Pharmacogn. Commn. 2016; 6(2) 93-99
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Growth Inhibitory Activity of Selected High Antioxidant Australian *Syzygium* Species Against the Food Poisoning and Tissue Necrotic Pathogen *Clostridium perfringens*

Mitchell Henry Wright,[1] Cameron Jay Lee,[1] Charmaine Estelle Pollock,[1] Anthony Carlson Greene,[1] Ian Edwin Cock,[1,2*]

[1]School of Natural Sciences, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, AUSTRALIA.
[2]Environmental Futures Research Institute, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, AUSTRALIA.

ABSTRACT

Introduction: *Clostridium perfringens* is a gram positive pathogen which is an etiological agent in Clostridial myonecrosis and enteritis necroticans. Unless promptly treated, *C. perfringens* infections may result in tissue necrosis and death. *Syzygium australe* (brush cherry) and *Syzygium luehmannii* (riberry) fruit and leaves have documented therapeutic properties as general antiseptic agents against an extensive panel of bacteria. Despite this, studies are yet to test the growth inhibitory activity of these species against *C. perfringens*. **Methods:** *S. australe* and *S. luehmannii* fruit and leaf extracts were investigated by disc diffusion assay for growth inhibitory activity against a clinical strain of *Clostridium perfringens*. Their MIC values were determined to quantify and compare their efficacies. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** Methanolic and aqueous *S. australe* and *S. luehmannii* fruit and leaf extracts as well as the corresponding fruit ethyl acetate extracts, displayed growth inhibitory activity in the disc diffusion assay against *C. perfringens*. The aqueous and methanolic extracts were particularly potent growth inhibitors, each with MIC values substantially <500 µg/mL. The *S. australe* fruit extracts were nontoxic in the *Artemia franciscana* bioassay (LC_{50} values <1000 µg/mL). All ethyl acetate extracts were also nontoxic. In contrast, the *S. luehmannii* aqueous and methanolic extracts (fruit and leaf), as well as the *S. australe* leaf extracts displayed substantial toxicity in the assay. **Conclusion:** The potent growth inhibitory bioactivity of the fruit and leaf aqueous and methanolic *Syzygium* spp. extracts against *C. perfringens* indicates their potential as medicinal agents in the treatment and prevention of clostridial myonecrosis and enteritis necroticans.

Key words: *Syzygium australe, Syzygium luehmannii*, Riberry, Brush cherry, antioxidant, Myonecrosis, Enteritis necroticans, Gas gangrene.

Correspondence:
Ian Edwin Cock,
School of Natural Sciences, Nathan Campus,
Griffith University, 170 Kessels Rd, Nathan,
Queensland 4111, AUSTRALIA.
Tel no.: +61 7 37357637; Fax no: +61 7 37355282.

E-mail: i.cock@griffith.edu.au (I. E. Cock).
DOI : 10.5530/pc.2016.2.7

INTRODUCTION

The genus *Clostridium* comprises a large group of sporulating, Gram-positive, fermentative saprophytes. Generally existing in nature as part of the natural bacterial flora, the genus is clinically significant as several members of the genus have been linked to a number of diseases in humans.These include *C. botulinum* (botulism), *C. difficile* (pseudomembranous colitis), *C. sordellii* (pneumonia, endocarditis) and *C. perfringens* (food poisoning, clostridial myonecrosis).[1,2] Although non-invasive, pathogenic clostridia are opportunistic and under suitable conditions can infect a host and cause disease through the production of various exotoxins.[3] The diseases vary greatly in both associated symptoms and degree of lethality, and some species can cause multiple diseases depending on the route of infection. The best known example within the genus is *C. perfringens*, which can cause food poisoning if ingested, or clostridial myonecrosis (gas gangrene) through the infection of deep-tissue wounds.[4]

C. perfringens is one of the most common causes of food poisoning, accounting for up to a million infections annually in the United States.[5] Present in air, soil, water, faeces and as part of the human gut microbiota, the bacterium commonly infects food and at ambient temperatures can propagate to infectious levels. Whilst proper food preparation can significantly reduce the likelihood of infection, the sporulating nature of *C. perfringens* can result in the organism's survival.[6] In fact, *C. perfringens* is reportedly the second most common cause of bacterial food poisoning, with only by nontyphoidal *Salmonella* causing more cases.[5] Antibiotic therapy can offer an effective means of treatment, however due to the potential development of bacterial resistance, the search is ongoing for alternative antimicrobial agents. In addition to the design and synthesis of new agents, the probing of natural resources for antimicrobial agents offers an appealing alternative to traditional means of drug development. The geographical isolation and unique flora positions Australia to be an ideal environment for such investigations with several previous studies reporting antimicrobial activity against a number of other microorganisms.[7] *Syzygium* (family Myrtaceae) is a genus of flowering plants which comprises approximately 500 species.[8] *Syzygium* spp. are widespread globally, occurring in tropical and sub-tropical regions of Africa, Southern and South-East Asia, and throughout Australia. The greatest diversity of *Syzygium* spp. occurs in South-East Asia and Northern Australia. Most species are evergreen trees or large shrubs, with glossy green foliage. Several species have culinary and therapeutic uses. In the commercially most important species *Syzygium aromaticum* (clove), the unopened flower bud is used as a spice. This plant also has uses in traditional medicine due to its anaesthetic properties.[9] The antibacterial activity of *S. aromaticum* is also well known. Numerous studies have reported on the antibacterial[10] and anti-fungal[11] activities of oils and extracts from this plant.

