Pharmacogn. Commn. 2020;10(1):37-43
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Brachychiton acerifolius (A.Cunn. ex G.Don) Macarthur and *C. moore* Leaf and Flower Extracts Inhibit the Growth of a Panel of Pathogenic Bacteria

Ian Edwin Cock[1,2,*]
[1]School of Environment and Science, Griffith University, Nathan, Brisbane, Queensland, AUSTRALIA.
[2]Environmental Futures Research Institute, Griffith University, Nathan, Brisbane, Queensland, AUSTRALIA.

ABSTRACT

Introduction: *Brachychiton acerifolius* (A.Cunn. ex G.Don) Macarthur and *C. moore* is a large tree that is native to subtropical regions of the east of Australia. Decoctions and infusions produced from the leaves of the related species *Brachychiton diversifolius* were used by the first Australians to treat a variety of bacterial diseases. Despite this, *B. acerifolius* leaf and flower extracts have not been rigorously examined for antibacterial properties against many pathogens. **Methods:** The antimicrobial activity of *B. acerifolius* leaf and flower 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:** Methanolic *B. acerifolius* leaf and flower extracts inhibited the growth of a wide range of bacterial species. Growth of both gram positive and gram-negative bacteria was inhibited by the *B. acerifolius* leaf and flower extracts, although the gram negative bacteria were substantially more susceptible to the extracts than the gram positive bacteria were. The leaf extracts were more potent growth inhibitors than the flower extracts against all bacteria tested. The *B. acerifolius* leaf extract was a strong inhibitor of *A. faecalis*, *K. pneumonia*, *P. fluorescens*, *S. newport*, *S. sonnei*, *B. cereus*, *S. aureus* and *S. pyogenes* growth, with MIC values substantially <1000µg/mL. The antibacterial activity of the methanolic *B. acerifolius* leaf extract was further investigated by growth time course assays which showed significant growth inhibition in cultures of *P. fluorsecens* and *B. cereus* within 1hr of exposure. Both extracts were determined to be nontoxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for internal use as well as for topical uses. **Conclusion:** The lack of toxicity of the *B. acerifolius* leaf and flower extracts and their growth inhibitory bioactivity against a panel of pathogenic bacteria partially validate the traditional usage of this species to treat bacterial diseases and indicates their potential in the development of antiseptic agents.

Key words: *Malvaceae*, Illawarra flame tree, Australian plants, Antibacterial activity, Toxicity, Medicinal plants.

Correspondence:
Dr. Ian Edwin Cock
[1]School of Environment and Science, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland-4111, AUSTRALIA.
[2]Environmental Futures Research Institute, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland-4111, AUSTRALIA.
Phone no: +61 7 37357637
E-mail: i.cock@griffith.edu.au
DOI: 10.5530/pc.2020.1.7

INTRODUCTION

In recent years, many bacterial strains have acquired resistance genes, rendering them either extremely (XDR) or totally drug resistant (TDR) to many antibiotics.[1] Few therapeutic options remain to treat the diseases caused by these pathogens and this situation is likely to worsen as bacteria exchange resistance genes and more strains become multi-drug resistant (MDR). For this reason, the development of alternative antibiotic chemotherapies is considered by the World Health Organisation (WHO) to be one of the most serious challenges facing medical science.[2] It is unlikely that the previous methods of antibiotic discovery/development will be as successful in the future and new therapeutic options are urgently required.

Traditional plant derived medicines are an attractive option for the development of new antibiotic therapies as they have been used for a variety of therapeutic purposes, including fighting microbial disease and their uses have often been well documented.[3] Indeed, the ability of plant extracts to block the growth of pathogenic bacteria has become the focus of much recent study.[4,5] Much of the research into traditional medicinal plant use has focused on Asian,[6,7] African[8-12] and South American[13,14] plants. Similarly, the medicinal properties of Australia flora has also received recent attention. Australian Aborigines understood the therapeutic properties of a wide variety of Australian plants and how to use them effectively.[15] Whilst studies have reported antibacterial activity for some Australian plant species,[16-19] the antibacterial activity of many Australian native plants remains unexamined.

