Swainsona Formosa (G.Don) Joy Thomp. Solvent Extractions Inhibit the Growth of a Panel of Pathogenic Bacteria

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ABSTRACT

Introduction: *Swainsona formosa* is a legumous plant which is endemic to the arid inland regions of Australia. Several *Swainsona* spp. were valued by the first Australian for their antiseptic properties and were used traditionally to treat a variety of bacterial diseases. Despite this, *S. formosa* solvent extractions have not been rigorously examined for antibacterial properties against many bacterial pathogens. **Methods:** The antimicrobial activity of *S. formosa* leaf extracts was investigated by disc diffusion and growth time course assays against a panel of pathogenic bacteria. The growth inhibitory activity was quantified by MIC determination. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *S. formosa* leaf extracts inhibited the growth of a wide range of gram positive and gram negative bacteria. The methanolic extracts were generally more potent than the aqueous extracts. The methanolic and aqueous *S. formosa* leaf extracts were particularly potent inhibitors of *A. faecalis*, *A. hydrophilia*, *K. pneumoniae*, *P. mirabilis*, *B. cereus*, *S. aureus* and *S. pyogenes* growth, with MIC values substantially <1000 µg/mL and as low as 150 µg/mL against some bacteria (methanolic extract against *P. mirabilis*). The antibacterial activity of the methanolic and aqueous *S. formosa* leaf extracts was fur-

ther investigated by growth time course assays which showed significant growth inhibition in cultures of all bacterial species within 1 h of exposure. All extracts were determined to be nontoxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for therapeutic uses. **Conclusions:** The lack of toxicity of the *S. formosa* leaf extracts and their growth inhibitory bioactivity against a panel of pathogenic bacteria indicate their potential in the development of antiseptic agents.

Key words: Sturts Desert Pea, Fabaceae, Swainsonine, N,N-Dimethyltryptamine, Australian Plants, Antibacterial Activity, Medicinal Plants.

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DOI : 10.5530/pc.2017.2.13

INTRODUCTION

Bacterial resistance to conventional antibiotics is a concern to public health.[1] The development of super resistant bacterial strains is resulting in currently used antibiotic agents failing to end many bacterial pathogen infections. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents. The growth inhibitory qualities of medicinal plants against pathogenic bacteria have been long recognised. Recently there has been a revival of interest in herbal medications due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals.[2] The first Australians had well developed ethnopharmacological systems and understood the therapeutic properties of a wide variety of aromatic Australian plants.[3] Despite this, relatively few studies have rigorously examined the antibacterial activity of many Australian native plants. However, recently there has been increased study in this field.[4-7]

Swainsona formosa (G.Don) Joy Thomps. (family Fabaceae; synonyms *Clianthus formosus* (G.Don) Ford & Vickery, *Clianthus dampieri* Lindl., *Clianthus oxleyi* A.Cunn. ex Lindl.; commonly known as Sturts desert pea) is a low growing or prostrate legume which is endemic to arid inland regions of the Australian continent. Its taxonomy is somewhat confused, with the species name changing at least 9 names since its original naming as *Clianthus dampieri* Lindl. in the early part of the 18th century. Since that time, it has been reclassified under 3 separate genera (*Clianthus*, *Donia* and *Swainsona*) and was recently proposed to be classified as *Willdampia formosa* (G.Don) A.S.George, although this reclassification has since been rejected. Despite the taxonomic ambiguity, this species is most closely aligned with the genus *Swainsona* on the basis of morphology, genetics and chemotaxonomic similarities. *S. formosa* has

a distinctive appearance, with showy blood red flowers with a bulbous black centre (Figure 1a). It is perhaps best known as the state floral emblem for the state of South Australia.

