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The Medicinal Potential of Australian Native Plants from Toohey Forest, Australia

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Abstract

Eleven methanolic extracts of ten Australian native plants from Toohey Forest, Brisbane, Australia were investigated for their potential medicinal value as antibacterial agents. All plants showed some antibacterial activity against at least one of the bacteria tested. *Alcaligenes faecalis*, *Aeromonas hydrophilia* and *Bacillus cereus* were the most susceptible bacteria, being inhibited by 9, 9 and 10 of the plant extracts respectively. *Davallia pyxidata* and *Marchantia polymorpha* extracts were least effective, inhibiting the growth of only 1 or 2 bacteria respectively. *Acrotriche aggregata*, *Petalostigma pubescens*, *Leptospermum trinervia* and *Planchonella queenslandica* leaf extracts were particularly effective bacterial agents being capable of inhibiting the growth of 8 (57%), 10 (71%), 9 (64%) and 9 (64%) of the bacteria tested respectively. *A. aggregata*, *P. pubescens* and *L. trinervia* leaf extracts displayed low toxicity in the *Artemia franciscana* nauplii bioassay, confirming their potential as antibacterial agents for medicinal use.

Key words: Australian plants, medicinal plants, antibacterial, toxicity, *Artemia franciscana*

Introduction

Plants have long been used by most if not all civilisations as medicines for treating a variety of different diseases and complaints. Phytotherapy in Asia is particularly widespread. Asian plant preparations and medications have been used in the treatment of numerous disorders including eczema, malaria and respiratory disorders (Hoareau

and DaSilva, 1999). Similarly, plant based medicinal systems continue to be the primary therapeutic system in many parts of Africa. For example, *Phytolacca dodecandra* is used as a moluscicide in the control of schistosomiasis (Lemma, 1991). African plant medicines have also found a place in modern Western medicinal systems. The antitumour agent's vinblastine and vincristine (derived from *Catharanthus roseus*) are currently used in the treatment of a variety of tumours (Sersa *et al*, 2001; Reich *et al*, 1999). Studies have also demonstrated the myriad of medicinal plant uses by indigenous North and Central Americans (Moerman, 1998) and South Americans (Roth and Lindorf, 2002). Approximately 1500 plant species are currently used in Europe to treat a wide variety of medical conditions (Hoareau and DaSilva, 1999). The South Pacific region also has well established history of medicinal plant usage. In Fiji for example, approximately 500 plant species are known to be used medicinally, with the majority of these used as ointments and dressings to treat wounds and skin disorders (Han, 1998). Receiving much recent interest is the anti-anxiety potential of the important cultural and medicinal plant kava (Piper methysticum) (Denham et al, 2002; Abebe, 2002).

Much of the research into traditional medicinal plant use has focused on Asian (Patwardhan *et al*., 2005), African (Hostettmann *et al*., 2000) and South American (Paz *et al*., 1995) plants. Recent studies have begun to examine the therapeutic potential of Australian medicinal plants (Cock, 2008; Palombo and Semple, 2001, Setzer *et al*, 2001). In particular, the antibacterial nature of Australian plants is receiving attention due to the development of super-resistant bacterial strains and the need to find new antibacterial agents. In recent studies in this laboratory, we have examined the antimicrobial activity (Cock, 2008) and toxicity (Cock, 2009) of some

Australian native plants, many from the Toohey Forest area of Brisbane, Australia. Several plants were identified as having promising antimicrobial activities. The current report extends these previous studies by examining other plant species common in Toohey Forest.

Some plant species tested in the current studies were targeted due to their taxonomic relationship to previously studied species. *Leptospermum confertus* and *Leptospermum trinervia* were selected because other *Leptospermum* species have previously been shown to have good antibacterial activity (Cock, 2008; Davis and Ward, 2003; Weston *et al*, 2000; Brophy *et al*, 1991). *Xanthorrhoea johnsonii* was targeted because of its growth characteristics. Due to its slow growth (Page and Olds, 2004; Bulow-Olsen *et al*, 1982; Lamont and Downes, 1979) and lack of apparent physical deterrants, it was thought that *X. johnsonii* may employ chemical protectants to deter foraging animals. All other species tested were randomly selected. All species were tested for antibacterial activity against a panel of fourteen bacteria. The *Artemia franciscana* nauplii bioassay was also used to determine the relative toxicity of the extracts.

