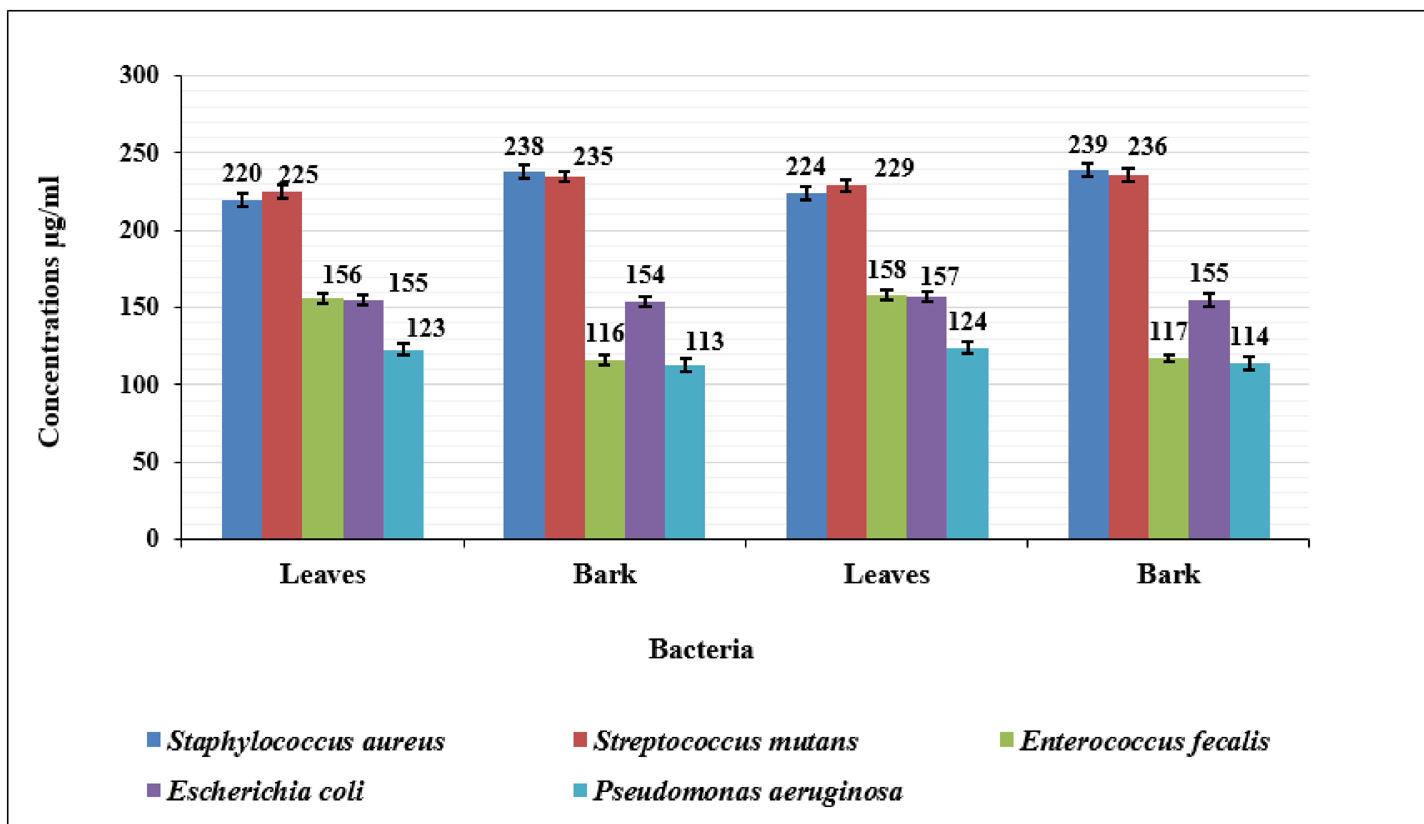

Figure 2: Shows Minimum inhibitory and bactericidal concentrations of leaves and bark of methanol extracts of Syzygium palghatense against various microorganisms including Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa.