Several *Swainsona* spp. were used by Australian Aborigines as traditional medicines.[3-8] *Swainsona galegifolia* (Andrews) R.Br. and *Swainsona pterostylis* (DC.) Bakh.f. were considered particularly useful as antiseptics and as bacteriocide chemotherapies against a broad spectrum of bacterial pathogens.[3-8] Interestingly, we were unable to find confirmed reports of Aboriginal medicinal use of *S. formosa* for similar purposes. However, a defining phytochemical characteristic of many *Swainsona* spp. is the presence of the indolizidine alkaloid phytotoxin swainsonine (Figure 1b).[9] Swainsonine has been associated with livestock intoxication via inhibition of the enzymes α-mannosidase and mannosidase II which are required for processing and maturation of N-linked oligosaccharides of newly synthesised glycoproteins. To date, most interest in the therapeutic properties of swainsonine have focussed on its potential as a cancer chemotherapeutic drug via a reduction of tumour cell metastatis, decreased proliferation and enhanced cellular immune responses.[10] Interestingly, many pathogenic bacteria also contain N-glycosylates attached to their surface proteins.[11,12] Despite the ability of swainsonine to disrupt this process, there is a lack of studies examining the growth inhibitory properties of swainsonine, or of extracts containing this compound.

S. formosa has also been reported to produce significant quantities of N,N-dimethyltryptamine (DMT; Figure 1c).[13] The presence of DMT in some Australian plants (including *S. formosa*) is interesting and has resulted in the failed attempt by the Australian government in 2011/2012 to introduce legislation banning the growing of many DMT containing plants, including the floral emblem of South Australia (*S. formosa*) and
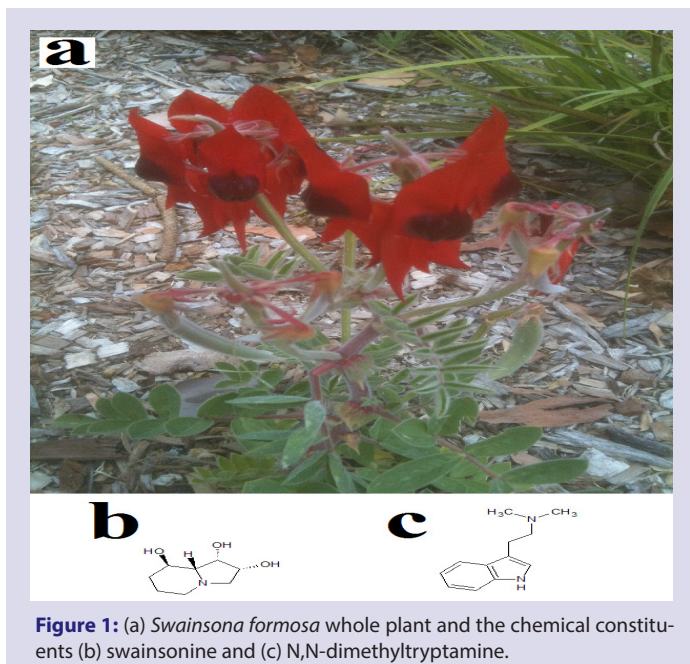
Figure 1: (a) *Swainsona formosa* whole plant and the chemical constituents (b) swainsonine and (c) N,N-dimethyltryptamine.

the Australian floral emblem (*Acacia pycnantha*; golden wattle). Whilst DMT is best known for its psychedelic properties, several DMT derivatives have potent antimicrobial activity.[14] Despite its interesting phytochemistry and the ethnobotanical usage of other *Swainsona* spp. as antiseptics, studies examining the bacterial growth inhibitory properties of *S. formosa* are lacking. The current report was undertaken to screen *S. formosa* leaf extracts for growth inhibitory properties against a panel of pathogenic bacteria.

MATERIALS AND METHODS

Plant collection and extraction

Swainsona formosa (G.Don) Joy Thomp. leaves were obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. The leaf samples were dried in a Sunbeam food dehydrator and stored at -30 °C. Prior to use, the dried leaves were freshly ground to a coarse powder and 1 g quantities were weighed into separate tubes. A volume of 50 mL methanol, sterile deionised water, ethyl acetate, chloroform or hexane was added to individual tubes and extracted for 24 hours at 4 °C with gentle shaking. All solvents were obtained from Ajax, Australia and were AR grade. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellets were dissolved in 10 mL sterile deionised water (containing 1 % DMSO). The extracts were passed through 0.22 μm filter (Sarstedt) and stored at 4 °C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *S. formosa* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[15-17]

