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Original Article

Citrus australasica F. Muell. Leaf Extracts are Ineffective Inhibitors of the Growth of a Panel of Bacterial Pathogens

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ABSTRACT

Introduction: Closely related plant species often share similar secondary metabolites and bioactivities and are therefore good targets for bioactivity testing when one or more species within a genus are known to possess therapeutic properties. The genus *Citrus* has a long history of ethnobotanbical usage in many areas of the world. Many species are known to have therapeutic properties, several species of which have well established antibacterial bioactivities. **Materials and Methods :** The ability of *Citrus australasica* F. Muell. leaf extracts to inhibit the growth of a panel of bacterial pathogens was investigated by disc diffusion assay. Toxicity was examined using the *Artemia franciscana* nauplii bioassay. **Results:** The *C. australasica* methanolic and aqueous extracts were ineffective at inhibiting the growth of gram-positive and gram-negative panels of bacteria and were nontoxic in the *Artemia* lethality assay following 24 hr exposure. **Conclusion:** Despite its close taxonomic relationship with several bioactive *Citrus* spp. with antibacterial activity, *C. australasica* leaf extracts were ineffective bacterial growth inhibitors. However, these extracts may have other therapeutic properties and testing against protozoa, fungi, virus and tumour cells is required.

Keywords: Rutaceae, Finger lime, Caviar lime, Pharmacognosy, Traditional medicine, Medicinal plants, Toxicity.

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INTRODUCTION

The use of natural plant therapeutics is as old as human civilisation and in many regions of the world is still the primary modality of health care. Ayuverdic medicine in India for example is still commonly practiced, with approximately 85% of Indians using crude plant preparations for the treatment of various diseases and ailments.[1] Even in Western civilisations, plants play an important role in medicine. At least 25 % of pharmaceuticals prescribed worldwide are directly obtained from plants with many more drugs being semi-synthetic derivatives of natural plant precursors.[2-4] Examples of medicinally important plant derived compounds include the anti-malarial drug quinine and its derivatives (from *Cinchona* spp.), the antitumour drugs vincristine and vinblastine (from *Catharanthus roseus*) along with the semi-synthetic analogue vindesine, the analgesics morphine and codeine (from *Papaver somniferum*), the anticholinogenic drug atropine derived from plants of the family *Solinaceae* (*Atropa belladonna*, *Datura stramonium* and *Mandragora officinarum*), the anticancer drug taxol (derived from *Taxus brevifolia*) and the cardiac glycoside digoxin (from *Digitalis purpurea*).[5]

Despite the potential of plants to provide us with useful pharmaceutical agents, the field is still poorly studied. Only an estimated 5-10 % of the approximately 300, 000-500, 000 plant species worldwide have been screened for 1 or more bioactivities.[5] With so many plant species yet to be tested, it is essential that plant selection processes narrow the field. The main selection criteria currently used is to select plants on the basis of ethnobotanical usage as traditional medicines. Other important selection criteria is to examine plants closely related to plants for which medicinal potential is well established, or plants with known physiochemical properties for which a therapeutic property has previously been reported. Plants with high antioxidant contents are considered particularly promising for the development of new therapies as many antioxidant plant compounds have protective and curative effects. Indeed, plants high antioxidant capacities have been previously reported to treat cancer,[6-10] cardiovascular diseases,[11] neural degeneration,[12] diabetes and obesity.[13] Interestingly, multiple recent studies have also reported noteworthy inhibitory activity against multiple bacteria, highlighting the potential of high antioxidant plant extracts as antibacterial chemotherapies.[14-20]

In recent years, the development of bacterial pathogens that are either extremely (XDR) or totally drug resistant (TDR) to common clinically used antibiotics[21] has resulted in the need to develop new antibiotic chemotherapies. There are now limited therapeutic options for many diseases caused by bacterial pathogens and the situation is expected to worsen in the future as bacteria exchange resistance genes. Indeed, the development of alternative antibacterial treatment modalities has become crucial and is considered by the World Health Organisation (WHO) to be one of the most serious challenges facing medical science.[22] For a number of reasons reviewed elsewhere,[21] it is unlikely that the previous methods of antibiotic discovery/development will be as successful in the future and new treatment modalities are urgently required. Traditional medicines and herbal remedies have great potential for antimicrobial drug development and there has recently been a substantial increase in interest in this field.[23-26]

Citrus is a genus of flowering trees and shrubs of the family Rutaceae. The genus consists of some of the most commercially important fruits, including oranges, lemons, limes, grapefruit and pomelos. Whilst all *Citrus* species are native to either South Asia, East Asia, Melanesia and Australia, they have been widely domesticated and are now grown in subtropical and tropical regions globally. According to the Swingle taxonomic system, the genus *Citrus* consists of sixteen distinct species, although other taxonomic systems recognise 156 (Tanaka system) or three species, with four hybrid groups (Mabberley system).[27] For this study, we used the Swingle system. Interestingly, a large portion of the *Citrus* genus are native to Australia, with eight species from that region. Furthermore, six of these *Citrus* species are endemic to Australia. Of these, *Citrus australasica* F.Muell. (Finger lime) (Figure 1), *Citrus australis* (Sweet) Planchon (Australian round lime) and *Citrus glauca* (Lindl.) Burkill (Australian desert lime) are perhaps the best known.

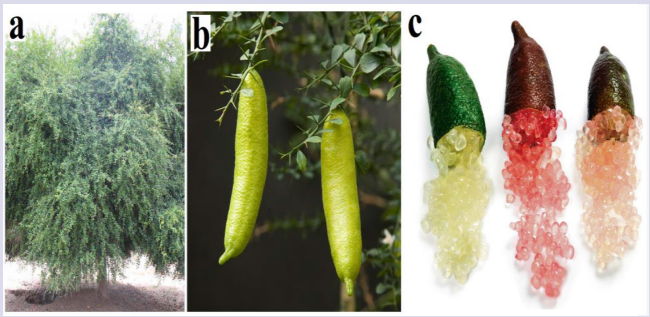

Figure 1: *C. australasica* (a) whole tree, (b) leaves and fruit, (c) open fruit showing vesicles.

