Supporting information 1

Effects of Active compounds from *Cassia fistula* on Quorum sensing mediated virulence and biofilm formation in *Pseudomonas aeruginosa*

Supplementary Method 1 (S Method 1)

Nematode culture maintenance

In-vivo bacterial pathogenesis was studied on the model organism *Caenorhabditis elegans*. The wild-type *Caenorhabditis elegans* (Bristol) N2 strain was utilised to employ paralytic, slow and fast killing assays. Before conducting the experiments, *Caenorhabditis elegans* N2 hermaphrodite worms were synchronized with hypochlorite (1% sodium hypochlorite). Nematodes were allowed to grow on NGM plates, with *E. coli* OP50 being used as their food. The hatched eggs were transferred onto the previously seeded *E. coli* OP50 on NGM plates (Brenner, 1974), following which incubation was done for 3-4 days at 21 °C. Finally, the synchronized L4-stage worms were used in subsequent assays. Worms were observed using a stereomicroscope (Magnus) [1–3].

References

(1)   Cezairliyan, B.; Vinayavekhin, N.; Grenfell-Lee, D.; Yuen, G. J.; Saghatelian, A.; Ausubel, F. M. Identification of Pseudomonas Aeruginosa Phenazines That Kill Caenorhabditis Elegans. *PLoS Pathog.* **2013**, *9* (1). https://doi.org/10.1371/journal.ppat.1003101.

(2)   Gallagher, L. A.; Manoil, C. Pseudomonas Aeruginosa PAO1 Kills Caenorhabditis Elegans by Cyanide Poisoning. *J. Bacteriol.* **2001**, *183* (21), 6207–6214. https://doi.org/10.1128/JB.183.21.6207.

(3)   Tan, M. W.; Mahajan-Miklos, S.; Ausubel, F. M. Killing of Caenorhabditis Elegans by Pseudomonas Aeruginosa Used to Model Mammalian Bacterial Pathogenesis. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96* (2), 715–720. https://doi.org/10.1073/pnas.96.2.715.

Results

Supplementary figure 1 (S Fig. 1)

Flash chromatography chromatogram-
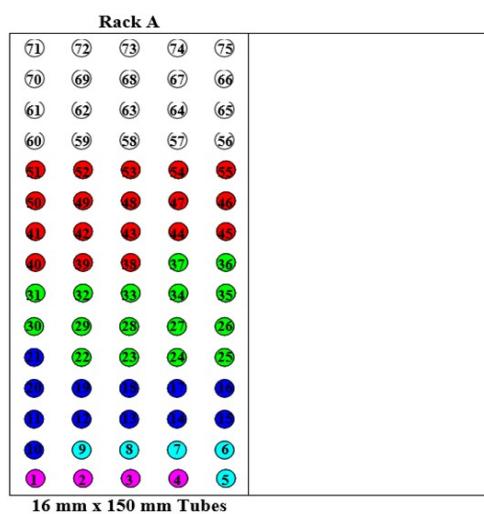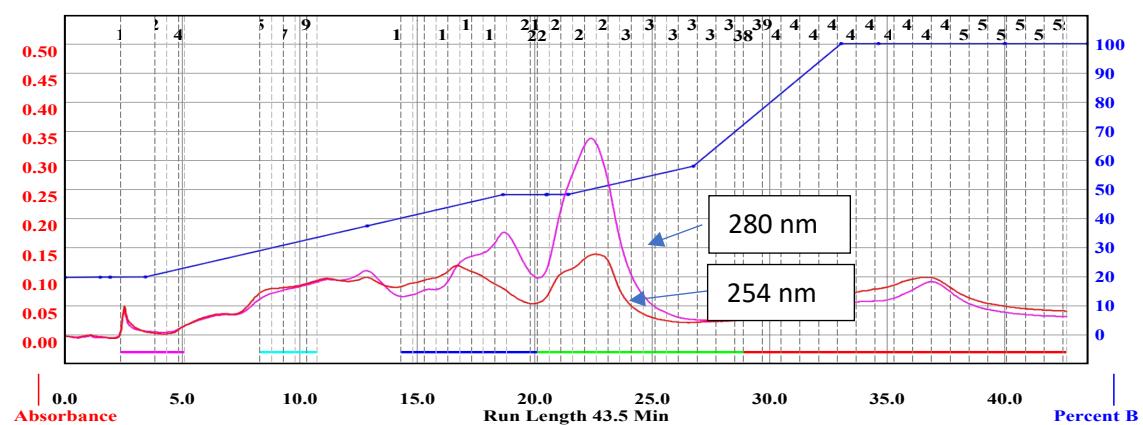
Supplementary Table 1 (S Table 1) - Flash chromatography mobile phase table -