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd., Australia. Clinical isolate microbial strains of *Aeromonas hydrophilia, Alcaligenes feacalis, Bacillus cereus, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas fluorescens, Salmonella newport, Serratia marcescens, Shigella sonneii, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyonenes* were obtained from Ms Michelle Mendell and Ms Jane Gifkins, Griffith University. All stock cultures 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 disc diffusion assay.[18-20] Briefly, 100 μL of each bacterial culture was grown in 10 mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. A volume of 100 μL of the bacterial suspension was spread onto nutrient agar plates and extracts were tested for antibacterial activity using 5 mm sterilised filter paper discs. Discs were infused with 10 μL of the plant extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4 °C for 2 h before incubation at 30 °C for 24 h. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was performed in at least triplicate. Mean values (± SEM) are reported in this study. Standard discs of ampicillin (10 μg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10 μL of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of each extract against susceptible bacteria was determined as previously described.[21,22] Briefly, the *S. formosa* extracts were diluted in deionised water and tested across a range of concentrations. Discs were infused with 10 μL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was completed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to determine the MIC values of each extract.

Bacterial growth time course assay

Bacterial growth time course studies were performed as previously described.[23,24] Briefly, 3 mL of the most susceptible bacterial cultures (*K. pneumoniae, P. mirabilis* and *B. cereus*) in nutrient broth were added to 27 mL nutrient broth containing 3 mL of 10 mg/mL methanolic plant extract to give a final concentration of 1000 μg/mL in the assay. The tubes were incubated at 30 °C with gentle shaking. The optical density was measured hourly at 550 nm for a 6 h incubation period. Control tubes were incubated under the same conditions but without the extract. All assays were performed in triplicate.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 4 mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.[25-27] Briefly, 400 μL of seawater containing approximately 62 (mean 61.7, n = 85, SD 11.6) *A. franciscana* nauplii were added to wells

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the *S. formosa* extracts.

Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/mL)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
M	186	18.6	+++	+++	+	-	++	-	-	++	+	+++	+++	-	-
W	142	14.2	+++	+++	+	-	+	-	-	+	+	+++	+++	-	-
E	83	8.3	++	+	-	-	+	-	-	+	-	++	++	-	-
C	127	12.7	-	-	-	-	-	-	-	-	-	++	+	-	-
H	62	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract.

of a 48 well plate and immediately used for bioassay. A volume of 400 µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A 400 µL seawater negative control was run in 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 detected within 10 seconds. After 24 h, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was determined using probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments. One way ANOVA was used to calculate statistical significance between control and treated groups with a *P* value < 0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of dried and powdered *S. formosa* leaves with solvents of varying polarity yielded dried extracts ranging from 62 mg (hexane extract) to 186 mg (methanol extract) (Table 1). The aqueous (142 mg) and chloroform extracts (127 mg) also yielded relatively high levels of extracted materials. The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO), resulting in the extract concentrations shown in Table 1.

Qualitative phytochemical studies showed that the higher polarity methanol and water solvents extracted the greatest diversity and highest levels of phytochemicals. Both contained high levels of phenolics, flavonoids and tannins as well as moderate levels of saponins and alkaloids. The ethyl acetate extract contained similar phytochemical classes, albeit generally at lower levels. Interestingly, despite extracting relatively large amounts of material, the chloroform and hexane extracts

Table 2: Minimum bacterial growth inhibitory concentration (µg/mL) of the *S. formosa* extracts.