Materials and Methods

Plant Material

Collection of Plant Samples

Acrotriche aggregata (leaves), *Davallia pyxidata* (leaves), *Daviesia squarrosa* (leaves), *Petalostigma pubescens* (leaves and fruit), *Leptospermum confertus* (leaves),

Leptospermum trinervia (leaves), *Marchantia polymorpha* (whole plant), *Parsonia straminea* (leaves), *Planchonella queenslandica* (leaves), and *Xanthorrhoea johnsonii* (leaves) were collected from Toohey Forest, Brisbane, Australia and were identified with reference to a taxonomic key to Toohey Forest plants (Coutts and Catterall, 1980).

Preparation of Crude Extracts

Plant samples were dried in a Sunbeam food dehydrator and the dried material was ground to a coarse powder. One gram of each of the samples of dried plant material was extracted extensively in 50 ml methanol (Ajax, AR grade) for 24 hours at 4 ^{o}C with gentle shaking. The extract was filtered through filter paper (Whatman No. 54) under vacuum followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellet was dissolved in 10 ml 20 % methanol. The extract was passed through 0.22 µm filter (Sarstedt) and stored at 4 ^{o}C.

Antibacterial Screening

Test Microorganisms

All microbial strains were obtained from Michelle Mendell and Tarita Morais, Griffith University, Australia. Stock cultures of *Aeromonas hydrophila, Alcaligenes feacalis, Bacillus cereus, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas fluorescens, Salmonella newport, Serratia marcescens, Shigella sonnei, Staphylococcus aureus, Staphylococcus*

epidermidis and *Streptococcus pyogenes* were subcultured and maintained in nutrient broth at 4 ° C.

Evaluation of Antimicrobial Activity

Antimicrobial activity of all plant extracts was determined using a modified Kirby-Bauer (Bauer *et al*, 1966) disc diffusion method. Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh nutrient broth until they reached a count of approximately 10^8 cells/ml as determined by direct microscopic determination. One hundred microliters of microbial suspension was spread onto nutrient agar plates.

The extract and fractionated components were tested using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4 ^{o}C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Alcaligenes feacalis, Aeromonas hydrophilia, Bacillus cereus, Citrobacter freundii, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas fluorescens, Serratia marcescens,* were incubated at 30 ^{o}C for 24 hours, then the diameters of the inhibition zones were measured in millimetres. Plates inoculated with *Escherichia coli, Salmonella newport, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes* were incubated at 37 ^{o}C for 24 hours, then the diameters of the inhibition zones were measured. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate. Mean values are reported in this study. Standard discs of ampicillin (2 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd. and served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water or 10 µl of 10% methanol were used as a negative controls.

Toxicity Screening

Reference Toxins for Toxicity Screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay. Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) was obtained from Sigma-Aldrich as a mixture of cis (76.6%) and trans (23.0%) isomers and prepared as a 4 mg/ml stock in distilled water. The stock was serially diluted in artificial seawater for use in the bioassay.

Artemia franciscana Nauplii Toxicity Screening

Toxicity was tested using the *Artemia franciscana* nauplii lethality assay developed by Meyer *et al* (1982) for the screening of active plant constituents with the following modifications. *Artemia franciscana* Kellogg cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/l distilled water were prepared prior to use. 2 g of *A. franciscana* cysts were incubated in 1 L synthetic seawater under artificial light at 25^{o}C, 2000 Lux with continuous aeration. Hatching commenced within 16-18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. Seawater

(400 µl) containing approximately 45 (mean 44.5, n = 392, SD 15.9) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted to 2 mg/ml in seawater for toxicity testing, resulting in a 1 mg/ml concentration in the bioassay. 400 µl of diluted plant extracts and the reference toxins were transferred to the wells and incubated at 25 ± 1^{o}C under artificial light (1000 Lux). A negative control (400 µl seawater) was run in at least triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 72 h all nauplii were sacrificed and counted to determine the total number per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis (Finney, 1971).