Brachychiton acerifolius (A.Cunn. ex G.Don) Macarthur and *C. moore* (Figure 1a; Family *Malvaceae*; commonly known Illawarra flame tree) is a large tree that grows in subtropical regions on the east coast of Australia. It has large variable leaves, with up to 7 deep lobes (Figure 1b) which it sheds in the dry winter season. *B. acerifolius* produces masses of red flowers (Figure 1c) in late spring which develop into dark brown pod-like fruits up to 10cm in length. The seeds are nutritious and were roasted and eaten by Aborigines. Whilst we were unable to find records of the medicinal usage of *B. acerifolius* to treat bacterial disease, the related species *Brachychiton diversifolius* R.Br. (commonly known as northern kurrajong) was used by the first Australians to treat wound infections, as well as diarrhoea and dysentery.[20] The antibacterial activity of *B. acerifolius* is also poorly studied. However, *B. diversifolius* has been reported to have moderate growth inhibitory activity against *Bacillus subtilis* and *Sarcina lutea*.[21] The same study reported that the extracts were completely ineffective against *Staphylocoxccus aureus* and *Pectobacterium carovorum* at 2000µg/mL. However, that study tested the bacteria against a single concentration and did not determine MIC values, making it difficult to compare the activity to that of other studies. A recent study also reported that *Brachychiton rupestris* (T.Mitch. ex Lindl.) K.Schum. leaf extracts are potent inhibitors of *Streptococcus pyogenes* growth.[22]

Several investigations have reported the chemical composition of *B. acerifolius* leaves and several interesting phytochemical components have been identified. The flavonoid content has been particularly well reported and apigenin-7-O-glucuronide (Figure 1d), rutin (Figure 1e), luteolin-7-O-D-glucuronide (Figure 1f), quercetin (Figure 1g), kaempferol

(Figure 1h), luteolin (Figure 1i) and apigenin (Figure 1j) have been identified.[23] Interestingly, many flavonoids (including some of those identified in that study) have good antibacterial activities[24] and may give the leaves bacterial growth inhibitory properties. Despite these promising studies, the antibacterial properties and phytochemistry of the leaves from *B. acerifolius* is poorly studied. We were also unable to find any studies that tested the flowers for antibacterial activity. The current report was undertaken to screen *B. acerifolius* leaf and flower extracts for growth inhibitory properties against a panel of pathogenic bacteria.

MATERIALS AND METHODS

Plant collection and extraction

Brachychiton acerifolius (A.Cunn. ex G.Don) Macarthur and *C. moore* leaves and flowers were obtained from verified trees on Logan campus of Griffith University, Brisbane, Australia. The leaf and flower samples were dried in a Sunbeam food dehydrator and stored at -30°C. Prior to use, the dried plant materials were freshly ground to a coarse powder and 1g quantities were weighed into separate tubes. A volume of 50mL methanol was added to individual tubes and extracted for 24 hr 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 and dried in an Eppendorf concentrator 5301. The resultant pellets were dissolved in 10mL sterile deionised water (containing 1% DMSO). The extracts were passed through 0.22µm filter (Sarstedt) and stored at 4°C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *B. acerifolius* leaf and flower extracts for the presence of phenolic compounds, flavonoids, saponins, triterpenoids, phytosteroids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[25,26]

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.[27,28] Briefly, 100µL of each bacterial culture was grown in 10mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. A volume of 100µL of the bacterial suspension was spread onto nutrient agar plates and extracts were tested for antibacterial activity using 5mm sterilised filter paper discs. Discs were infused with 10µL of the plant extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2hr before incubation at 37°C for 24hr. 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 (10µg) and chloramphenicol (2µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10µL of distilled water (containing 1% DMSO) 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.[6,29] Briefly, the *B. acerifolius* extracts were diluted in deionised water (containing 1% DMSO) and tested across a range of concentrations. Discs were infused with 10µL 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. Ln 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.[30] Briefly, 3mL of the log phase *P. fluorescens* and *B. cereus* bacterial cultures in nutrient broth were added individually to 27mL nutrient broth containing 3mL of 10mg/mL methanolic leaf extract to give a final concentration of 1000µg/mL in the assay. The tubes were incubated at 37°C with gentle shaking. The optical density was measured hourly at 550nm for a 6hr 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 4mg/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.[31,32] Briefly, 400µL of seawater containing approximately 63 (mean 63.3, n=75, SD 11.3) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400µL 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 400µL 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 were counted. The nauplii were considered dead if no movement of the appendages was detected within 10 sec. After 24hr, 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 1g of dried and powdered *B. acerifolius* leaves and flowers with methanol yielded 320mg and 375mg respectively (Table 1). The dried extracts were resuspended in 10mL of deionised water (containing 1% DMSO), resulting in 32 and 37.5mg/mL concentrations respectively. Qualitative phytochemical studies showed that both the leaf and flower extracts similar phytochemical profiles. Both contained high levels of polyphenolics, saponins and phytosterols, moderate levels of flavonoids, as well as low levels of triterpenpoids and tannins.

