Antimicrobial testing

***Method* overview:** Methanolic extracts (resuspended in water) obtained from the leaves of a native Australian plant (denoted species 8472) were screened against Gram positive and Gram negative bacteria. Specifically, Methicillin-sensitive *Staphylococcus aureus* (MSSA) (NCTC 6571), methicillin-resistant *Staphylococcus aureus* (MRSA) (two clinical isolates denoted 1092 and 1113), *Bacillus cereus* (ATCC 10987), *Klebsiella pneumoniae* (ATCC 27736), *Escherichia coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. pyogenes* (ATCC 19615), *Staphylococcus epidermidis* (QUT code 0613), *Proteus vulgaris* (ATCC 7002), *Proteus mirabilis* (ATCC 6380), *Acinetobacter baumannii* (ATCC 19606), *Enterococcus gallinarum* (ATCC 49608), *Enterococcus casseliflavus* (ATCC 700668), *Enterococcus faecalis* (ATCC 49532) and *Enterococcus faecium* (ATCC 700221). Individual strains were streaked onto nutrient agar (Oxoid Ltd, Australia) and incubated at 37 °C for 24 hours.

S. pyogenes (ATCC 19615) was streaked onto horse blood agar and incubated at 37 °C for 24 hours. Culture plates were stored at 4 °C until required.

***Well diffusion assay*:** Mueller-Hinton (MH) plates were evenly inoculated with individual microorganisms using a sterile disposable spreader. Wells were aseptically punched into the agar using a 6 mm biopsy punch and filled with 80 µL of the various extracts. Antibiotic discs were used as positive controls whereby, trimethoprim (1.25 µg) + sulfamethoxasole (23.75 µg) acted as the control for both MRSA isolates, *S. epidermidis, P. vulgaris* and *P. mirabilis*; penicillin G (10 µg) was used for MSSA; erythromycin (15 µg) was used for *S. pyogenes* and *B. cereus;* gentamicin (10 µg) was used for *K. pneumoniae*, *E. coli, P. aeruginosa* and

A. baumannii. Teicoplanin (30 µg) was used for *E. faecalis*, *E. casseliflavus and E. gallinarum* whilst Linezolid (30 µg) was used for *E. faecium*. Plates were then incubated at 37 °C for 24 hours and the subsequent zones of inhibition (radius from the edge of the well to outer margin of clear zone) were measured (mm). Each assay was performed in triplicate and final values were expressed as mean values ± SEM.

***Results*:**

Table 1. Well diffusion assay: Determination of the antimicrobial activity of species 8472. Of the 16 bacteria screened against methanolic extracts derived from species 8472, only *K. pneumoniae* was shown to be impervious to the plant. In contrast, both MRSA isolates, *S. pyogenes*, *S. epidermidis* and vancomycin-resistant bacteria *E. gallinarum*, *E. faecium* and *E. casseliflavus* were found to be highly sensitive to species 8472 as the zones of inhibition were almost equivalent or greater than the antibiotic controls. (*All work was performed by the Indigenous Medicines Group (IMG), located at the Institute of Health and Biomedical Innovation, Queensland University of Technology. For further information, please contact the leader of the IMG, Dr. Trudi Collet at* t.collet@qut.edu.au).

Bacteria	Controls		Species
	+ve	-ve	8472
MRSA 1113	7.2± 0.8	-	
	9.0±0.8	**MRSA 1092**	
	9.1± 0.7	-	
	9.0±0.1	**MSSA**	17.6± 2.1
	-	5.0±0.5	
B. cereus	7.3± 1.6	-	6.5± 0.5
S. pyogenes	8.0± 0.0	-	9.0± 0.3
S. epidermidis	9.0± 0.2	-	
	9.0± 0.0	***E. coli***	

	4.0± 0.0	-	
	2.0± 0.4	***K. pneumoniae***	
	4.5± 0.0	-	
	0.0± 0.0	***P. vulgaris***	
	9.0± 0.0	-	
	3.0± 0.2	***P. mirabilis***	
	9.0± 0.0	-	
	3.0± 0.1	***P. aeruginosa***	
	5.0± 0.0	-	
	2.5± 0.1	***E. gallinarum***	
	3.0± 0.1	-	
	5.0± 0.0	***E. faecalis***	
	7.0± 0.0	-	
	6.0± 0.2	***E. faecium***	
	6.0± 0.2	-	
E. casseliflavus	7.0± 0.6		
	1.0± 0.0	-	6.0± 0.4
A. baumannii	6.0± 0.3	-	2.0± 0.0