Mono-microbial biofilm model

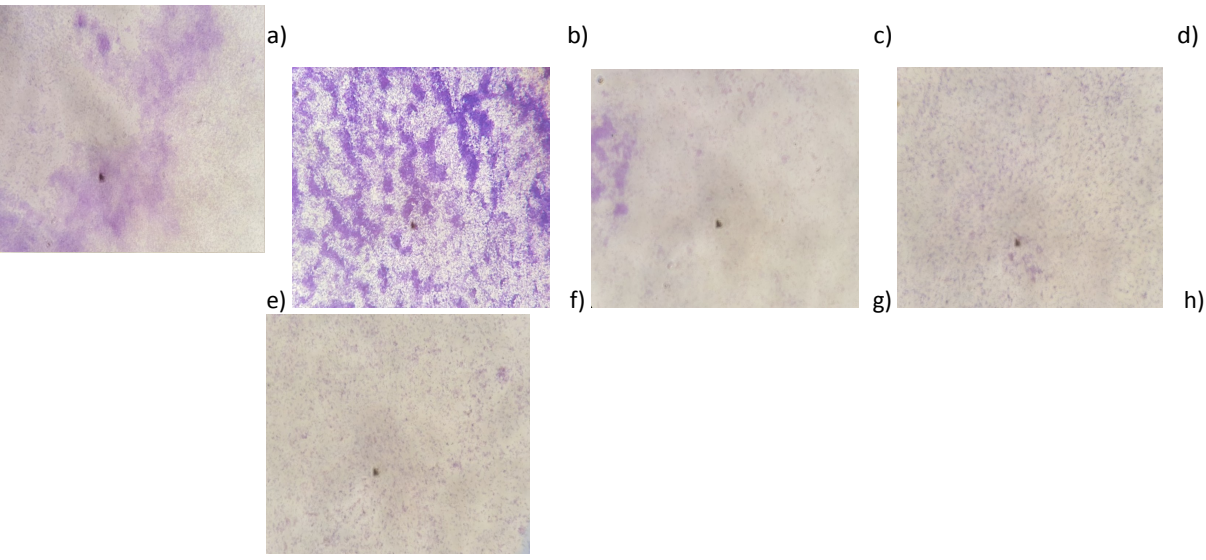
***Experimental plan* overview:** Methanolic extracts (resuspended in water) obtained from the leaves of a native Australian plant (denoted species 8472) were screened against established single bacterial biofilms in triplicate. Extracts were tested at a concentration range of 10, 20 and 30 mg/mL. Bacteria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

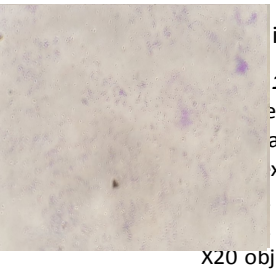
***Method overview*:** Bacterial cultures were grown overnight within the 96 well plate (100 µL) and then treated with 20 µL of each extract at various concentrations as stated above. Subsequent to incubation, all wells were heat fixed, stained with crystal violet (CV) and then inoculated with 95% ethanol 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. Of the three different concentrations tested, 10 mg/mL elicited the greatest degradation regardless of bacterial source of the biofilm. Plant extracts at a concentration of 10 mg/mL were found to totally degrade (100%) the biofilm produced by MRSA. In contrast, *E. coli* was shown to be the least susceptible to the plant extract irrespective of concentration.

	Bacteria	**Degradation of biofilm (%)**	
		mg/mL	
	10	**20**	**30**
Pseudomonas aeruginosa	87.13	78.36	69.40
Escherichia coli	77.09	73.12	67.77
MRSA	100.00	100.00	92.41





i) j) k) l)

1. Verification of biofilm formation and degradation. Biofilms generated by *P. aeruginosa*, *E. coli* and MRSA were ed against extracts obtained from species 8472 for their degradation ability. Untreated biofilms from each of the three a (a, e and i) clearly show the matrix formation. The biofilm formed by MRSA was found to be totally degraded by the xtract (figure j). (b) *P. aeruginosa*+10 mg/mL of extract; c) *P. aeruginosa*+20 mg/mL of extract; d) *P. aeruginosa*+10 of extract; f) *E. coli*+10 mg/mL of extract; g) *E. coli*+20 mg/mL of extract; h) *E. coli*+30 mg/mL of extract; j) MRSA+10 of extract; k) MRSA+20 mg/mL of extract; l) MRSA+30 mg/mL of extract. Images were taken with a Leica microscope X20 objective. NB: the dark triangular mark