Results and Discussion

Eleven samples from ten Australian native plant species were extracted in methanol, dried and the weight of the dried extracted material recorded (Table 1). The weight of dried extractable material varied across samples, ranging from 39 mg (*X. johnsonii* leaves) extracted per 1 g starting plant material up to 276 mg (*D. pyxidata* leaves) from the original 1 g of ground dried plant material. All extracts were resuspended in 10 ml of 20 % methanol, resulting in the crude test extract concentrations reported in Table 1.

Table 1: Botanical names of plant species extracted, weight of dried extractable material and the concentrations of each extract.

Plant species	Plant part extracted	Dried extract (mg)	Extract conc. (mg/ml)
Acrotriche aggregata	leaves	271	27.1
Davallia pyxidata	leaves	276	27.6
Daviesia squarrosa	leaves	236	23.6
Leptospermum confertus	leaves	79	7.9
Leptospermum trinervia	leaves	271	27.1
Marchantia polymorpha	whole plant	95	9.5
Parsonia straminea	leaves	109	10.9
Petalostigma pubescens	leaves	251	25.1
Petalostigma pubescens	flowers	216	21.6
Planchonella queenslandica	leaves	149	14.9
Xanthorrhoea johnsonii	leaves	39	3.9

Antimicrobial activity of the extracts was determined by disc diffusion assays. All eleven extracts showed antibacterial activity against one or more bacteria (table 2). Four extracts (*Acrotriche aggregata*, *Petalostigma pubescens*, *Leptospermum trinervia* and *Planchonella queenslandica* leaf extracts) were particularly versatile, being capable of inhibiting the growth of more than half of the bacteria tested. Both Gram-positive and Gram-negative bacteria were susceptible to these extracts. *A. aggregate*, *L. trinervia* and *P. pubescens* leaf extracts inhibited both Gram-positive and Gram-negative bacteria to similar extents. In contrast, *P. queenslandica* extract was more effective at inhibiting the growth of Gram-positive bacteria compared to Gram-negative bacteria. *P. queenslandica* extract inhibited the growth of all (100%) Gram-positive bacteria compared to 5 (50%) of the Gram-negative bacteria tested.

The greater susceptibility of Gram-positive bacteria has been previously reported for South American (Paz *et al*., 1995), African (Kudi *et al*, 1999; Vlietinck *et al*, 1995) and Australian (Cock, 2008; Palombo and Semple, 2001) plant extracts. Susceptibility differences between Gram-positive and Gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The Gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics (Tortora *et al*, 2001).

Table 2: Antibacterial activity of plant extracts.

	A. aggregata	***D. pyxidata***	***D. squarrosa***	***P. pubescens (L)***	***P. pubescens (F)***	***L. confertus***	***L. trinervia***	***M. polymorpha***	***P.queenslandica***	***P. straminea***	***X. johnsonii***	**Amp**	**Chl**
Gram negative rods													
A. faecalis	11.3 ± 0.6*	0 ± 0	9.0 ± 0	15.7 ± 0.6	6.3 ± 0.6	7.0 ± 0	13.7 ± 1.5	0 ± 0	9.3 ± 1.2	6.7 ± 0.6	6.7 ± 0.6	15.2 ± 1.2	6.3 ± 0.6
A. hydrophilia	6.3 ± 0.6	0 ± 0	6.3 ± 0.6	7.3 ± 0.6	6.3 ± 0.6	0 ± 0	7.3 ± 0.6	6.0 ± 0	7.7 ± 0.6	6.7 ± 0.6	6.0 ± 0	12.0 ± 1.0	28.7 ± 1.6
C. freundi	9.3 ± 0.6	0 ± 0	0 ± 0	12.3 ± 0.6	0 ± 0	0 ± 0	9.7 ± 0.6	0 ± 0	8.7 ± 0.6	0 ± 0	0 ± 0	8.3 ± 0.6	15.7 ± 1.2
E. coli	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14.7 ± 0.6	17.3 ± 0.6
K.pneumoniae	9.7 ± 0.6	0 ± 0	0 ± 0	13.3 ± 0.6	0 ± 0	6.3 ± 0.6	11.7 ± 0.6	0 ± 0	7.0 ± 1.0	7.0 ± 0	6.7 ± 1.2	10.3 ± 0.6	21.3 ± 1.5
P. mirabilis	13.0 ± 1.0	0 ± 0	0 ± 0	17.3 ± 0.6	7.0 ± 0	7.3 ± 0.6	15.0 ± 0	0 ± 0	9.7 ± 0.6	0 ± 0	8.3 ± 0.6	17.3 ± 0.6	8.7 ± 0.6
P. fluroscens	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	18.3 ± 0.6	20.6 ± 0.6
S. newport	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	18.7 ± 0.6	20.3 ± 0.6