Table 2: Semi-quantitative adherence assay of methanolic extracts of Syzygium palghatense leaves and bark against Pseudomonas aeruginosa

Concentration (µg/mL)	Optical density (OD, 570 nm)		Percentage of inhibition (%)	
	SPL	SPB	SPL	SPB
Control	0.3447 ± 0.0156	0.3210 ± 0.1054	0	0
62.5	0.2760 ± 0.0041	0.2341 ± 0.0035	19.68 ± 1.0210	20.24 ± 1.0240
125	0.2300 ± 0.0041	0.2101 ± 0.0038	33.27 ± 1.2045	34.65 ± 1.2062
250	0.1975 ± 0.0028	0.1825 ± 0.0027	42.70 ± 1.2001	47.36 ± 1.0547
500	0.1630 ± 0.0010	0.1547 ± 0.0090	52.70 ± 1.1590	53.01 ± 1.1254
1000	0.1297 ± 0.0011	0.1018 ± 0.0015	62.35 ± 1.3024	64.44 ± 1.1305
			IC_{50}: 2.05 µg/mL	IC_{50}: 1.25 µg/mL

SPL: Syzygium palghatense leaves; SPB: Syzygium palghatense bark; mean ± SD, n=6

Table 2: Shows semi-quantitative adherence assay of methanolic extracts of Syzygium palghatense leaves and bark against Pseudomonas aeruginosa with IC_{50} as 2.05 µg/mL and 1.25 µg/mL.

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Table 3: Inhibition assay of initial and pre-formed biofilm using methanolic extracts of Syzygium palghatense leaves and bark against Pseudomonas aeruginosa

Concentration (µg/mL)	Optical density (OD, 570 nm)		Percentage of inhibition (%)	
	SPL	SPB	SPL	SPB
Control	1.5787 ± 0.3201	1.3015 ± 0.0.214	0	0
62.5	1.2094 ± 0.2110	1.2011 ± 0.2101	23.39 ± 1.0235	25.12 ± 1.0332
125	1.0149 ± 0.1504	1.0024 ± 0.1401	35.71 ± 1.1102	36.15 ± 1.1232
250	0.8312 ± 0.1025	0.6842 ± 0.1021	47.34 ± 1.2025	48.32 ± 1.2060
500	0.4626 ± 0.0019	0.2581 ± 0.0014	70.69 ± 1.2240	75.21 ± 1.2251
1000	0.2792 ± 0.0010	0.1756 ± 0.0005	82.31 ± 1.2256	86.33 ± 1.2300
			IC_{50}: 1.48 µg/mL	IC_{50}: 1.22 µg/mL

SPL: Syzygium palghatense leaves; SPB: Syzygium palghatense bark; mean ± SD, n=6

Table 3: Shows Inhibition assay of initial and pre-formed biofilm using methanolic extracts of Syzygium palghatense leaves and bark against Pseudomonas aeruginosa with IC_{50}: 1.48 and 1.22 µg/mL

Table 4: Quantitative analysis of biofilm quantification of methanolic extracts of Syzygium palghatense leaves and bark against Pseudomonas aeruginosa

Concentration (µg/mL)	Optical density (OD, 570 nm)		Percentage of inhibition (%)	
	SPL	SPB	SPL	SPB
Control	0.9755 ± 0.0850	0.8254 ± 0.0714	0	0
62.5	0.7667 ± 0.0562	0.6547 ± 0.0410	21.40 ± 1.2003	35.01 ± 1.2017
125	0.5120 ± 0.0401	0.4112 ± 0.0350	47.51 ± 1.3015	49.32 ± 1.5101
250	0.3760 ± 0.0308	0.2580 ± 0.0276	61.45 ± 1.5140	64.23 ± 1.5204
500	0.3083 ± 0.0305	0.1026 ± 0.0147	68.39 ± 1.5221	68.56 ± 1.5650
1000	0.2157 ± 0.0014	0.1018 ± 0.0011	77.88 ± 1.6008	79.27 ± 1.6102
			IC_{50}: 1.60 µg/mL	IC_{50}: 1.46 µg/mL

SPL: Syzygium palghatense leaves; SPB: Syzygium palghatense bark; mean ± SD, n=6

Table 4: Shows quantitative analysis of biofilm quantification of methanolic extracts of Syzygium palghatense leaves and bark against Pseudomonas aeruginosa with IC_{50}: 1.60 µg/mL and 1.46 µg/mL.