Bacterial Species	M	W	E	C	H
Gram negative bacteria					
A. faecalis	468	783	-	-	-
A. hydrophilia	640	1044	926	-	-
C. freundii	-	-	-	-	-
E. coli	-	-	-	-	-
K. pneumoniae	166	375	880	-	-
P. mirabilis	141	168	743	947	-
P. fluorescens	-	-	-	-	-
S. newport	-	-	-	-	-
S. marcescens	-	-	-	-	-
S. sonneii	-	-	-	-	-
Gram positive bacteria					
B. cereus	328	855	1163	1870	-
S. aureus	575	927	1045	-	-
S. epidermidis	-	-	-	-	-
S. pyonenes	622	1449	-	-	-

Numbers indicate the mean MIC and LC_{50} values of triplicate determinations. - indicates no inhibition.
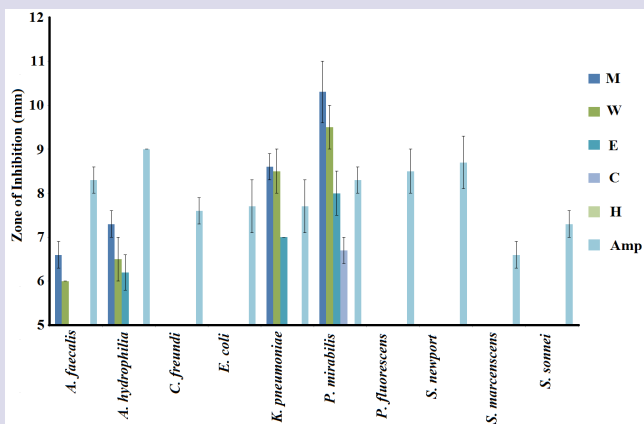
Figure 2: Growth inhibitory activity of *S. formosa* leaf extracts against gram negative bacterial species. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 μg) control. All determinations were performed in at least triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.
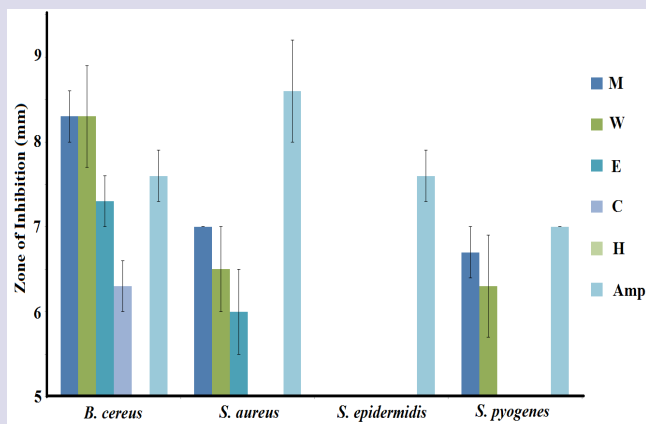

Figure 3: Growth inhibitory activity of *S. formosa* leaf extracts against gram positive bacterial species. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 μg) control. All determinations were performed in at least triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.
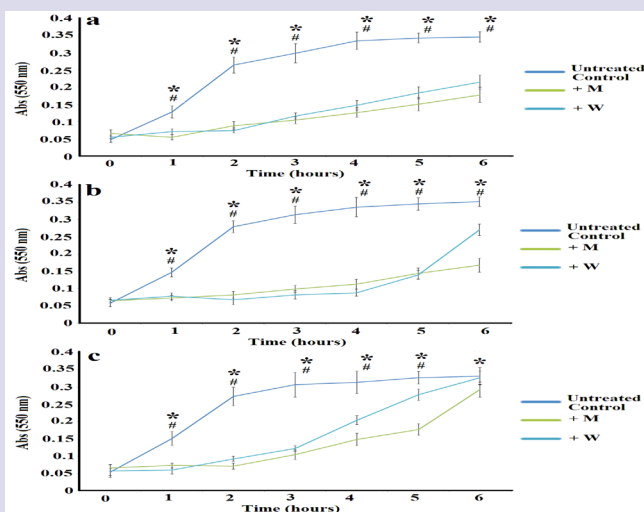