Several other *Syzygium* spp. (eg. *Syzygium australe, Syzygium jambos, Syzygium luehmannii*) produce edible fruit which have very high antioxidant capacities.[12-14] Consumption of high levels of antioxidants has been associated with a decreased incidence of chronic diseases.[12,15,16] High antioxidant levels have also been shown to act as a preventative against

the development of degenerative diseases such as cancer,[17] cardiovascular diseases,[18] neural degeneration,[19] diabetes and obesity.[20] Furthermore, recent studies have also reported antibacterial activity in extracts from high antioxidant Australian plants.[21-24] Particularly noteworthy, the Australian species *S. australe* (Bush Cherry) and *S. luehmannii* (Riberry) have potent growth inhibitory activity against a broad panel of bacteria.[25,26] However, despite the reported broad spectrum growth inhibitory activity of Australian *Syzygium* spp., numerous pathogens are yet to be evaluated for their susceptibility to Australian *Syzygium* spp. extracts. The current study was undertaken to test the ability of to *S. australe* and *S. luehmannii* fruit and leaf extracts to inhibit the growth of the Gram-positive anaerobic bacterium *Clostridium perfringens*.

MATERIALS AND METHODS

Plant source and extraction

S. luehmannii and *S. australe* fruit and leaves were collected from verified trees in suburban regions of Brisbane, Australia and voucher specimens deposited in the School of Natural Sciences, Griffith University, Australia. The plant materials were comprehensively dried using a Sunbeam food dehydrator and the dried material stored at -30°C until required. Prior to use, the plant materials were thawed and ground into a coarse powder. Individual 1 g quantities of the ground plant material were weighed into separate tubes and 50 mL of deionised water, methanol or ethyl acetate were added. All solvents were obtained from Ajax, Australia and were AR grade. The ground plant materials were independently extracted in each solvent for 24 h at 4°C through gentle shaking. The extracts were subsequently filtered through filter paper (Whatman No. 54) under vacuum, followed by drying using rotary evaporation in an Eppendorf concentrator 5301. The resultant dry extract was weighed and dissolved in 10 mL deionised water (containing 1% DMSO).

Qualitative phytochemical studies

Phytochemical analysis of the extracts for the presence of alkaloids, cardiac glycosides, tannins, saponins, phenolic compounds, flavonoids, polysteroids and triterpenoids was achieved as previously described.[27-29]

Antioxidant capacity

The antioxidant capacity of each sample was assessed using a modified DPPH free radical scavenging method.[30,31] Ascorbic acid (0-25 µg per well) was used as a reference and the absorbances were measured and recorded at 515 nm. All tests were completed alongside controls on each plate and all were performed in triplicate. The antioxidant capacity based on DPPH free radical scavenging ability was determined for each extract and expressed as µg ascorbic acid equivalents per gram of original plant material extracted.

Antibacterial screening

Clinical *Clostridium perfringens* screening

A clinical strain of *Clostridium perfringens* was supplied by Ms. Jane Gifkins (Griffith University) which was originally isolated and verified by Dr. John Bates (Department of Health, Queensland, Australia). Cultures were grown and maintained using a thioglycollate liquid media (Oxoid Ltd., Australia). All growth studies were performed using nutrient agar (Oxoid Ltd., Australia) under induced anaerobic conditions through the use of anaerobic jars and AnaeroGen™ 3.5 L atmospheric generation systems (Thermo Scientific). All studies were performed at 30°C and the stock culture was subcultured and maintained in thioglycollate liquid media at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of *Syzygium* spp. extracts was determined using a modified disc diffusion assay.[23,30] Briefly, 100 µL of *C. perfringens* was grown in 10 mL of fresh thioglycollate media until an approximate count of 10^8 cells/mL was reached. A volume of 100 µL of the bacterial suspension was spread onto nutrient agar and extracts were tested for antibacterial activity using 6 mm sterilised filter paper discs. Discs were impregnated with 10 µL of the extracts, allowed to dry and placed onto the inoculated plates. The plates were left 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 penicillin (2 µg) and ampicillin (10 µg) were obtained from Oxoid Ltd., Australia and used as positive controls to compare antibacterial activity. Filter discs impregnated with 10 µL of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentrations (MIC) of the extracts was determined as previously described.[32,33] Briefly, the plant extracts were diluted in deionised water and tested across a range of concentrations. Discs were impregnated with 10 µL of the extract dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted. Determination of MIC values were achieved using linear regression.

Toxicity screening

Reference toxin for toxicity screening

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

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artermia franciscana* nauplii lethality assay.[34-36] Briefly, 400 µL of seawater containing ~43 (mean 43.2, n=155, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used in the bioassay. Volumes of 400 µL of reference toxin or the diluted plant extracts were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). For each plate, a negative control (400 µL seawater) was run in triplicate. All treatments were also performed in at least triplicate. The wells were assessed at regular intervals and the number of dead counted. The nauplii were deemed dead if no movement of the appendages was detected within 10 sec. 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 calculated using probit analysis.