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Table 5: LC-MS QTOF analysis of methanol extract of Syzygium palghatense leaves

Compound	Name	Formula	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score
1	Gallic acid	$C_7H_6O_5$	2.137	170.0225	170.0215	5.56	80.18
2	Ellagic acid	$C_{14}H_6O_8$	3.018	302.0063	302.0063	-0.02	99.80
3	Quercetin	$C_{15}H_{10}O_7$	2.120	302.0423	302.0436	-1.26	81.57
4	Stearic acid	$C_{18}H_{36}O_2$	29.900	284.2721	284.2715	2.01	98.21
5	Lauric acid	$C_{12}H_{24}O_2$	13.302	200.1776	200.1776	0.05	99.73
6	Myristic acid	$C_{14}H_{28}O_2$	22.722	228.2087	228.2089	-0.82	98.22
7	Palmitic acid	$C_{16}H_{32}O_2$	26.760	256.2408	256.2402	2.23	98.47
8	Oleic acid	$C_{18}H_{34}O_2$	35.715	282.2558	282.2559	-0.12	98.63
9	Linoleic acid	$C_{18}H_{32}O_2$	29.052	280.2402	280.2402	-0.44	99.53
10	Oleanolic acid	$C_{30}H_{48}O_3$	31.760	456.3587	456.3603	-3.60	92.40
11	Chlorogenic acid	$C_{16}H_{18}O_9$	21.493	354.0975	354.0951	6.77	87.10
12	Cinnamic acid	$C_9H_8O_2$	31.262	148.0527	148.0507	1.88	99.63
13	Vanillin	$C_8H_8O_3$	9.497	152.0473	152.0473	0.00	98.07
14	Rhamnetin	$C_{16}H_{12}O_7$	2.519	316.0533	316.0583	-15.70	72.68
15	Kaempferol	$C_{15}H_{10}O_6$	21.775	286.0480	286.0477	0.79	79.95
16	Myricetin	$C_{15}H_{10}O_8$	17.140	318.0374	318.0376	-0.52	98.10
17	Maslinic acid	$C_{30}H_{48}O_4$	32.674	472.3544	472.3553	-1.83	98.46
18	Ferulic acid	$C_{10}H_{10}O_4$	9.962	194.0573	194.0602	-3.29	85.61

Table 5: Shows LC-MS QTOF analysis (liquid chromatography coupled with quadrupole time-of-flight mass spectrometry analysis) of methanol extract of Syzygium palghatense leaves

Table 6: LC-MS QTOF analysis of methanol extract of Syzygium palghatense bark

Compound	Name	Formula	RT	Mass	Mass (Tgt)	Diff (Tgt, ppm)	Score
1	Gallic acid	$C_7H_6O_5$	2.137	170.0225	170.0215	5.56	80.18
2	Ellagic acid	$C_{14}H_6O_8$	3.018	302.0063	302.0063	-0.02	99.80
3	Quercetin	$C_{15}H_{10}O_7$	2.120	302.0423	302.0436	-1.26	81.57
4	Stearic acid	$C_{18}H_{36}O_2$	29.900	284.2721	284.2715	2.01	98.21
5	Lauric acid	$C_{12}H_{24}O_2$	13.302	200.1776	200.1776	0.05	99.73
6	Myristic acid	$C_{14}H_{28}O_2$	22.722	228.2087	228.2089	-0.82	98.22
7	Palmitic acid	$C_{16}H_{32}O_2$	26.760	256.2408	256.2402	2.23	98.47
8	Oleic acid	$C_{18}H_{34}O_2$	35.715	282.2558	282.2559	-0.12	98.63
9	Linoleic acid	$C_{18}H_{32}O_2$	29.052	280.2402	280.2402	-0.44	99.53
10	Oleanolic acid	$C_{30}H_{48}O_3$	31.760	456.3587	456.3603	-3.60	92.40

