

Polymicrobial biofilm model

Experimental plan overview: Methanolic extracts (resuspended in water) obtained from the leaves of a native Australian plant (denoted species 8472) were screened against polymicrobial biofilms in triplicate. Extracts were tested at a concentration range of 10, 20 and 30 mg/mL. Bacteria tested: *Pseudomonas aeruginosa*, *Escherichia coli* and MRSA which were established in various combinations. Specifically, *P. aeruginosa* and *E. coli*; *P. aeruginosa* and MRSA; *E. coli* and MRSA; *P. aeruginosa*, *E. coli* and MRSA.

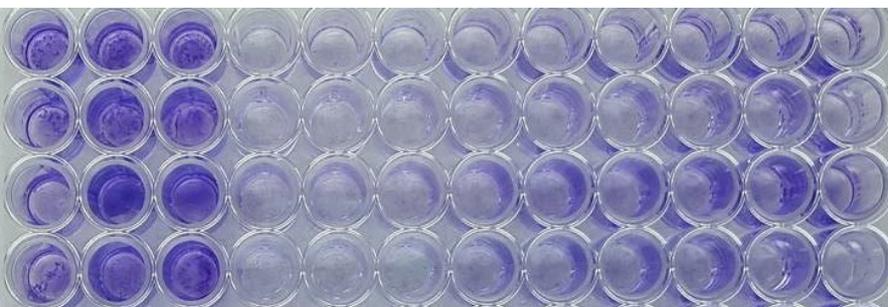
Method overview: Bacterial cultures were grown overnight within the 96 well plate (100 µL) and then treated with 20 µL of each extract at various concentrations as stated above. Subsequent to incubation, all wells were heat fixed, stained with crystal violet (CV) and then inoculated with 95% ethanol to solubilise the CV which was then read at an absorbance of 550nm (PolarStar plate reader). The averaged blank (as defined by the plate reader) was subtracted from the raw absorbance data. Values were then represented as the average reduction percentage *i.e.* the inhibition/degradation of the extract treated biofilms compared to the untreated biofilms (control).

Results:

Table 1. Biofilm degradation by plant extracts obtained from species 8472. Three bacterial species which are known to routinely generate biofilms were tested for their susceptibility to Australian native plant extracts obtained from species 8472. Further, since biofilms very often exist in a polymicrobial form, the bacteria were grown together in various combinations. Overall, 10 mg/mL of plant extract was the most effective concentration against all four polymicrobial biofilms. The combination of *P. aeruginosa* and MRSA was shown to be the most susceptible to species 8472 as 94.29% of the biofilm was degraded. Similarly, 93.42% of the *P. aeruginosa*, *E. coli* and MRSA biofilm was also found to be degraded by the plant extract. Given the results, *E. coli* appears to be the least susceptible to species 8472.

Bacterial combination	Degradation of biofilm (%)
	<u>mg/mL</u>

	10	20	30
<i>P. aeruginosa</i> and <i>E. coli</i>	83.36	55.19	41.19
<i>P. aeruginosa</i> and MRSA	94.29	86.36	75.99
<i>E. coli</i> and MRSA	87.45	78.29	67.77
<i>P. aeruginosa</i> , <i>E. coli</i> and MRSA	93.42	67.85	72.06



Of the three concentrations tested, 10 mg/mL biofilms regardless of bacterial combination. (All located at the Institute of Health and Biomedical ; Kelvin Grove campus. For further information, qut.edu.au).