Statistical analysis

Data is expressed as the mean ± SEM of at least three independent experiments.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of Australian *Syzygium* spp. plant materials (1 g) with various solvents yielded dried plant extracts ranging from 62 mg (*S. luehmannii* leaf ethyl acetate extract) to 560 mg (*S. luehmannii* fruit methanolic extract) (Table 1). Methanolic extracts provided significantly greater yields of extracted material relative to the corresponding aqueous and

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities (mg equivalence of ascorbic acid/g dried plant material) of plant extracts

		S. luehmannii fruit			*S. luehmannii* leaf			*S. australe* fruit			*S. australe* leaf		
		W	M	E	W	M	E	W	M	E	W	M	E
	Mass of dried extract (mg)	120	560	130	88	190	62	240	360	110	180	360	110
	Concentration of resuspended extract (mg/ml)	12	56	13	9	19	6	24	36	11	18	36	11
Phenolics	Total phenolics	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	++
	Water soluble phenolics	+++	+++	-	+++	+++	-	+++	+++	-	+++	+++	-
	Water insoluble phenolics	++	+++	++	++	+++	++	++	+++	++	++	+++	++
Alkaloids	Cardiac glycosides	-	-	-	-	-	-	-	-	-	-	-	-
	Saponins	+	+	-	+	+	-	+	-	-	+	+	-
	Triterpenes	+	+	-	+	+	-	-	+	-	+	+	-
	Polysteroids	-	-	-	-	-	-	-	-	-	-	-	-
	Meyer test	-	-	-	-	-	-	-	-	-	-	-	-
	Wagner test	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	Flavonoids	+++	+++	+	++	+++	++	+++	+++	+	++	+++	++
	Tannins	+	+	-	+	+	-	+	+	-	+	+	-
	Free	-	-	-	-	-	-	-	-	-	-	-	-
	Combined	-	-	-	-	-	-	-	-	-	-	-	-
	Antioxidant content by DPPH reduction (expressed as mg AA equivalence per 1 g plant material extracted)	59.2	94.6	1.5	44.7	43.4	5.5	40.7	55.2	9.2	25.3	40.2	2.58

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. W=aqueous extract; M=methanolic extract; E=ethyl acetate extract; AA=ascorbic acid.

Table 2: MIC against *C. perfringens* growth (µg/mL) and *Artemia* nauplii bioassay LC_{50} values (µg/mL) of *S. australe* and *S. luehmannii* fruit and leaf extracts

Extract	MIC	LC_{50}
SAFW	94	3310
SAFM	306	1879
SAFE	> 10,000	-
SALW	> 10,000	244
SALM	881	294
SALE	-	-
SLFW	275	478
SLFM	161	414
SLFE	> 10,000	-
SLLW	341	813
SLLM	462	450
SLLE	-	-

Numbers indicate the mean MIC or LC_{50} values of triplicate determinations. - indicates that MIC or LC_{50} values were not obtained as the bacterial growth inhibition or % mortality did exceed 50% at any dose tested.

ethyl acetate extracts, which gave low to moderate yields. The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO), resulting in the concentrations presented in Table 1.

Antioxidant content

Antioxidant capacity for the plant extracts (Table 1) ranged from 1.5 mg (*S. luehmannii* fruit ethyl acetate extract) to a high of 94.6 mg ascorbic acid equivalence per gram of dried plant material extracted (*S. luehmannii* fruit methanolic extract). The antioxidant capacities for the aqueous and methanolic extracts of each plant part were generally similar for both species and were substantially higher than the corresponding ethyl acetate extracts.

Antimicrobial activity

To determine the ability of the crude fruit and leaf extracts to inhibit *C. perfringens* growth, 10 µL of each extract were screened using a disc diffusion assay. Bacterial growth was inhibited by 10 of the 12 extracts screened (83%) (Figure 1). The methanolic extracts were the most potent inhibitor of growth (as judged by zone of inhibition), with inhibition zones as much as 19.3 ± 0.6 mm (*S. australe* fruit methanolic extract). This compares favourably with the penicillin (2 µg) and ampicillin controls (10 µg), with the zones of inhibition of 12.3 ± 0.3 and 13 ± 1.0 mm respectively. Indeed, the methanolic fruit and leaf extracts of both *Syzygium* spp. displayed inhibition zones > 12 mm. The aqueous *S. australe*