S. marcenscens	9.0 ± 0	5.8 ± 0.3	6.3 ± 0.6	14.0 ± 0	0 ± 0	0 ± 0	7.7 ± 1.2	0 ± 0	0 ± 0	6.0 ± 0	0 ± 0	0 ± 0	14.7 ± 0.6
S. sonnei	0 ± 0	0 ± 0	0 ± 0	14.7 ± 0.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	14.0 ± 0	14.3 ± 0.6
Gram positive rods													
B. cereus	7.7 ± 0.6	0 ± 0	7.3 ± 0.6	7.7 ± 0.6	6.0 ± 0	6.7 ± 0.6	9.0 ± 1.0	6.0 ± 0	8.3 ± 0.6	6.3 ± 0.6	7.3 ± 0.6	26.7 ± 0.6	13.3 ± 1.2
Gram positive cocci													
S. aureus	11.7 ± 0.6	0 ± 0	0 ± 0	14.0 ± 1.0	0 ± 0	7.7 ± 0.6	14.0 ± 1.0	0 ± 0	9.3 ± 0.6	0 ± 0	0 ± 0	11.7 ± 2.1	16.0 ± 1.0
S. epidermidis	0 ± 0	0 ± 0	10.0 ± 0	0 ± 0	0 ± 0	0 ± 0	6.3 ± 0.6	0 ± 0	8.7 ± 0.6	0 ± 0	0 ± 0	26.3 ± 1.5	12.3 ± 0.6
S. pyogenes	0 ± 0	0 ± 0	8.3 ± 1.5	7.0 ± 0	0 ± 0	7.3 ± 0.6	0 ± 0	0 ± 0	8.0 ± 0	0 ± 0	0 ± 0	17.0 ± 1.0	24.0 ± 1.0

*Numbers indicate the mean diameters of inhibition of triplicate experiments ± standard deviation.

All extracts were diluted to 2000 µg/ml in artificial seawater for toxicity testing, resulting in a 1000 µg/ml concentration in the *Artemia franciscana* lethality bioassay. The results of *A. franciscana* bioassay screening of the Australian plant methanolic extracts are shown in table 3. Previous reports (Santos Pimenta *et al*, 2003; Wickens and Pennacchio, 2002) express LC_{50} values of toxins following 24 h of exposure. Of the 11 extracts tested, 2 (18.2 %) showed greater than 50 % mortality at 24 h. Of these, *X. johnsonii* leaves showed the highest toxicity, resulting in 100% mortality within 24 h. *P. pubescens* flowers also showed high mortality at 24 h with 66 % *A. franciscana* death. Of the positive controls, only potassium dichromate displayed 100% mortality at 24 h. Mevinphos treatment resulted in no evident mortality at 24 h.

Therefore, mortality at later times (48 and 72 h) are also reported here. A further 2 extracts showed greater than 50 % mortality at 48 h compared to 24 h (*M. polymorpha* whole plant extract and *P. queenslandica* leaf extract). This equates to approximately 36 % of the tested extracts showing toxicity at 48 h.