Figure 4: Bacterial growth curves for the methanolic and aqueous S. formosa extracts against (a) *K. pneumoniae*, (b) *P. mirabilis* and (c) *B. cereus*. All bioassays were performed in at least triplicate and are expressed as mean ± SEM. * = growth results in the presence of the methanolic extract that are significantly different to the untreated control growth (p<0.01); # = growth results in the presence of the aqueous extract that are significantly different to the untreated control growth (p<0.01).
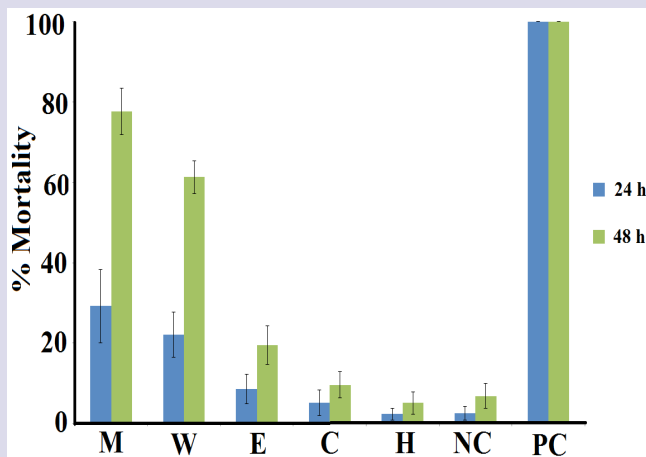

Figure 5: The lethality of the *S. formosa* leaf extracts (2000 μg/mL), potassium dichromate (1000 μg/mL) and a seawater control. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; NC = negative (seawater) control; PC = positive control (1000 μg/mL potassium dichromate). All bioassays were performed in at least triplicate and are expressed as mean ± SEM.

were devoid of all classes of phytochemicals screened. Due to their nonpolar nature, these extracts would be expected to contain high levels of lipids, hydrocarbons etc. As our qualitative phytochemical studies did not screen for these compounds, they were not detected and other techniques are required to further examine the nature of these nonpolar components.

Antimicrobial activity

To determine the growth inhibitory activity of the *S. formosa* leaf extracts against the panel of pathogenic bacteria, aliquots (10 μL) of each extract were screened in the disc diffusion assay. The aqueous and methanolic *S. formosa* leaf extracts inhibited a broadest range of gram negative (Figure 2) and gram positive bacteria (Figure 3). The methanolic *S. formosa*

extract was a more potent growth inhibitor than the aqueous extract against most gram negative bacterial species (as assessed by the sizes of the zones of inhibition; Figure 2). The inhibition of the methanolic and aqueous extracts was particularly noteworthy against *K. pneumoniae*, *P. mirabilis* and *B. cereus*, with inhibition zones of approximately 8.5, 10 and 8.3 mm respectively for both extracts. This compares favourably to the inhibition by the ampicillin control (10 μg: inhibition zones of approximately 7.7, 8.3 and 7.6 mm against *K. pneumoniae*, *P. mirabilis* and *B. cereus* respectively). The ethyl acetate and chloroform extracts also inhibited the growth of a range of bacteria (5 (36 %) and 2 (14 %) of the 14 bacterial species tested respectively), albeit generally with substantially smaller inhibition zones than were recorded for methanolic and aqueous extracts. The hexane extract was devoid of growth inhibitory activity.

Gram positive bacteria were also susceptible to the *S. formosa* leaf extracts (Figure 3). Of the 4 gram positive bacterial strains tested, 3 (75

%) were inhibited by both the methanolic and aqueous *S. formosa* leaf extracts. With the exception of *S. pyogenes*, all of these bacterial species were also inhibited by the ethyl acetate *S. formosa* leaf extract. The chloroform extract only inhibited *B. cereus* of the gram positive bacteria. Furthermore, the chloroform extract only produced a small zone of inhibition indicative of weak growth inhibitory activity. The hexane extract was devoid of bacterial growth inhibitory activity against all gram positive bacterial species.