Peak #	Start Tube	End Tube
1	A:1	A:4
2	A:5	A:9
3	A:10	A:21
4	A:22	A:37
5	A:38	A:55

Duration	% B	Solvent A	Solvent B
0.0	20.0	A1 hexane	B1 ethyl acetate
1.5	20.1	A1 hexane	B1 ethyl acetate
0.4	20.1	A1 hexane	B1 ethyl acetate
0.0	20.1	A1 hexane	B1 ethyl acetate
1.5	20.2	A1 hexane	B1 ethyl acetate
9.4	37.6	A1 hexane	B1 ethyl acetate
5.8	48.3	A1 hexane	B1 ethyl acetate
1.8	48.3	A1 hexane	B1 ethyl acetate
0.0	48.3	A1 hexane	B1 ethyl acetate
0.0	48.4	A1 hexane	B1 ethyl acetate
...	...	...	...

Supplementary figure 2 (S Fig. 2) Short-chain assay
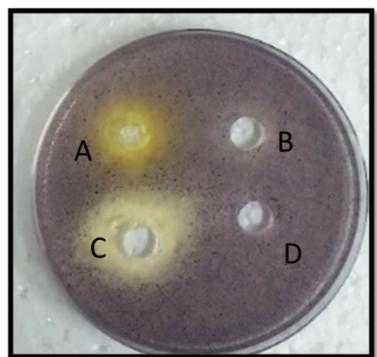
S Figure 2. *Plate based Short chain assay where (A) F-1 (1 mg ml^{-1}), (B) DMSO, (C) AZM (4 µg l^{-1}), (D) PBS:EtoH (1:1)*

Supplementary figure 3 (S Fig. 3)

LC MS linearity and quantification was performed and a calibration curve was plotted for each concentration (ranging from 32 ng ml^{-1} to 2500 ng ml^{-1} in F-1), comparing the peak area ratio to the internal standard (Rhein).

RHEIN LINEARITY

Data#	Data Filenar	Ret. Time	Sample Type	Level#		Area	S/N	Sample Nam	Accuracy[%]	Cal. Point	Area Ratio	Conc. (ppm)	Std. Conc.	Area%
1	RHEIN 32NG	2.358	Standard(Ca		1	31,313	37.17	RHEIN 32NG	184.7	1	-----	59.11	32	100
2	RHEIN 64NG	2.365	Standard(Ca		2	65,904	67.21	RHEIN 64NG	130.9	1	-----	83.775	64	100
3	RHEIN 125N(	2.366	Standard(Ca		3	139,031	144.86	RHEIN 125N(	108.7	1	-----	135.918	125	100
4	RHEIN 250N(	2.36	Standard(Ca		4	328,472	324.79	RHEIN 250N(	108.4	1	-----	270.999	250	100
5	RHEIN 500N(	2.36	Standard(Ca		5	573,056	493.8	RHEIN 500N(	89.1	1	-----	445.4	500	100
6	RHEIN 1000I	2.358	Standard(Ca		6	1,276,929	810.06	RHEIN 1000I	94.7	1	-----	947.296	1000	100
7	RHEIN 2500I	2.361	Standard(Ca		7	3,494,454	1,290.49	RHEIN 2500I	101.1	1	-----	2,528.50	2500	100

MRM EVENTS   RHEIN MRM PRECURSSOR

MRM   Acq. Time: 0 - 5   min   Compound Name: RHEIN

Ch	Precursor m/z	Product m/z	Dwell Time (msec)	Q1 Pre Bias(V)	CE	Q3 Pre Bias(V)
Ch1	283.20	239.00	100.0	20.0	14.0	25.0
Ch2	283.20	183.00	100.0	20.0	30.0	30.0
Ch3	283.20	210.95	100.0	20.0	25.0	21.0
Ch4						
Ch5						

Event Time: 0.309 sec   Q1 Resolution: Unit   Advanced Settings...