Antimicrobial activity

To determine the growth inhibitory activity of the *B. acerifolius* leaf and flower extracts against the panel of pathogenic bacteria, aliquots (10µL) of each extract were screened in the disc diffusion assay. The *B. acerifolius* leaf extract inhibited the growth of several gram negative bacterial species (Figure 2). Of the 10 g negative bacterial strains tested, 5 (50%) were inhibited by the methanolic *B. acerifolius* leaf extract. *A. faecalis, P. fluorescens* and *S. newport* were most susceptible to the *B. acerifolius* leaf extract, with ZOIs of 16.7, 18.2 and 14mm respectively. The inhibition of these bacteria by this extract was similar or better than the inhibition by the positive controls ampicillin and chloramphenicol. Strong activity was also recorded against *K. pneumonia* and *S. sonnei,* each with 10mm ZOIs. In contrast, the flower extracts were completely ineffective against all gram negative bacterial species. Similarly, the *B. acerifolius* leaf extract was much more effective than the flower extract against a panel of gram positive bacteria (Figure 3). The leaf extract was particularly potent against *S. aureus*, with a ZOI of 16.6mm measured. In contrast, a 7.2mm ZOI was measured for the flower extract against the same bacterium. The leaf extract was also a good inhibitor of *B. cereus* and *S. pyogenes,* with ZOIs of 9.6 and 8.2mm respectively. These results are comparable to the inhibition seen for the ampicillin and chloramphenicol controls. In contrast, all extracts were completely ineffective against a panel of fungi (Figure 4).

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 *B. acerifolius* leaf extracts were good growth inhibitors of several bacterial species, although the flower extracts were only effective against the gram positive bacteria (as judged by MIC; Table 2). *P. fluorescens* was the most susceptible bacteria to the *B. acerifolius* leaf extract leaf extracts, with an MIC value of 25µg/mL (approximately 0.25µg infused into the disc). The MIC values determined for the leaf extract against *A. faecalis* (76µg/mL), *S. newport* (84µg/mL) and *B. cereus* (58µg/mL) are also indicative of potent growth inhibition. The MIC values of the leaf extract against *K. pneumonia*, *S. sonnei*, *S. aureus* and *S. pyogenes* were also <1000µg/mL and therefore also indicate good growth inhibitory activity. In contrast, the flower extract only inhibited the growth of the gram-positive bacteria and the MIC values (>1000µg/mL) indicate only moderate growth inhibitory activity.

Bacterial growth time course assay

The most susceptible gram negative (*P. fluorescens*) and gram positive (*B. cereus*) bacterial species were selected for further study by growth time course assays in the presence and absence of the leaf extract. Only the effect of the leaf extract on the bacterial growth time courses was evaluated as this extract was generally the most potent extract at inhibiting bacterial growth. The starting concentration of the extract used in these assays was 1000µg/mL. The methanolic *B. acerifolius* leaf extract significantly inhibited *P. fluorescens* (Figure 5a) and *B. cereus* (Figure 5b) growth within 1h, indicating a rapid antimicrobial action. Notably, the growth of both bacterial species was inhibited for the entire growth time-course, indicating that the growth inhibition of these bacteria may be bacteriocidal at the concentrations tested against these bacteria.

Quantification of toxicity

The toxicity of the *B. acerifolius* leaf and flower extracts was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000µg/mL (Figure 6). Both extracts induced >50% mortality at 24, 48 and 72hr and were thus deemed to be non-toxic. The potassium dichromate positive control also induced mortality within 4h (results not shown), with 100% mortality induction seen by 24hr. To further quantify the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay (Table 2). For comparison, serial dilutions of potassium dichromate were also tested. All extracts were determined to be nontoxic, with LC_{50} values >1000µg/mL following 24hr exposure. Extracts with an LC_{50} >1000µg/mL towards *Artemia* nauplii have previously been defined as being nontoxic.[32]

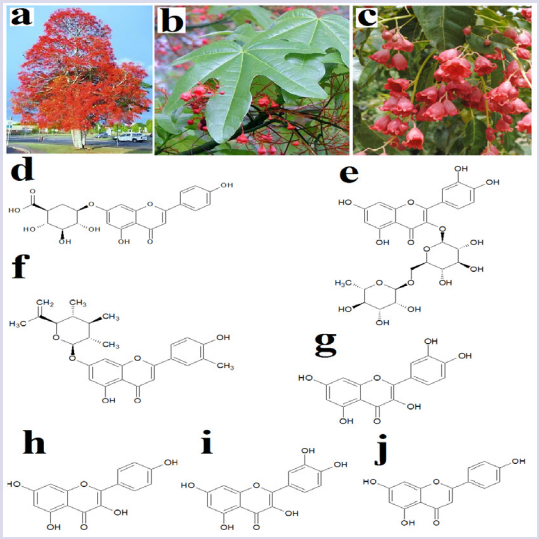


Figure 1: *B. acerifolius* (a) whole tree, (b) leaves and (c) flowers, as well as the chemical structures of (d) apigenin-7-O-glucuronide, (e) rutin, (f) luteolin-7-O-b-D-glucuronide, (g) quercetin, (h) kaempferol, (i) luteolin, (j) apigenin.[23]