The antimicrobial efficacy was further quantified by determining the MIC values for each extract against the microbial species which were determined to be susceptible. The methanolic, aqueous and ethyl acetate *S. formosa* leaf extracts were potent growth inhibitors of several bacterial species (as judged by MIC; Table 2). *P. mirabilis* was the most susceptible bacteria to the *S. formosa* leaf extracts, with MIC values <175 μg/mL (<2 μg infused into the disc) recorded for the aqueous and methanolic extracts. The ethyl acetate and chloroform extracts was also good *P. mirabilis* growth inhibitor, with MIC values of <1000 μg/mL. As *P. mirabilis* infection is a common cause of urinary tract infections and has also been identified as a trigger of rheumatoid arthritis,[28,29] the aqueous and methanolic *S. formosa* leaf extracts have potential for the prevention of these diseases in genetically susceptible individuals. The methanolic and aqueous extracts were similarly potent inhibitors of *K. pneumoniae* growth with MIC values 150-375 μg/mL. *K. pneumoniae* infections have been identified as a trigger for ankylosing spondylitis in genetically susceptible people,[24] further highlighting the potential for the prevention and treatment of ankylosing spondylitis.

Furthermore, the aqueous and methanolic extracts were also potent *A. faecalis*, *A. hydrophilia* , *S. aureus* and *S. pyogenes* growth inhibitors, with MIC values generally in the 500-1000 μg/mL range. The ethyl acetate extract was also a potent *A. hydrophilia* growth inhibitor (MIC 926 μg/mL). The chloroform *S. formosa* extract also inhibited *P. mirabilis* and *B. cereus* growth, albeit with MIC values >1000 μg/mL, indicating moderate growth inhibition. Moderate to low growth inhibition (or no inhibition) was noted for all other extract/bacterium combinations.

Bacterial growth time course assay

The antibacterial activity of the *S. formosa* extracts was further investigated in *K. pneumoniae*, *P. mirabilis* and *B. cereus* by bacterial growth time course assays in the presence and absence of the extract. Only the effect of the methanolic and aqueous extracts on the bacterial growth time course were evaluated as these extracts were generally the most potent bacterial growth inhibitors. The starting concentration of the extract used in these assays was 1000 μg/mL. The methanolic *S. formosa* extract significantly inhibited *K. pneumoniae* (Figure 4a), *P. mirabilis* (Figure 4b) and *B. cereus* growth (Figure 4c) within 1 h, indicating a rapid antimicrobial action. Whilst *B. cereus* growth was inhibited for at least the first 5 hours of the time course, the bacteria were generally able to overcome this inhibition by 6h, with the recorded turbidity not significantly different to that of the untreated control. This indicates that the growth inhibition of these bacteria was bacteriostatic for the methanolic and aqueous *S. formosa* extracts at the concentrations tested. In contrast, inhibition of *K. pneumoniae* and *P. mirabilis* by the methanolic and aqueous *S. formosa* extracts was substantially more profound, with growth still significantly inhibited by the end of the 6 h time course study for both bacteria. This may indicate that these extracts have bactericidal activity against these bacterial species at the dose tested. Indeed, the turbidity at 6 h was not greatly increased from the starting turbidity.

Quantification of toxicity

The toxicity of the *S. formosa* extracts was screened in the *Artemia franciscana* nauplii bioassay at a concentration of 2000 μg/mL (Figure 5).

All extracts induced low levels of mortality at 24 h, similar to the % mortality seen for the seawater control. By 48 h, the aqueous and methanolic extracts had begun to induce mortality significantly higher than that in the untreated control. As only the methanolic extract induced > 50 % toxicity at 48 h, all extracts were deemed to be nontoxic. Extracts with an LC_{50} of greater than 1000 μg/mL towards *Artemia* nauplii have previously been defined as being nontoxic,[27] In contrast, the potassium dichromate positive control induced mortality within 4 h (results not shown), with 100 % mortality induction seen by 24 h.