Q3 Resolution: Unit
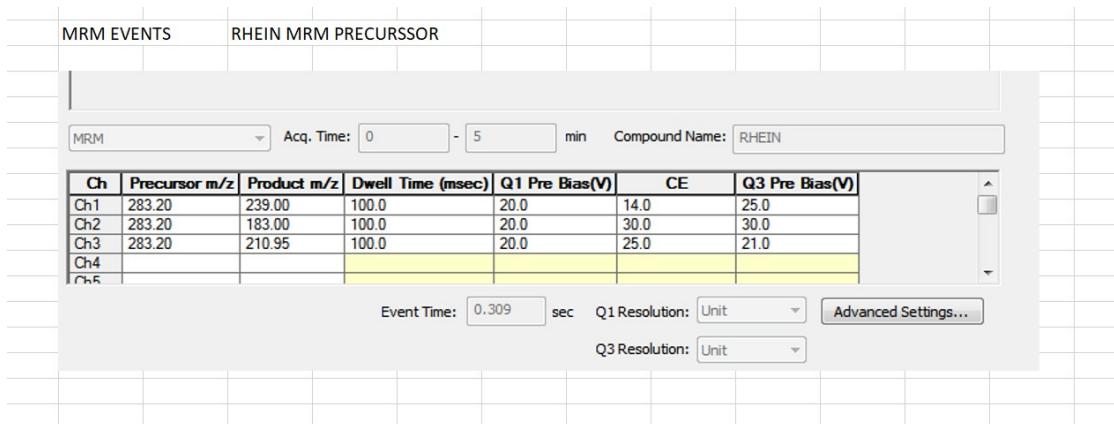
Supplementary Table 2 (S Table 2) PCR primers used for RT-PCR

Gene Name	Primer sequence	Annealing temp	Amplicon size (bp)	
lasI F	GGCTGGGACGTTAGTGTCAT	55 ° C	104	——
lasI R	AAAACCTGGGCTTCAGGAGT			——
lasR F	ACGCTCAAGTGGAAAATTGG	59 ° C	111	——
lasR R	TCGTAGTCCTGGCTGTCCTT			——
rhlI F	AAGGACGTCTTCGCCTACCT	60.5 ° C	130	——
rhlI R	GCAGGCTGGACCAGAATATC			——
rhlR F	CATCCGATGCTGATGTCCAACC	65 ° C	101	——
rhlR R	ATGATGGCGATTTCCCCGGAAC			——
rpsL F	GCAACTATCAACCAGCTGGTG	65 ° C	2321	——
rpsL R	GCTGTGCTCTTGCAGGTTGTG			——

Supplementary Table 3 (S Table 3) Peptone-Glucose-Sorbitol (PGS) Media Composition

Chemicals	For 100 ml
Bacto-Peptone	1%
Glucose	1%
Sodium Chloride	1%
Sorbitol	0.15%

Supplementary Table 4 (S Table 4) Nematode Growth Medium (NGM) Composition

Chemicals	For 1 litre
NaCl	3 gm
Agar	17 gm
Peptone	2.5 gm
5 mg ml^{-1} cholesterol in ethanol (Do not autoclave)	1 ml
1 M KPO$_4$ buffer pH 6.0 (108.3 g KH$_2$PO$_4$, 35.6 g K$_2$HPO$_4$, H$_2$O to 1 litre)	25 ml
Calcium chloride	1 ml
1M Magnesium sulfate	1 ml

Supplementary Table 5 (S Table 5): Docking interaction score for active constituents of C*assia fistula* and standard (Rhein) used in the study against *LasI* and *LasR*

Compounds	Binding energy with *LasI* (1RO5) (kcal mol^{-1})	Binding energy with *LasR* (2UV0) (kcal mol^{-1})
Rhein	-10.258	-10.21
3-Aminodibenzofuran	-6.052	-8.923
9-Octadecenamide	-4.32	-7.062
Methyl Stearate	-3.987	-6.599
Methyl Linoleate	-3.96	-6.548
5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	-6.67	-5.762
Dihydrorhodamine	-6.521	-5.587
1- Eicosene	-5.016	-4.461
Methyl Oleate	-5.746	-4.427
Methyl Palmitate	-3.15	-4.081
palmitic acid	-4.259	-3.937
1-Octadecene	-1.453	-3.827
Tetradecane	-1.876	-2.504
2,4-Di-tert-butylphenol	-2.783	-2.112
1-Nonanol	-1.612	-1.819