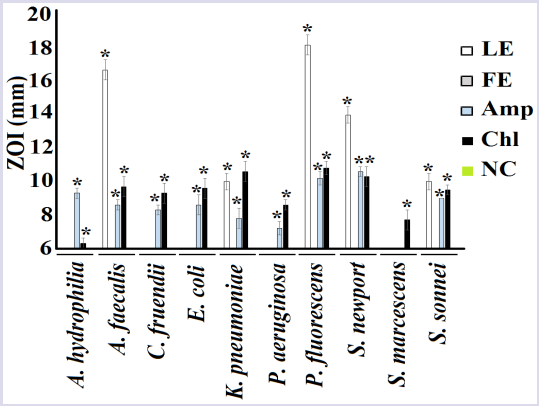


Figure 2: Antibacterial activity of *B. acerifolius* leaf and flower extracts and ampicillin (10µg) and chloramphenicol (10µg) controls measured as zones of inhibition (mm) against Gram negative bacteria. LE = leaf extract; FE = flower extract; Amp = ampicillin; Chl = chloamphenicol. Results are expressed as mean ± SEM of three determinations in triplicate (n=9). * indicates results that are significantly different to the untreated control (p<0.01).

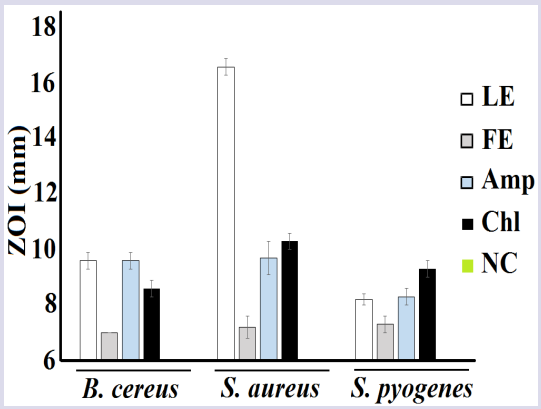


Figure 3: Antibacterial activity of *B. acerifolius* leaf and flower extracts and ampicillin (10µg) and chloramphenicol (10µg) controls measured as zones of inhibition (mm) against Gram positive bacteria. LE = leaf extract; FE = flower extract; Amp = ampicillin; Chl = chloamphenicol. Results are expressed as mean ± SEM of three determinations in triplicate (*n*=9). * indicates results that are significantly different to the untreated control ($p<0.01$).

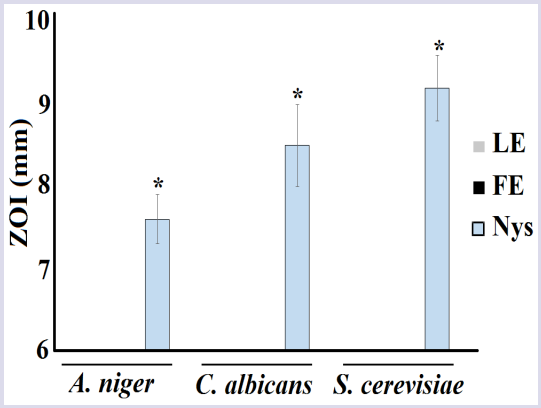


Figure 4: Antifungal activity of the *B. acerifolius* leaf and flower extracts and a nystatin control (100µg) measured as zones of inhibition (mm). LE = leaf extract; FE = flower extract; Nys = nystatin. Results are expressed as mean ± SEM of three determinations in triplicate (*n*=9). * indicates results that are significantly different to the untreated control ($p<0.01$).

DISCUSSION

Recent increases in bacterial resistance has made the development of new antibiotic therapies a high priority.[1,2] A concurrent decrease in the development of new antibiotic therapies has further increased this problem and new antibiotic therapies are urgently needed. Interest in re-evaluating medicinal plants for new antibiotic chemotherapies has increased substantially in recent years. Although we were unable to find evidence that *B. acerifolius* was also used for these purposes, the first Australians used several other *Brachychiton* spp. to treat multiple diseases and infections caused by bacterial pathogens.[15,20] Furthermore, limited scientific evaluations have rigorously evaluated the antibacterial properties of *B. acerifolius*.