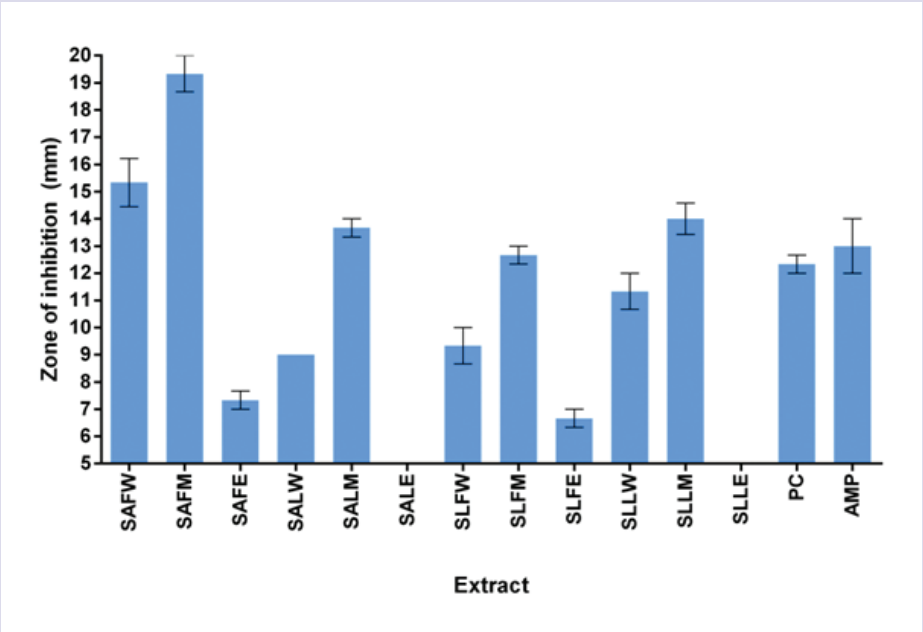


Figure 1: Growth inhibitory activity of *S. australe* and *S. Luehmannii* fruit and leaf extracts against the *C. perfringens* environmental isolate measured as zones of inhibition (mm). SAFW=aqueous *S. australe* fruit extract; SAFM=methanolic *S. australe* fruit extract; SAFE=ethyl acetate *S. australe* fruit extract; SALW=aqueous *S. australe* leaf extract; SALM=methanolic *S. australe* leaf extract; SALE=ethyl acetate *S. australe* leaf extract; SLFW=aqueous *S. luehmannii* fruit extract; SLFM=methanolic *S. luehmannii* fruit extract; SLFE=ethyl acetate *S. luehmannii* fruit extract; SLLW=aqueous *S. luehmannii* leaf extract; SLLM=methanolic *S. luehmannii* leaf extract; SLLE=ethyl acetate *S. luehmannii* leaf extract; PC=penicillin (2 µg); AMP=ampicillin (10 µg). Results are expressed as mean zones of inhibition ± SEM.

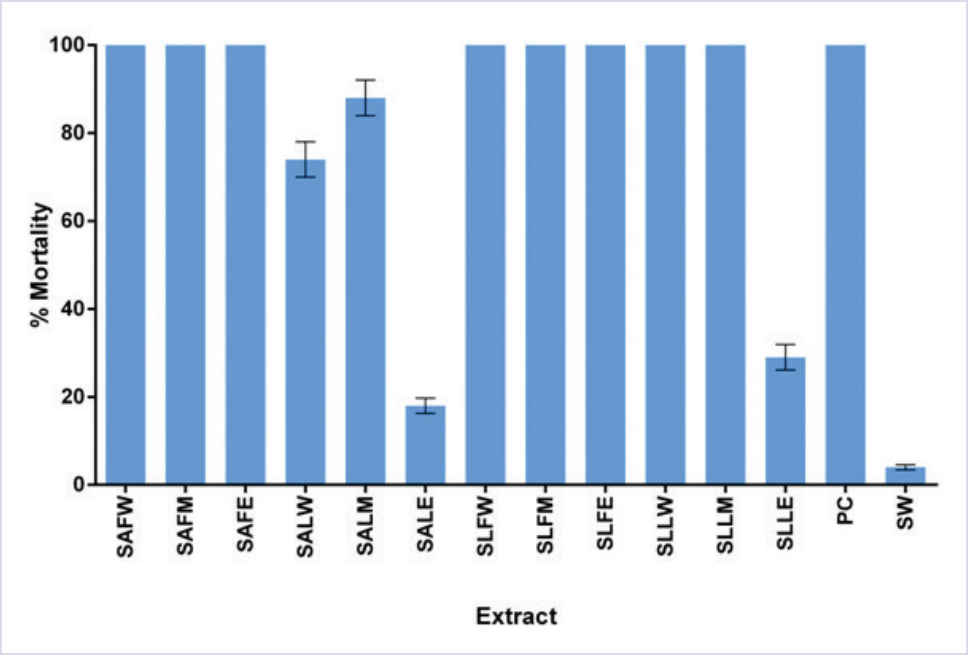


Figure 2: The lethality of the *S. australe* and *S. luehmannii* fruit and leaf extracts (2000 µg/mL) and the potassium dichromate control (1000 µg/mL) towards *Artemia franciscana* nauplii after 24 h exposure. SAFW=aqueous *S. australe* fruit extract; SAFM=methanolic *S. australe* fruit extract; SAFE=ethyl acetate *S. australe* fruit extract; SALW=aqueous *S. australe* leaf extract; SALM=methanolic *S. australe* leaf extract; SALE=ethyl acetate *S. australe* leaf extract; SLFW=aqueous *S. luehmannii* fruit extract; SLFM=methanolic *S. luehmannii* fruit extract; SLFE=ethyl acetate *S. luehmannii* fruit extract; SLLW=aqueous *S. luehmannii* leaf extract; SLLM=methanolic *S. luehmannii* leaf extract; SLLE=ethyl acetate *S. luehmannii* leaf extract; PC=potassium dichromate control; SW=seawater control. Results are expressed as mean % mortality ± SEM.

fruit and *S. luehmannii* leaf extracts also displayed good inhibition of *C. perfringens* growth with >10 mm zones of inhibition.