Table 3: Toxicity of Australian plant extracts (1 mg/ml) to *Artemia franciscana*.

Plant species	Plant part extracted	24 h	48 h	72 h
Acrotriche aggregata	leaves	0 ± 0*	1.8 ± 1.6	12.6 ± 13.9
Davallia pyxidata	leaves	5.2 ± 2.3	8.9 ± 5.9	16.8 ± 8.1
Daviesia squarrosa	leaves	0 ± 0	9.1 ± 5.6	19.3 ±8.0
Petalostigma pubescens	leaves	0 ± 0	0 ± 0	48.6 ± 28.4
Petalostigma pubescens	flowers	66.0 ± 2.3	90.5 ± 0.8	100.0 ± 0
Leptospermum confertus	leaves	0 ± 0	25.7 ± 4.9	42.5 ± 11.1
Leptospermum trinervia	leaves	0 ± 0	1.2 ± 1.2	3.4 ± 1.5
Marchantia polymorpha	whole plant	29.2 ± 3.2	66.5 ± 4.0	79.3 ± 1.5
Parsonia straminea	leaves	0 ± 0	20.1 ± 15.1	74.9 ± 3.9
Planchonella queenslandica	leaves	0 ± 0	87.2 ± 5.1	100.0 ± 0
Xanthorrhoea johnsonii	leaves	100.0 ± 0	76.7 ± 3.9	87.9 ± 8.5
Mevinphos		0 ± 0	100.0 ± 0	100.0 ± 0
Potassium Dichromate		100.0 ± 0	100.0 ± 0	100.0 ± 0
Negative control		0 ± 0	0 ± 0	0 ± 0

*Numbers indicate the mean % mortality of at least triplicate experiments ± standard deviation.

Five plant extracts induced greater than 50% mortality by 72 h (*P. pubescens* flowers, *M. polymorpha* whole plant, *P. straminea* leaves, *P. queenslandica* leaves, and *X. johnsonii* leaves). These were considered sufficiently toxic to warrant further investigation to determine the dependence of mortality on the concentration of the extract the *A. franciscana* is exposed to. Table 4 shows the LC_{50} values of these extracts towards *A. franciscana*. *X. johnsonii* leaf extract was the most toxic of the plant extracts tested with 24, 48 and 72 h LC_{50} values of 361.0 ± 41.8 µg/ml, 402.1 ± 15.7 µg/ml and 199.8 ± 60.5 µg/ml respectively. An interesting feature of *X. johnsonii* toxicity was the apparent ability of the *A. franciscana* nauplii to overcome the toxicity. Some of the nauplii that appeared dead (as defined by no movement for 10 seconds) at 24 h had recovered by 48 h. Also, many of the nauplii that did not appear dead displayed obvious reactions to the extract (loss of control of forward movement). For this reason a dose at which 50 % were affected (EC_{50}) is also reported for *X. johnsonii* leaf extract (table 4).

Table 4: LC_{50} and EC_{50} (95% confidence interval) for *A. franciscana* nauplii exposed to Australian plant extracts and the reference toxins Mevinphos and potassium dichromate.

		LC50 value in µg mL^{-1}		
Plant species	**Plant part extracted**	**24 h**	**48 h**	**72 h**
Petalostigma pubescens	flowers	508.3 ± 29.0	477.0 ± 28.7	295.7 ± 19.5
Marchantia polymorpha	whole plant	795.8 ± 117.4	364.3 ± 34.1	308.2 ± 38.0
Parsonia straminea	leaves	-	-	909.6 ± 66.2
Planchonella queenslandica	leaves	-	506.5 ± 83.3	430.2 ± 39.1
Xanthorrhoea johnsonii	leaves	361.0 ± 41.8	402.1 ± 15.7	199.8 ± 60.5
Xanthorrhoea johnsonii *	leaves	220.1 ± 8.6 *	250.0 ± 9.8 *	187.5 ± 19.8 *

Mevinphos	1346.2 ± 80.1	523.3 ± 39.0	114 ± 12.8
Potassium Dichromate	87.1 ± 5.2	81.9 ± 3.8	79.6 ± 4.6

Values indicate the mean of triplicate determinations ± standard deviation. - indicates that LC_{50} values were unable to be obtained as no increase in mortality above seawater controls was evident. * indicates EC50 determinations (where effect is defined as lack of controlled forward movement).