Our study examined the ability of *B. acerifolius* leaf and flower extracts to inhibit the growth of a panel of medicinally important bacterial pathogens. The *B. acerifolius* leaf extract was a particularly potent inhibitor of *P. fluorescens* with an MIC value of 25µg/mL. The leaf extract was also

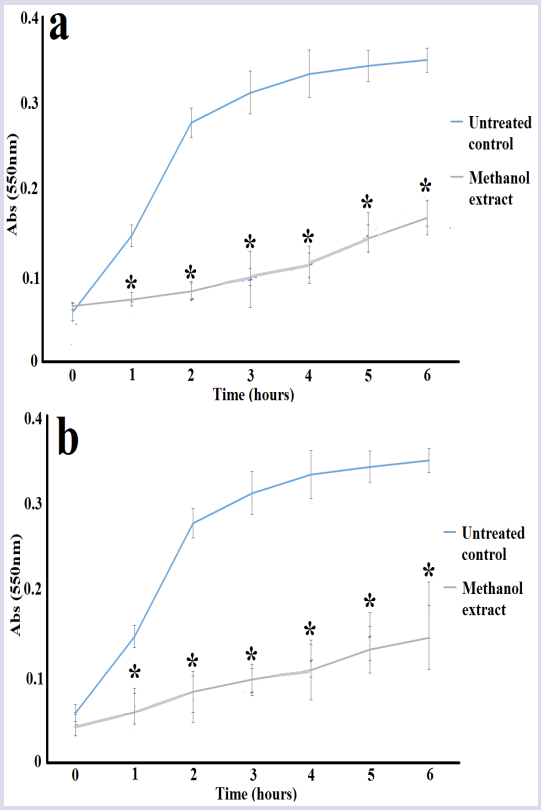


Figure 5: Bacterial growth curves for the *B. acerifolius* leaf extract against (a) *P. fluorescens* and (b) *B. cereus*. All bioassays were performed in at least triplicate and are expressed as mean ± SEM. * = growth results in the presence of the leaf extract that are significantly different to the untreated control growth ($p<0.01$).

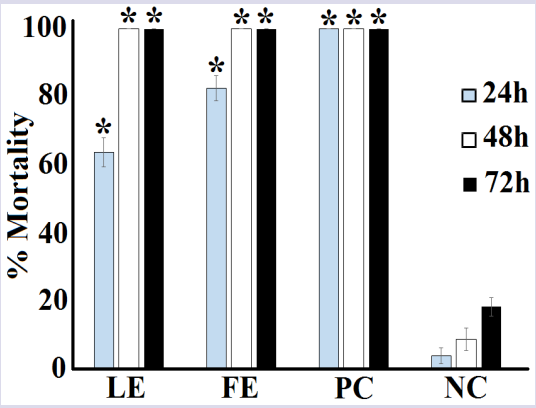


Figure 6: The lethality of the *B. acerifolius* leaf and flower extracts (2000µg/mL), potassium dichromate (1000µg/mL) and a seawater control. Blue bars represent the % mortality following 24hr exposure to the extract/toxin; white bars represent the % mortality following 48h exposure to the extract/toxin; black bars represent the % mortality following 72hr exposure to the extract/toxin; NC = negative (seawater) control; PC = positive control (1000µg/mL potassium dichromate). All bioassays were performed in at least triplicate and are expressed as mean ± SEM.* indicates results that are significantly different to the negative controls ($P<0.01$).

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

Extract	Mass of Dried Extract (mg)	Resuspended Extract (mg/mL)	Phenols			Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids			Flavanoids	Tannins			Anthraquinones	
			Total Phenolics	Water Soluble	Water Insoluble	Keller-Kiliani Test	Froth Persistence	Emulsion test	Salkowski Test	Acetic Anhydride Test	Meyers Test	Wagners Test	Draggendoffs Test	Shinoda Test	Kumar test	Ferric Chloride Test	Lead Acetate Test	Free	Combined
Leaf	320	32.0	+++	+++	+++	-	+++	++	+	+++	+	-	-	++	++	++	+	-	-
Flower	375	37.5	+++	+++	++	-	+++	++	+	+	-	-	-	++	++	+	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay.

a potent inhibitor of several bacterial pathogens associated with food poisoning and diarrhoea. Strong growth inhibitory activity was also noted for the *B. acerifolius* leaf extract against *A. facealis* (MIC 76µg/mL), *S. newport* (MIC 84µg/, L) and *B. cereus* (MIC 58µg/mL). The leaf extract was also a good inhibitor of *K. pneumonnia* (MIC 120µg/mL), *S. sonnei* (183µg/mL), *S. aureus* (672µg/mL) and *S. spyogenes* (870µg/mL). Therefore, the leaf extract has potential for the treatment of diarrhoea and gastrointestinal disease.