The antimicrobial efficacy of the extracts was further quantified through the determination of MIC values (Table 2). The aqueous and methanolic fruit extracts of both *Syzygium* spp., were particularly effective at inhibiting *C. perfringens* growth with MIC values generally <300 µg/mL (<3 µg impregnated in the disc). Similarly, the aqueous and methanolic *S. luehmannii* leaf extracts, as well as the methanolic *S. australe* leaf extract, were good growth inhibitors with MIC values <900µg/mL (<9 µg infused into the disc). These results compare well with the growth inhibitory activity of the penicillin and ampicillin controls which were tested at 2 µg and 10 µg respectively. All other extracts were either of only low efficacy in the assay, or did not inhibit *C. perfringens* growth at all.

Quantification of toxicity

All extracts were initially screened in the assay at 2000 µg/mL (Figure 2). As a reference toxin, potassium dichromate was also tested in the bioassay. The potassium dichromate reference toxin was rapid in its onset of mortality, inducing nauplii death within the first 3 h of exposure and 100% mortality was evident within 4-5 h (unpublished results). With the exception of the ethyl acetate leaf extracts of both species, all extracts showed > 50% mortality rates at 24 h.

To further quantify the effects of toxin concentration on *Artemia* nauplii mortality, the extracts were serially diluted in artificial seawater to test across a series of concentrations in the *Artemia* nauplii bioassay. The 24 h LC_{50} values of the *Syzygium* spp. extracts towards *A. franciscana* are presented in Table 2. No LC_{50} values are reported for the fruit or leaf ethyl acetate extracts of either species as <50% mortality was seen in all tested concentrations. Extracts with an LC_{50}>1000 µg/mL towards *Artemia* nauplii have been defined as being nontoxic.[37] The *S. australe* fruit methanolic and aqueous extracts also both had LC_{50} values substantially >1000 µg/mL and were therefore deemed to be non-toxic. Interestingly, the aqueous and methanolic extracts of *S. australe* leaf and of *S. luehmannii* fruit and leaf, displayed LC_{50} values substantially <1000 µg/mL and were therefore considered toxic. This was a surprising result as *S. australe* and *S. luehmannii* fruit are considered nutritious, high antioxidant foods. However, it is noteworthy that the bioassay organism (*Artemia franciscana*) is sensitive to extreme pH ranges. Our study reported that all of the extracts with LC_{50} values < 1000 µg/mL had relatively high antioxidant capacities. Furthermore, previous studies[14] reported that the high antioxidant capacities of these *Syzygium* spp. is largely due to their high ascorbic acid contents. Therefore, it is possible that the apparent toxicity reported in this study may instead be a result of the extracts high ascorbic acid contents.

DISCUSSION

Previous studies have reported potent growth inhibitory activities for *S. australe* and *S. leuhmannii* fruit and leaf extracts against a panel of pathogenic bacterial species.[25,38,39] Both Gram-positive and Gram-negative bacteria were screened in previous studies. Whilst the *Syzygium* spp. extracts had broad spectrum growth inhibitory activity, a greater susceptibility of Gram-positive bacteria was noted. Indeed, the previous studies reported that the aqueous and methanolic *S. australe* and *S. leuhmannii* extracts inhibited the growth of 40-80% of the gram positive bacterial species tested, with the methanolic extracts generally having a broader specificity. In light of this broad spectrum gram positive growth inhibitory activity, the potent *C. perfringens* growth inhibition evident in our study is perhaps not surprising.

Whilst an investigation of the phytochemistry of the *Syzygium* spp. extracts was beyond the scope of our study, high flavonoid, terpenoid and tannin contents are characteristics of this genus.[40,41] Flavonoids have well established bacterial growth inhibitory activities.[42] Relatively high levels of kaempferol and myricetin have been reported in leaf extracts from the taxonomically related species *Syzygium aromaticum* (clove).[9] The same study also reported potent growth inhibitory activity for pure kaempferol, myricetin and a methanolic *S. aromaticum* leaf extract against a panel of bacteria. Similarly, quercetin, rutin and their corresponding glycosides inhibit the growth of *Pseudomonas maltophilia* and *Enterobacter cloacae*.[43] The antimicrobial activity of terpenoids hasbeen extensively documented. Monoterpenoids including α-pinene, β-pinene, sabinene, mycrene, terpinene, limonene, piperitone and β-phellandrene inhibit the growth of a panel of bacteria including several nitrofurantoin resistant strains of Enterobacteriaceae.[42] Similarly, the antibacterial activities for several sesquiterpenoids including α-cubebene, copaene and caryophyllene have been reported.[42] Furthermore, many tannin compounds have bacterial growth inhibitory activity. Gallotannins inhibit the growth of a broad spectrum of bacterial species[44] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins,[45,46] and by inhibiting glucosyltransferase enzymes.[47] Elligitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/ml.[44,46] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[44,46] Thus, it is likely that multiple compounds within the *Syzygium* spp. extracts are contributing to the growth inhibition of *C. perfringens*.