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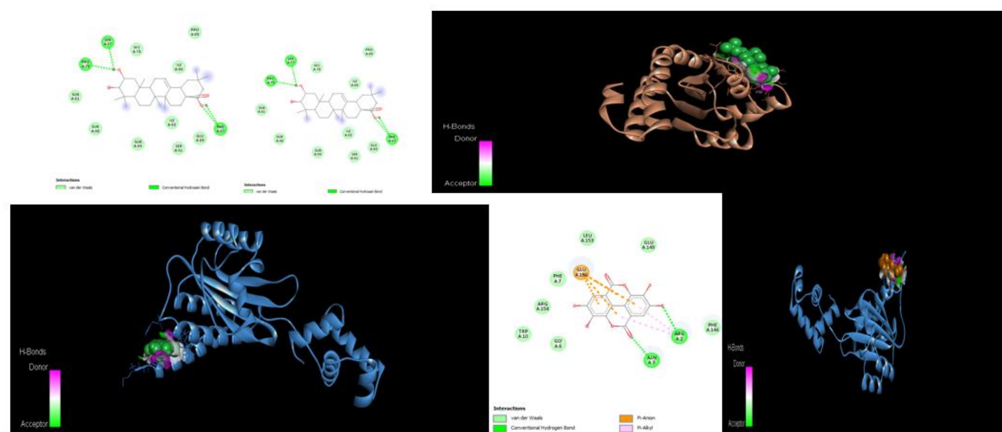
11	Chlorogenic acid	$C_{16}H_{18}O_9$	21.493	354.0975	354.0951	6.77	87.10
12	Cinnamic acid	$C_9H_8O_2$	31.262	148.0527	148.0507	1.88	99.63
13	Vanillin	$C_8H_8O_3$	9.497	152.0473	152.0473	0.00	98.07
14	Rhamnetin	$C_{16}H_{12}O_7$	2.519	316.0533	316.0583	-15.70	72.68
15	Kaempferol	$C_{15}H_{10}O_6$	21.775	286.0480	286.0477	0.79	79.95
16	Myricetin	$C_{15}H_{10}O_8$	17.140	318.0374	318.0376	-0.52	98.10
17	Maslinic acid	$C_{30}H_{48}O_4$	32.674	472.3544	472.3553	-1.83	98.46
18	Ferulic acid	$C_{10}H_{10}O_4$	9.962	194.0573	194.0602	-3.29	85.61
19	Gentisic acid	$C_7H_6O_4$	1.871	154.0266	154.0266	-0.28	96.75

Table 6: Shows LC-MS QTOF(liquid chromatography coupled with quadrupole time-of-flight mass spectrometry) analysis of the methanol extract of Syzygium palghatense bark.

Figure 3 : Docking results of Syzygium palghatense leaves

This figure displays both 2D and 3D visualizations of key leaf-derived phytocompounds—such as ellagic acid and maslinic acid—docked with the active site of the RhlR quorum sensing protein in Pseudomonas aeruginosa. Panels illustrate hydrogen bonding and molecular interactions (highlighted in green and magenta), demonstrating that these compounds form stable complexes in the RhlR binding pocket and potentially inhibit bacterial quorum sensing and biofilm formation.



Leaves (RhlR Target)

- Top-ranked Ligands (with binding energy ≤ –7.0 kcal/mol):
    - 1,2,6a,6b,9,9,12a-Heptamethyl-...octahydropicene-4a-carboxylic acid, methyl ester: ΔG = –8.4 kcal/mol (no H-bond)
    - Testosterone cypionate: ΔG = –8.0 kcal/mol (1 H-bond with Asn3)
    - Oleanolic acid: ΔG = –7.9 kcal/mol (no H-bond)
    - Ellagic acid: ΔG = –7.8 kcal/mol (2 H-bonds: Arg2, Asn3)
    - Maslinic acid: ΔG = –7.7 kcal/mol (1 H-bond: Asn3)
    - Rutin: ΔG = –7.0 kcal/mol (multiple H-bonds: His78, Ser77, Gln81, Glu89, Ile92)

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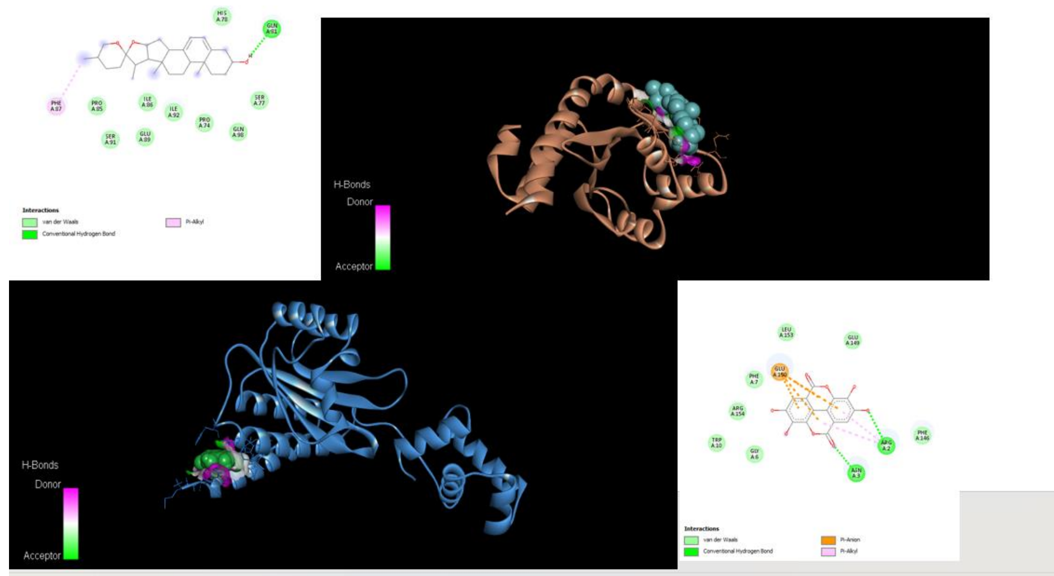
Ellagic acid and maslinic acid formed multiple hydrogen bonds with RhlR active site residues, indicating strong and specific binding. Some triterpenes showed higher binding affinities, but lacked polar interactions.