The ability of the *B. acerifolius* leaf extract to inhibit the growth of both gram-positive and gram-negative bacteria is in agreement with previous reports of the antibacterial activity of other Australian plant species.[33] In this study, the gram-negative and gram-positive bacteria were both susceptible to the *B. acerifolius* leaf extracts. In contrast, many previous studies have reported substantially greater susceptibility for gram-positive bacteria to South American,[34] African[11,12] and Australian[33] plant extracts. Results within our laboratory have also confirmed the greater susceptibility of gram-positive bacteria towards many other Australian plant extracts.[35,36] The gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including several antibiotics.[37] In contrast, other studies have demonstrated that gram-negative bacteria are often as susceptible (or more susceptible) to plant extracts from different Australian plant species.[38]

Whilst a detailed investigation of the phytochemistry of the *B. acerifolius* leaf extracts was beyond the scope of our study, qualitative screening studies were used to determine the classes of compounds present. Both the leaf and flower extracts contained relatively high levels of total phenolics and saponins. It is likely that these and other phytochemical classes, may contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies also indicated that flavonoid, triterpenoids and phytosterols were present in the *B. acerifolius* leaf extract. Many studies have reported potent antibacterial activities for a wide variety of these compounds.[24] Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism of bacterial growth inhibition.

It is likely that other phytocompounds may also contribute to the antibacterial properties of the *B. acerifolius* extracts. Several monoterpenoids including α-pinene, β-pinene, sabinene, mycrene, terpinene,

Table 2: Minimum bacterial growth inhibitory concentration (µg/mL) of the *B. acerifolius* leaf and flower extracts against susceptible bacterial species and the LC_{50} in the Artemia nauplii assay.

Bacterial species	*B. acerifilius* Extract	
	LE	FE
Gram negative		
A. hydrophilia	-	-
A. faecalis	76	-
C. freundi	-	-
E. coli	-	-
K.pneumoniae	120	-
P. aeruginosa	-	-
P. fluroscens	25	-
S. newport	84	-
S. marcenscens	-	-
S. sonnei	183	-
Gram positive		
B. cereus	58	1220
S. aureus	672	1853
S. pyogenes	870	1025
Fungi		
A. niger	-	-
C. albicans	-	-
S. pyogenes	-	-
***Artemia* nauplii toxicity (LC50)**		
24 hr exposure	1680	1247

Numbers indicate the mean MIC values of triplicate determinations. - indicates no inhibition.

limonene, piperitone and β-phellandrene inhibit the growth multiple bacteria.[24] Antibacterial activities have also been reported for several sesquiterpenoids including α-cubebene, copaene and caryophyllene.[24] The phytochemical studies described in this report did not test for these classes of compound. However, it is likely that they are present and that they may contribute to the antibacterial activity reported here. Tannins were also detected in our study. Interestingly, tannins have bacterial growth inhibitory activity against a broad panel of bacteria via a variety of mechanisms.[39] Thus, it is likely that multiple compounds within the *B. acerifolius* extracts are contributing to the antibacterial activity reported here.

The findings of this study also demonstrate that the *B. acerifolius* extracts extracts were nontoxic towards *Artemia franciscana* nauplii, with LC_{50} values substantially >1000µg/mL. Extracts with LC_{50} values >1000µg/mL towards *Artemia* nauplii are defined as being nontoxic.[32] 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, whilst these studies have demonstrated the potential of the *B. acerifolius* extracts in the development of future antibiotic chemotherapeutics, more work is required to isolate the inhibitory components and determine the mechanism of inhibition.

CONCLUSION

The results of this study demonstrate the potential of the *B. acerifolius* extracts as inhibitors of pathogenic bacteria growth. Furthermore, their lack of toxicity indicates that they are safe for internal treatment. Further studies aimed at the purification and identification of bioactive components are required to examine the mechanisms of action of these agents.

ACKNOWLEDGEMENT

The authors are grateful to Michelle Mendell and Jane Gifkins for the gift of the clinical isolate bacterial strains. Financial support for this work was provided by the Environmental Futures Research Institute and the School of Natural Sciences, Griffith University, Australia.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

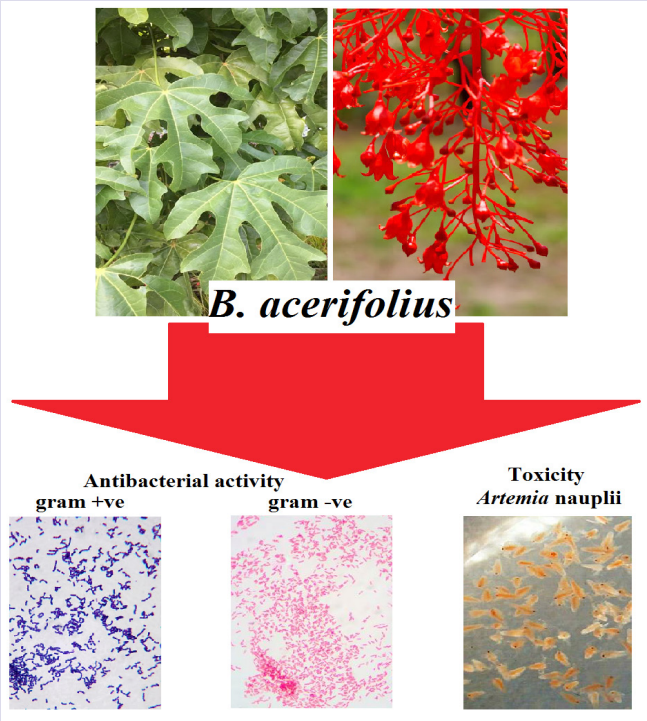
DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50 % mortality; **MIC:** Minimum inhibitory concentration.