The findings reported here also indicate that the majority of the *Syzygium* spp. extracts examined in this study displayed significant toxicity (<1000 µg/mL) in the *Artemia* nauplii bioassay. Indeed, the most promising aqueous and methanolic *S. luehmannii* fruit extracts (MIC values of 275 and 161 µg/mL respectively) had LC_{50} values of approximately 450 µg/mL, indicating moderate to high toxicity. This toxicity would impact on the usefulness of these extracts as a medicinal antiseptic agent. Similar extracts prepared from fruits of these species have previously been reported to have high ascorbic acid levels.[13,14] Whilst these ascorbic acid levels may have beneficial therapeutic effects, they may also be responsible (at least in part) for the toxicity reported here. Whilst *A. franciscana* have generally been reported to be a robust and hardy organism for toxicity screening, they are susceptible to pH changes.[37] The levels of ascorbic acid previously reported in *S. australe* and *S. leuhmannii* extracts[13,14] would be expected to have a significant impact on the pH of the seawater in the tests and this change may be responsible for the mortality induction reported in our study.Indeed, studies in our laboratory have shown that testing pure ascorbic acid in the concentrations previously reported to be in these extracts results in mortality similar to that reported in our study (unpublished results).

Whilst toxicity was assessed in this study with the test organism *A. franciscana*, toxicity towards *A. franciscana* has previously been shown to correlate well with toxicity towards human cells for many toxins.[37] However, further studies are required to determine whether this is also true for the *Syzygium* spp. extracts examined in these studies. Toxic antibacterial extracts may still be useful as non-medicinal antibacterial agents (eg. surface disinfectants and topical antiseptics). Likewise, toxic plant extracts may also still have medicinal potential even if they are not antimicrobial. Previous studies have demonstrated that toxicity in the *A. franciscana* bioassay may indicate anti-cancer potential.[37] Indeed, the anti-proliferative activity of *S.australe* and *S. luehmannii* fruit and leaf extracts has been reported against CaCo2 and HeLa human cancer cell lines,[12] indicating that these extracts may also have potential in the development of new anticancer drugs.

The results of this study indicate that the *Syzygium* spp. extracts examined warrant further study due to their *C. perfringens* growth inhibitory activity. Conversely, the toxicity detected for these extracts indicates

that further toxicity studies are required to evaluate the safety of these extracts for medicinal usage. Whilst the extracts examined in this report have potential as *C. perfringens* growth inhibitory agents, caution is needed before these compounds can be applied to medicinal purposes. Purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents.

CONCLUSION

The results of this study demonstrate the potential of *S. australe* and *S. leuhmannii* fruit and leaf extracts to inhibit *C. perfringens* growth. As the fruit and leaves of these *Syzygium* species are edible and are used as a culinary agent, they have potential in the prevention and treatment of clostridial myonecrosis and enteritis necroticans. However, before being acceptable for therapeutic uses, further cell line toxicity studies are required to verify the safety of these extracts. Furthermore, studies aimed at the purification and identification of the bioactive components arerequired to examine the mechanisms of action of these extracts.

ACKNOWLEDGEMENTS

Financial support for this work was provided by the Environmental Futures Research Institute and the School of Natural Sciences, Griffith University, Australia.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ABBREVIATION USED

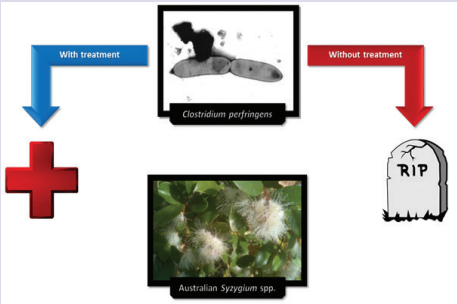
DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50% mortality; **MIC:** Minimum inhibitory concentration.