These studies demonstrate the antibacterial activity of Toohey Forest plants. In particular, *A. aggregate, P. pubescens* and *L. trinervia* leaf extracts are particularly promising as antibacterial agents, inhibiting 8 (57%), 10 (71%) and 9 (64%) of the bacteria tested respectively, whilst also having low toxicity in the *A. franciscana* bioassay. *P. queenslandica* also had good antibacterial activity inhibiting the growth of 9 (64%) of the bacteria tested. However, *P. queenslandica* was toxic in the *A. franciscana* bioassay. This assay has been previously shown to correlate well with toxicity towards human cells (Meyer *et al*, 1982: McLaughlin *et al*, 1998). Further toxicity studies using human cell lines is required to determine the extent of this extracts toxicity.

Toxic antibacterial extracts may still be useful as non-medicinal antibacterial agents (eg surface disinfectants). Likewise, toxic plant extracts may also still have medicinal potential even if they are not antimicrobial. McLaughlin *et al* (1998) have demonstrated that toxicity in the *A. franciscana* bioassay may indicate anti-cancer potential. These toxic extracts should also be tested against human cancer cell lines to

determine their potential as anticancer drugs. Furthermore, *X. johnsonii* extract showed interesting toxicity trends. Not only was this the most toxic extract tested, but the *Artemia* nauplii were able to recover from its toxicity, at least temporarily. Nauplii that were initially thought to be dead at 24 h (as determined by lack of movement) were seen to recover at 48 h, only to die again by 72 h.

It is possible that the *X. johnsonii* extract may function in a similar manner as the neuromuscular relaxant curare (derived from the South American plant *Chondrodendron tomentosum*). Curare is the common name for a preparation from *C. tomentosum* that are used by South American natives as arrow poisons (Chevallier, 1999). When curare directly enters the bloodstream the affected animal is paralysed and appears dead (Raghavendra, 2002; Brodie, 1812). Indeed, at higher doses the animal does die. However, very early research into curare function showed that affected animals often completely recover from the toxin, especially when the animals respiration is artificially maintained (Brodie, 1812; Brodie, 1811). Curare functions as a neuromuscular relaxant by blocking acetylcholine receptors (Strecker and Jackson, 1989) thus causing paralysis. Tubocurarine, one of the many alkaloids of curare, has a long history of use as an anaesthetic/muscle relaxant during operations (Gray and Halton, 1946; Griffith and Johnson, 1942) although it is no longer generally used following the development of safer synthetic alternatives such as atracurium and cisatracurium (Raghavendra, 2002; Bryson and Faulds, 1997). The similarities between the toxic effects of curare and *X. johnsonii* extract suggest this extract may also be acting as a neuromuscular blocker and may therefore hold promise as an anaesthetic agent although any similarities are tenuous and need further investigation. Further work is required to determine whether this is indeed the case.

In conclusion, the results of this study indicate that *A. aggregate*, *L. trinervia* and *P. pubescens* and *P. queenslandica* leaf extracts are worthy of further study due to the range of bacteria they are capable of inhibiting. Further evaluation of the antibacterial properties of these extracts against a more extensive panel of microbial agents is warranted. Likewise, purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents. Whilst the extracts examined in this report appear promising as antimicrobial agents, caution is needed before these compounds can be applied to medicinal purposes and as food additives to inhibit spoilage. In particular, further toxicity studies using human cell lines are needed to determine the suitability of these extracts for these purposes. Similarly, the toxic effects of *X. johnsonii* leaf extract warrants further investigation, particularly the possible anaesthetic/neuromuscular blocking affect of this extract.

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