REFERENCES

1. Cheesman MJ, Ilanko A, Blonk B, *et al.* Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?. Pharmacogn Rev. 2017;11(22):57-72. DOI: 10.4103/phrev.phrev_21_17
2. WHO. Antimicrobial Resistance. World Health Organization. 2016. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/. [Cited on 2017 May 10].
3. Cowan MM. Plant products as antibacterial agents. Clin Microbiol Rev. 1999;12(4):564-82.
4. Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria. Curr Opin Microbiol. 2000;3(5):528-34.
5. Chiariandy CM, Seaforth CE, Phelps RH, *et al.* Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. J Ethnopharmacol. 1999;64(3):265-70.
6. Hart C, Ilanko P, Sirdaarta J, *et al. Tasmannia stipitata* as a functional food/natural preservative: Antimicrobial activity and toxicity. Pharmacogn Commun. 2014;4(4):33-47. DOI: 10.5530/pc.2014.4.4
7. Wright MH, Arnold MSJ, Lee CJ, *et al.* Qualitative phytochemical analysis and antibacterial activity evaluation of Indian *Terminalia* spp. against the pharyngitis causing pathogen *Streptococcus pyogenes*. Pharmacogn Commun. 2016;6(2):85-92. DOI: 10.5530/pc.2016.2.6
8. Hübsch Z, Zyl RLV, Cock IE, *et al.* Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. S Afr J Bot. 2014;93:185-97. DOI: 10.1016/j.sajb.2014.04.005
9. Arkhipov A, Sirdaarta J, Rayan P, *et al.* An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacogn Commun. 2014;4(3):62-76. DOI: 10.5530/pc.2014.3.7
10. Rabadeaux C, Vallette L, Sirdaarta J, *et al.* An examination of the antimicrobial and anticancer properties of *Khaya senegalensis* (Desr.) A. Juss. bark extracts. Pharmacogn J. 2017;9(2):175-84. DOI: 10.5530/pj.2017.4.82
11. Cock IE, van Vuuren SF. Anti-*Proteus* activity of some South African medicinal plants: Their potential for the treatment and prevention of rheumatoid arthritis. Inflammopharmacol. 2014;22(1):23-36. DOI 10.1007/s10787-013-0179-3
12. Cock IE, Vuuren VSF. The potential of selected some South African plants with anti-*Klebsiella* activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacol. 2015;23(1):21-35. DOI: 10.1007/s-10787-013-0179-3
13. Mohanty S, Cock IE. Evaluation of the antibacterial activity and toxicity of *Myrciaria cauliflora* methanolic leaf and fruit extracts. Int J Microbiol. 2009;7(2):1-8.
14. Paz EA, Cerdeiras MP, Fernandez J, *et al.* Screening of Uruguayan medicinal plants for antimicrobial activity. J Ethnopharmacol. 1995;45(1):67-70.
15. Cock IE. Medicinal and aromatic plants–Australia. In Ethnopharmacology, Encyclopedia of Life Support Systems (EOLSS). 2011. Developed under the auspices of UNESCO. Oxford, UK: EOLSS Publishers. 2011. Available from: http://www.eolss.net.
16. Sirdaarta J, Matthews B, Cock IE. Inhibitory activity of *Kakadu* plum fruit extracts against microbial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. J Funct Foods. 2015;17:610-20. DOI: 10.1016/j.jff.2015.06.019
17. Winnett V, Sirdaarta J, White A, *et al.* Inhibition of *Klebsiella pneumonia* growth by selected Australian plants: Natural approaches for the prevention and management of ankylosing spondylitis. Inflammopharmacol. 2017;25(2):223-35. DOI: 10.1007/s10787-017-0328-1
18. Wright MH, Matthews B, Arnold MSJ, *et al.* The prevention of fish spoilage by high antioxidant Australian culinary plants: *Shewanella putrefaciens* growth inhibition. Int J Food Sci and Technol. 2016;51(3):801-13. DOI: 10.1111/ijfs.13026
19. Ilanko A, Cock IE. The interactive antimicrobial activity of conventional antibiotics and *Petalostigma* spp. Extracts against bacterial triggers of some autoimmune inflammatory diseases. Pharmacogn J. 2019;11(2):292-309. DOI: 10.5530/pj.2019.11.45
20. Lassak EV, McCarthy TM. Australian medicinal plants. A complete guide tom identification and usage. Reed New Holland Publishers, Sydney Australia. 2011.