REFERENCES

1. Aldape MJ, Bryant AE, Stevens DL. *Clostridium sordellii* infection: Epidemiology, clinical findings, and current perspectives on diagnosis and treatment. Clinical Infectious Diseases. 2006;43(11):1436-46.
2. Fröschle B, Messelhäusser U, Höller C. Fate of *Clostridium botulinum* and incidence of pathogenic clostridia in biogas processes. Journal of Applied Microbiology. 2009;119(4):936-47.
3. Lyristis M, Bryant AE, Sloan J. Identification and molecular analysis of a locus that regulates extracellular toxin production in *Clostridium perfringens*. Molecular Microbiology. 1994;12(5):761-77.
4. Hauschild AHW, Thatcher FS. Experimental gas gangrene with food-poisoning *Clostridium perfringens* type A. Canadian Journal of Microbiology. 1968;14(6):705-9.
5. Scallan E, Hoekstra RM, Angula FJ. Foodborne illness acquired in theUnited States-major pathogens. Emerging Infectious Diseases. 2011;17(1):7-15.
6. Brynestad S, Granum PE. *Clostridium perfringens* and foodborne infections. International Journal of Food Microbiology. 2002;74(3):195-202.
7. Cock IE. Medicinal and aromatic plants–Australia. In Ethnopharmacology section, Biological, Physiological and Health Sciences. Encyclopedia of Life Support Systems (EOLSS) 2011; Developed under the auspices of the UNESCO: EOLSS Publishers, Oxford, UK (http://www.eolss.net).
8. Page S, Olds M. Botanica: The illustrated A-Z of over 10,000 garden plants for Australian gardens and how to cultivate them. Random House 2004; Australia.
9. Cai L, Wu CD. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. Journal of Natural Products. 1996;59(10):987-90.
10. Arora DS, Kaur GJ. Antibacterial activity of some Indian medicinal plants. Journal of Natural Medicine. 2007;61:313-7.
11. Park MJ, Gwak KS, Yang I. Antifungal activities of the essential oils of *Syzygium aromaticum* (L.) Mer. Et Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. Journal of Microbiology. 2007;45(5):460-5.
12. Jamieson N, Sirdaarta J, Cock IE. The anti-proliferative properties of Australian plants with high antioxidant capacities against cancer cell lines. Pharmacognosy Communications. 2014;4(4):71-82. DOI: 10.5530/pc.2014.4.8
13. Konczak I, Zabaras D, Dunstan M. Antioxidant capacity and hydrophilic phytochemicals in commercially grown Australian fruits. Food Chemistry. 2010;123(4):1048-54.
14. Netzel M, Netzel G, Tian Q, Schwartz S, Konczak I. Native Australian fruits–a novel source of antioxidants for food. Innovative Food Science and Emerging Technologies. 2007;8(3):339-46.
15. Cock IE, Winnett V, Sirdaarta J. The potential of selected Australian medicinal plants with anti-Proteus activity for the treatment and prevention of rheumatoid arthritis. Pharmacognosy Magazine. 2015;11(42 Suppl):S190-S208. DOI: 10.4103/0973-1296.157734
16. Mohanty S, Cock IE. The chemotherapeutic potential of *Terminalia ferdinandiana*: Phytochemistry and bioactivity. Pharmacognosy Reviews. 2012;6(11):29-36. DOI: 10.4103/0973-7847.95855
17. Hertog MG, Bueno-de-Mesquita HB, Fehily AM, Sweetnam PM. Fruit and vegetable consumption and cancer mortality in the caerphilly study. Cancer Epidemiology Biomarkers and Prevention. 1996;5(9):673-7.
18. Vita JA. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. American Journal of Clinical Nutrition. 2005;81(1):292S-7S.
19. Youdim KA, Spencer JPE, Schroeter H. Dietary flavonoids as potential neuroprotectans. Biological Chemistry. 2002;383:503-19.
20. Tsuda T, Horio F, Uchida K. Dietary cyanidin 3-O-b-D-glucoside-rich purple corn colour prevents obesity and ameliorates hyperglycemia in mice. Journal of Nutrition. 2003;133:2125-30.
21. Sirdaarta J, Matthews B, White A, *et al*. GC-MS and LC-MS analysis of Kakadu plum fruit extracts displaying inhibitory activity against microbial triggers of multiple sclerosis. Pharmacognosy Communications. 2015;5(2):100-15. DOI: 10.5530/pc.2015.2.2
22. Sirdaarta J, Matthews B, Cock IE. Kakadu plum fruit extracts inhibit growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. Journal of Functional Foods. 2015;17:610-20. DOI: 10.1016/j.jff.2015.06.019
23. Winnett V, Boyer H, Sirdaarta J, *et al*. The potential of *Tasmannia lanceolata* as a natural preservative and medicinal agent: antimicrobial activity and toxicity. Pharmacognosy Communications. 2014;4(1):42-52. DOI: 10.5530/pc.2014.1.7
24. Cock IE, Mohanty S. Evaluation of the antibacterial activity and toxicity of *Terminalia ferdinandiana* fruit extracts. Pharmacognosy Journal. 2011;3(2):72-9. DOI: 10.5530/pj.2011.20.14
25. Sautron C, Cock IE. Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium luehmannii* fruit extracts. Pharmacognosy Communications. 2014;4(1):53-60. DOI: 10.5530/pc.2014.1.8
26. Chikowe G, Mpala L, Cock IE. Antibacterial activity of selected Australian Syzygium species. Pharmacognosy Communications. 2013;3(4):77-83.
27. Arkhipov A, Sirdaarta J, Rayan P, *et al*. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacognosy Communications. 2014;4(3):62-76. DOI: 10.5530/pc.2014.3.7
28. Kalt FR, Cock IE. Gas chromatography-mass spectroscopy analysis of bioactive Petalostigma extracts: Toxicity, antibacterial and antiviral activities. Pharmacognosy Magazine. 2014;10(37 Suppl):S37-S49. DOI: 10.4103/0973-1296.127338
29. Rayan P, Matthews B, McDonnell PA, *et al*. *Terminalia ferdinandiana* extracts as inhibitors of *Giardia duodenalis* proliferation: a new treatment for giardiasis. Parasitology Research. 2015;114(7):2611-20. DOI 10.1007/s00436-015-4465-4
30. Courtney R, Sirdaarta J, Matthews B, *et al*. Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. Pharmacognosy Journal. 2015;7(1):18-31. DOI: 10.5530/pj.2015.7.2
31. Maen A, Cock IE. Inhibitory activity of Australian culinary herb extracts against the bacterial triggers of selected autoimmune diseases. Pharmacognosy Communications. 2015;5(2):130-9. DOI: 10.5530/pc.2015.2.4
32. Wright MH, Matthews B, Greene AC, *et al*. Growth inhibition of the zoonotic bacteria *Bacillus anthracis* by high antioxidant Australian plants: New leads for the prevention and treatment of anthrax. Pharmacognosy Communications. 2015;5(3):173-89. DOI: 10.5530/pc.2015.3.3
33. Vesoul J, Cock IE. The potential of Bunya nut extracts as antibacterial functional food agents. Pharmacognosy Communications. 2012;2(1):72-9. DOI: 10.5530/pc.2012.1.13
34. Cock IE, van Vuuren SF. Anti-Proteus activity of some South African medicinal plants: their potential for the prevention of rheumatoid arthritis. Inflammopharmacology. 2014;22(1):23-36. DOI 10.1007/s10787-013-0179-3
35. Cock IE. Antimicrobial activity of *Acacia aulacocarpa* and *Acacia complanta* menthanolic extracts. Pharmacognosy Communications. 2012;2(1):66-71. DOI: 10.5530/pc.2012.1.12
36. Ruebhart D, Wickramasinghe W, Cock IE. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa*, microcystin-LR and menadione toxicity in *Artemia franciscana* nauplii. Journal of Toxicology and Environmental Health Part A. 2009;72(24):1567-75. DOI: 10.1080/15287390903232459
37. Cock IE, Ruebhart DR. Comparison of the brine shrimp nauplii bioassay and the ToxScreen-II test for the detection of toxicity associated with Aloe vera (*Aloe barbadensis* Miller) leaf extract. Pharmacognosy Research. 2009;1(2):98-101.
38. Cock IE. Antimicrobial activity of *Syzygium australe* and *Syzygium luehmannii* leaf methanolic extracts. Pharmacognosy Communications. 2012;2(2):71-7. DOI: 10.5530/pc.2012.2.11