Figure 4: Docking results of Syzygium palghatense bark

This figure displays both 2D and 3D visualizations of key bark-derived phytocompounds—such as maslinic acid and ellagic acid—docked with the active site of the LasR quorum sensing protein in Pseudomonas aeruginosa. Panels illustrate hydrogen bonding and molecular interactions (highlighted in green and magenta), demonstrating that these compounds form stable complexes in the LasR binding pocket and potentially inhibit bacterial quorum sensing and biofilm formation.



Bark **(LasR Target)**

- Top-ranked Ligands (with binding energy ≤ –7.0 kcal/mol):
  - 7-Dehydrodiosgenin: ΔG = –8.6 kcal/mol (no H-bond)
  - Lupeol: ΔG = –8.2 kcal/mol (no H-bond)
  - Oleanolic acid: ΔG = –7.9 kcal/mol (no H-bond)
  - Ellagic acid: ΔG = –7.8 kcal/mol (2 H-bonds: Arg2, Asn3)
  - Maslinic acid: ΔG = –7.7 kcal/mol (1 H-bond: Asn3)
  - Quercetin: ΔG = –6.8 kcal/mol (multiple H-bonds, but less overall affinity)

Ellagic acid and maslinic acid from bark again formed hydrogen bonds with LasR, mirroring leaf results. 7-Dehydrodiosgenin and lupeol had the strongest binding energies, but lacked key H-bonding. Ellagic acid and maslinic acid (from both extracts) form critical H-bond interactions with the biofilm regulatory proteins of Pseudomonas aeruginosa, suggesting biofilm inhibitory and anti-virulence potential. Bark and leaves both supply strong hits, but bark ligands (especially 7-dehydrodiosgenin and lupeol) yielded slightly higher binding affinities against LasR, while leaves excelled against RhlR.

Conclusion

Both leaves and bark extracts of Syzygium palghatense exhibit broad-spectrum antimicrobial and antibiofilm activities, with bioactive polyphenols and triterpenoids as key contributors. Bark extract consistently shows slightly higher

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antimicrobial zones, lower MIC/MFC/IC_{50} values, and marginally greater biofilm inhibition than leaves. Docking results indicate that key compounds from both extracts (notably ellagic acid and maslinic acid) strongly interact with biofilm-related proteins, supporting their anti-biofilm mechanism. Therefore, while both are effective, the bark extract is slightly superior as an antimicrobial and antibiofilm agent.

Discussion

Antimicrobial resistance has become a global challenge [17]. As a multi-antibiotic resistant Gram-negative pathogen, Pseudomonas aeruginosa constitutes a major clinical threat [18,19]. The present study demonstrated that methanolic extracts of both leaves and bark of Syzygium palghatense possess notable antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungal pathogens, with Pseudomonas aeruginosa exhibiting the highest susceptibility. Biofilm inhibition assays indicated a dose-dependent effect, with bark extract showing marginally higher inhibition and lower IC_{50} values than leaves, suggesting greater antibiofilm potency. LC-MS QTOF profiling revealed the presence of bioactive polyphenols (ellagic acid, quercetin, kaempferol, myricetin), triterpenoids (maslinic acid, oleanolic acid, lupeol), and fatty acids, many of which are reported to have quorum sensing and biofilm inhibitory activity [Sommer et al., 2020[20]; Sonmezer et al., 2016[18]]. Molecular docking further supported these findings, as key compounds such as ellagic acid and maslinic acid formed stable hydrogen bonds with the RhlR and LasR quorum sensing proteins of P. aeruginosa, potentially interfering with QS-regulated biofilm formation [Tian et al., 2018[21]; Trott & Olson, 2010[15]]. These results indicate that S. palghatense, particularly its bark extract, could serve as a promising natural source of anti-biofilm agents, warranting further mechanistic and in vivo validation.

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