21. Salem MZ, Ali HM, Mansour MM. Fatty acid methyl esters from air-dried wood, bark and leaves of *Brachychiton diversifolius* R. Br: Antibacterial, antifungal and antioxidant activities. Bioresources. 2014;9(3):3835-45.
22. Mpala L, Chikowe G, Cock IE. *Brachychiton rupestris* (T.Mitch. ex Lindl.) K. Schum. Extracts inhibit the growth of *Streptococcus pyogenes*. Pharmacogn Commun. 2019;9(1):27-33. DOI: 10.5530/pc.2019.1.6
23. Zeid AH, Farag MA, Hamed MA, *et al.* Flavonoid chemical composition and antidiabetic potential of *Brachychiton acerifolius* leaves extract. Asian Pac J Trop Biomed. 2017;7(5):389-96.
24. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmania lanceolata* (*Tasmanian pepper*): A review. Pharmacogn Commun. 2013;3(4):1-13. DOI: 10.5530/pc.2013.4.3
25. Kalt FR, Cock IE. Gas chromatography-mass spectroscopy analysis of bioactive Petalostigma extracts: Toxicity, antibacterial and antiviral activities. Pharmacogn Mag. 2014;10 (Suppl 1):S37-49. DOI: 10.4103/0973-1296.127338
26. Vesoul J, Cock IE. The potential of Bunya nut as an antibacterial food agent. Pharmacogn Commun. 2012;2(1):72-9. DOI: 10.5530/pc.2012.1.13
27. Wright MH, Shalom J, Matthews B, *et al. Terminalia ferdinandiana* Exell: Extracts inhibit *Shewanella* spp. growth and prevent fish spoilage. Food Microbiol. 2019;78:114-22. DOI: 10.1016/j.fm.2018.10.006
28. Cock IE. Antimicrobial activity of *Callistemon citrinus* and *Callistemon saligna* methanolic extracts. Pharmacogn Commun. 2012;2(3):50-7. DOI: 10.5530/pc.2012.3.11
29. Cock IE, Winnett V, Sirdaarta J, *et al.* The potential of selected Australian medicinal plants with anti-*Proteus* activity for the treatment and prevention of rheumatoid arthritis. Pharmacogn Mag. 2015;11(Suppl 1):S190-208. DOI: 10.4103/0973-1296.157734
30. Bryant K, Cock IE. *Eupomatia laurina* R.Br. fruit solvent extractions inhibit the growth of a panel of pathogenic bacteria. Pharmacogn Commun. 2017;7(1):16-23. DOI: 10.5530/pc.2017.1.3
31. Ruebhart DR, Wikramasinghe WA, Cock IE. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. J Toxicol Environ Health Part A. 2009;72(24):1567-75.
32. 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. Pharmacogn Res. 2009;1(2):98-101.
33. Cock IE. Antibacterial activity of selected Australian plant species. The Int J Microbiol. 2008;6:2.
34. Mohanty S, Cock IE. Evaluation of the antibacterial activity and toxicity of *Myrciaria cauliflora* methanolic leaf and fruit extracts. Int J Microbiol. 2009;7(2):1-8.
35. Cock IE. Antimicrobial activity of *Eucalyptus major* and *Eucalyptus baileyana* methanolic extracts. Int J Microbiol. 2009;6(1).
36. Chikowe G, Mpala L, Cock IE. Antibacterial activity of selected Australian *Syzygium* species. Pharmacogn Commun. 2013;3(4):77-83. DOI: 10.5530/pc.2013.4.11
37. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction, Benjamin Cummings, San Francisco. 2001.
38. Cock IE. Antimicrobial activity of *Acacia aulacocarpa* and *Acacia complanta* methanolic extracts. Pharmacogn Commun. 2012;2(1):66-71. DOI: 10.5530/pc.2012.1.12
39. Buzzini P, Arapitsas P, Goretti M, *et al.* Antimicrobial activity of hydrolysable tannins. Mini-Rev Med Chem. 2008;8(12):1179-87.

PICTORIAL ABSTRACT



SUMMARY

- *B. acerifolius* leaf and flower extracts were screened for the ability to block the growth of a panel of bacterial pathogens.
- The growth inhibition of both gram-positive and gram-negative bacteria was tested.
- The antibacterial activity was quantified by determining the MIC values of each extract.
- Growth time course studies were also undertaken against *P. fluorescens* and *B. cereus*.
- Toxicity of the *B. acerifolius* leaf and flower extracts was determined using the *Artemia* nauplii toxicity bioassay.

ABOUT AUTHORS

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.