39. Cock IE. Antibacterial activity of selected Australian native plant extracts. Internet Journal of Microbiology. 2008;4(2):1-8.
40. Tian LW, Xu, M, Wang D. Phenolic constituents from the leaves of *Syzygium forrestii* Merr. And Perry. Biochemical Systematics and Ecology. 2011;39(2): 156-8.
41. Pino JA, Marbot R, Rosado A. Volatile constituents of Malay rose apple [*Syzygium malaccense* (L.) Merr. and Perry]. Flavour and Fragrance Journal. 2004;19(1):32-5.
42. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmannia lanceolata* (Tasmanian pepper): A review. Pharmacognosy Communications. 2013:3(4):13-25. DOI: 10.5530/pc.2013.4.3
43. Waage SK, Hedin PA. Quercetin 3-O-galactosyl-(1, 4, 6)-glucoside, a compound from narrowleaf vetch with antibacterial activity. Phytochemistry. 1985; 24(2):243-5.
44. Buzzini P, Arapitsas P, Goretti M. Antimicrobial activity of hydrolysable tannins. Mini-Reviews in Medicinal Chemistry. 2008;8(12):1179-87.
45. Wolinsky LE, Sote EO. Isolation of natural plaque-inhibiting substances from 'Nigerian chewing sticks'. Caries Research. 1984;18(3):216-25.
46. Hogg SD, Embery G. Blood-group-reactive glycoprotein from human saliva interacts with lipoteichoic acid on the surface of *Streptococcus sanguis* cells. Archives in Oral Biology. 1982;27(3):261-8.
47. Wu-Yuan CD, Chen CY, Wu RT. Gallotannins inhibit growth, water-soluble glucan synthesis, and aggregation of *Streptococci mutans*. Journal of Dental Research. 1988;67(1):51-5.

PICTORIAL ABSTRACT



SUMMARY

- *S. australe* and *S. luehmannii* leaf and fruit extracts inhibit *C. perfringens* growth.
- Aqueous and methanolic extracts were particularly potent with MIC's generally <500 µg/mL.
- *S. australe* fruit extracts and the ethyl acetate extracts from both *Syzygium* spp. were nontoxic. (LC_{50}'s>1000 µg/mL).
- All *S. luehmannii* aqueous and methanolic extracts as well as the *S. australe* leaf extracts, displayed substantial toxicity.
- *Syzygium* spp. extracts have therapeutic potential in the prevention and treatment of clostridial myonecrosis and food poisoning.

ABOUT AUTHORS

Dr. Mitchell Henry Wright: Received his PhD in 2014, for his work investigating the manganese reduction and oxidation characteristics of environmental bacteria. He is currently a postdoctoral researcher at Griffith University, Australia, where he is working on several projects both in the areas of Geomicrobiology and Pharmacognosy. His present research interests are the use of bacteriogenic manganese oxides in the bioremediation of metal-contaminated sites as well as the use of Australian native plants in the treatment and prevention of various pathogenic bacteria. Dr Wright's interests include wearing silly hats and supporting bad football teams.

Dr. Anthony Greene: Is a senior lecturer and researcher at Griffith University, Brisbane Australia. He obtained his PhD in Microbiology from the University of New South Wales and focuses on extreme environments, bioremediation and Geomicrobiology. His specific interests include the microbial ecology of thermophilic, saline and alkaliphilic environments and the mechanisms and industrial potential of extremophilic bacteria contained therein.

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 scientific publications in a variety of peer reviewed journals. Unlike Dr Wright who enjoys wearing silly hats, Dr Cock is wearing the hat in this photo due to a lost wager.