DISCUSSION

Plant derived remedies are becoming increasingly sought after in the treatment of a myriad of diseases and disorders due both to their perception of greater safety than synthetic drugs, and the failure of current drug regimens to effectively treat many diseases. This study reports on the growth inhibitory properties of *S. formosa* leaf extracts against a panel of pathogenic bacteria, and on their toxicity. Both gram positive and gram negative bacteria tested in this study were susceptible to the *S. formosa* leaf extracts, although the gram positive bacteria were more susceptible (as judged by the number of bacteria inhibited). Indeed, the methanolic extract inhibited 75 % of the gram positive bacterial species screened, compared to 40 % of the gram negative bacteria. This is consistent with many previous studies with other plant species which report a greater susceptibility of gram positive bacteria towards solvent extracts for South American,[30] African,[31,32] and Australian [33] plant extracts. Results within this laboratory [10-34-36] have also confirmed the greater susceptibility of gram positive bacteria towards many other Australian plant extracts, although examples of Australian plants having a greater effect on gram negative bacteria have also been reported.[37,38]

Our study examined the ability of *S. formosa* leaf extracts to inhibit the growth of a panel of medicinally important bacterial pathogens. The methanolic and aqueous extracts were identified as being particularly potent inhibitors of *P. mirabilis* with MIC values of 141 and 168 μg/mL respectively. As *P. mirabilis* can trigger rheumatoid arthritis in genetically susceptible individuals.[28,29] these extracts have potential for the development of rheumatoid arthritis preventative therapies. The methanolic and aqueous extracts were also potent *K. pneumoniae* growth inhibitory properties, with MIC values 166 and 375 μg/mL respectively. As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,[24] this extract may also be useful in the prevention of ankylosing spondylitis.

The methanolic and aqueous *S. formosa* leaf extracts also were moderate to good inhibitors of several other bacterial pathogens. Both the aqueous and methanolic *S. formosa* leaf extracts were also good inhibitors of *A. faecalis*, *A. hydrophilia*, *B. cereus*, *S. aureus* and *S. pyogenes* growth with MIC values generally 300-1000 μg/mL. As these bacteria have been implicated in a number if gastrointestinal and skin diseases, the aqueous and methanolic *S. formosa* leaf extracts also may have applications in the treatment of these diseases.

Whilst a detailed investigation of the phytochemistry of the *S. formosa* leaf extracts was beyond the scope of our study, qualitative screening studies were used to determine the classes of compounds present. Several commonalities were noted: the most potent aqueous, methanolic and ethyl acetate extracts all contained relatively high levels of phenolics, tannins and flavonoids. Many studies have reported potent growth inhibitory activities for a number of tannin compounds. Gallotannins have been reported to inhibit the growth of a broad spectrum of bacterial species[39] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins,[40,41] and by inhibiting glucosyltransferase enzymes.[42] Elligitannins are also highly potent inhibitors of bacterial growth,

with MIC values as low as 62.5 μg/mL.[39-41] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[39,41] Thus, it is likely that *S. formosa* leaf tannins may contribute to the inhibition of bacterial growth reported in our study.

It is likely that other phytochemical classes may also contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies indicate that polyphenolics and flavonoids were present in the *S. formosa* leaf extracts in high levels. Many studies have reported potent antibacterial activities for a wide variety of polyphenolic compounds, including many flavonoids.[43] Furthermore, alkaloids were detected in low to moderate levels in the inhibitory extracts. This is noteworthy as the alkaloids swainsonine and DMT have both previously been reported in *Swainsona* spp. Extracts.[9-13] Swainsonine induces toxicity by inhibiting α-mannosidase and mannosidase II activity,[10] which subsequently inhibits glycoprotein processing and maturation. Many pathogenic bacteria incorporate N-glycosylically linked surface proteins[11,12] and thus swainsonine may affect bacterial growth. However, this is yet to be adequately tested. Of further note, several DMT derivatives have potent antimicrobial activity [14] and thus may also contribute to the inhibitory activity reported in our study. Further studies are required to test these compounds for growth inhibitory activity against the bacterial pathogens screened in our study. It would be particularly interesting to test swainsonine and DMT for the growth inhibition against *B. cereus*, *K. pneumoniae* and *P. mirabilis*. Furthermore, bioactivity driven isolation of active components is required to confirm the bioactive components and to further evaluate the mechanism of bacterial growth inhibition.

The findings reported here also demonstrate that all of the *S. formosa* leaf extracts were nontoxic towards *Artemia franciscana* nauplii, with 24 h LC_{50} values substantially > 1000 μg/mL. Extracts with LC_{50} values > 1000 μg/mL towards *Artemia* nauplii are defined as being nontoxic.[27] Whilst our preliminary toxicity studies indicate that these extracts may be safe for therapeutic use, studies using human cell lines are required to further evaluate the safety of these extracts. Furthermore, as both swainsonine and DMT have psychoactive properties, studies are needed to evaluate the potential negative side effects of these extracts. Whilst these studies have demonstrated the potential of the *S. formosa* leaf extracts in the development of future antibiotic chemotherapeutics for the prevention and treatment of urinary tract infections, autoimmune diseases (particularly rheumatoid arthritis and ankylosing spondylitis) and some gastrointestinal and skin diseases, more work is required to isolate the inhibitory components and determine the mechanism of inhibition.

CONCLUSIONS

The results of this study demonstrate the potential of the *S. formosa* leaf extracts as inhibitors of pathogenic bacteria growth. Furthermore, their lack of toxicity indicates than they may be safe for therapeutic treatment. Further studies aimed at the purification and identification of bioactive components are needed to examine the mechanisms of action of these agents.

ACKNOWLEDGEMENTS

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; LC_{50}: The concentration required to achieve 50 % mortality; MIC: minimum inhibitory concentration.

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PICTORIAL ABSTRACT

S. formosa — **Growth Inhibition** — **Extracts** — **Non-toxic**
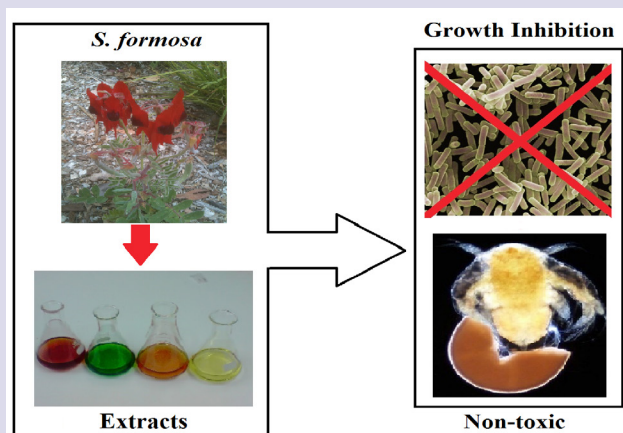
SUMMARY

- *S. formosa* leaf extracts displayed broad spectrum antibacterial activity against gram positive and gram negative bacteria.

- The gram negative bacteria *A. faecalis, A. hydrophilia, K. pneumoniae* and *P. mirabilis* were particularly susceptible (MICs substantially <700 µg/mL).

- The gram positive bacteria *B. cereus, S. aureus* and *S. pyogenes* were also highly susceptible (MICs <1000 µg/mL).

- All *S. formosa* leaf extracts were nontoxic in the *Artemia* nauplii bioassay (LC50 > 1000 µg/mL).

ABOUT AUTHORS

Ms Getmore Chikowe completed at BSc at Griffith University in life sciences. Following graduation, she undertook a research project in Dr Ian Cock's laboratory in the School of Natural Sciences at Griffith University. The project examined the growth inhibitory properties of a variety of AUSTRALIAn native plants against an extensive panel of bacterial pathogens.

Dr Ian Cock leads a research team in the Environmental Futures Research Institute and the School of Natural Sciences at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin, including *Aloe vera*, South Asian and South American tropical fruits, as well as Australia plants including *Scaevola spinescens, Pittosporum phylliraeoides, Terminalia ferdinandiana* (Kakadu plum), Australian *Acacias, Syzygiums, Petalostigmas* and *Xanthorrhoea johnsonii* (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.

Ms Lindiwe Mpala completed at BSc at Griffith University in life sciences. Following graduation, she undertook a research project in Dr Ian Cock's laboratory in the School of Natural Sciences at Griffith University. The project examined the growth inhibitory properties of a variety of Australian native plants against an extensive panel of bacterial pathogens.