Many *Citrus* species have a history of traditional use to prevent and treat a variety of diseases. Due to the high ascorbic acid contents of all species of this genus, the fruit were traditionally used to treat and prevent scurvy.[27] The pharmacognosy of *Citrus limon* (L.) Osebeck (commonly known as lemon) has been particularly well documented, as reviewed in.[29] The juice of *C. limon* fruit has been used to treat high blood pressure, colds and influenza, sore throats and coughs, rheumatism, fevers, asthma, respiratory diseases, arthritis and inflammation.[29] It has also been used to protect against infections. Notably, several of these diseases are caused by bacterial pathogens. In contrast to other *Citrus* species, the pharmacognosy of the Australian species has been relatively neglected.

Citrus australasica (synonym Microcitrus australasica*)* is a thorny shrub to small tree (Figure 1a) that is endemic to the eastern rainforest regions of Australia. It has thorny branches and produces elongated cylindrical fruit (Figure 1b) that are commonly known as caviar lime due to the appearance of the distinctive vesicles inside the fruit (Figure 1c). Notably, the fruit have been reported to have high antioxidant capacities (TEAC ~16μmol TE/g fruit).[29] That study also reported that the ascorbic acid levels were particularly high (~3.5μmol/g fruit), which was approximately 46 times higher than the levels in the equivalent mass of blueberries. However, despite the traditional uses of other Citrus spp. to treat bacterial infections and the high antioxidant capacity of *C. australasica*, it has yet to be rigorously examined for the ability to inhibit bacterial growth. This study screened *C. australasica* leaf extracts for the ability to inhibit the growth of a panel of bacterial pathogens of human importance.

MATERIALS AND METHODS

Plant Material

Collection of Plant Material and Extraction

Citrus australasica F. Muell. leaves were supplied by Mervyn Cooper of the Queensland Bushfoods Association, Australia from a verified tree in Brisbane, Australia. Freshly harvested leaves were washed in deionised water and processed within 4 hr of collection. The plant was monitored for 3 months prior to harvesting to ensure that it received no pesticides or fertilisers, and none were used in a 3 metre radius of the plant. Voucher specimens of the leaves (GU2014CALa) are stored in the School of Environment and Science, Griffith University. The harvested leaves were dried in a Sunbeam food dehydrator and the dried material was ground to a coarse powder. Individual 1g masses of the dried plant material was extracted extensively in 50mL methanol (Ajax Fine Chemicals Australia, AR grade) or deionised water for 24 hr at 4°C with gentle shaking. The extract was filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation. The resultant pellet was dissolved in 10mL deionised water (containing 0.5% DMSO). The extract was passed through 0.22μm filter (Sarstedt) and stored at 4°C.

Qualitative Phytochemical Studies

Phytochemical analysis of the *C. australasica* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by standard assays.[30]

Antibacterial Screening

Test Microorganisms

All media was purchased from Oxoid Ltd., Australia. The reference strains of *E. coli* (ATCC157293), *Klebsiella pneumoniae* (ATCC31488), *Proteus mirabilis* (ATCC21721) and *Streptococcus pyogenes* (ATCC19615) were purchased from American Tissue Culture Collection (ATCC), USA. Clinical isolate microbial strains of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Bacillus cereus, Citrobacter freundii*, *Pseudomonas fluorescens*, *Salmonella newport, Serratia marcescens, Shigella sonneii, Staphylococcus aureus* and *Staphylococcus epidermidis* strains 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 the *C. australasica* leaf extracts was determined using a modified disc diffusion assay.[31-33] Briefly, 100μL of the each bacterial suspension in log phase was spread onto individual nutrient agar plates and the extracts were tested for antibacterial activity using 6mm sterilised filter paper discs. The discs were each infused with 10μL of the individual plant extract, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2 hr before incubation at 37°C for 24 hr. The diameters of the zones of inhibition (ZOIs) were measured to the closest whole millimetre. Each assay was performed three times in triplicate (n=9). Mean values (± SEM) are reported in this study. Standard discs of ampicillin (10μg) and chloramphenicol (10μg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10μL of distilled water were used as a negative control.

Artemia franciscana Nauplii Toxicity Screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay (ALA).[34,35] Briefly, *A. franciscana* nauplii were incubated in the presence of the extracts, reference toxin (1mg/mL potassium dichromate) or artificial seawater (negative control) at 25±1°C under artificial light. All treatments were performed three times in triplicate (n=9). The number of dead were counted in each well at 24hr and 48hr. At the completion of the 48hr exposure period, the remaining live nauplii were sacrificed and the total number of nauplii in each well were counted and used to calculate the % mortality per well. LC_{50} values were calculated for each treatment using probit analysis.

Statistical Analysis

Data are expressed as the mean ± SEM of three independent experiments with internal triplicates (n=9). 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 *C. australasica* leaves with methanol and water yielded 328 and 284mg of extracted material respectively (Table 1). The extracts were resuspended in 10mL of

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

			Methanolic extract	Aqueous extract
	Mass of extracted material (mg)		328	284
	Concentration of resuspended extract (mg/mL)		32.8	28.4
Qualitative Phytochemical Tests	Phenols	Total phenols	+++	+++
		Water soluble phenols	++	++
		Insoluble phenols	-	+
	Saponins	Froth persistence	+	++
		Emulsion test	+	+
	Cardiac glycosides	Keller-Kiliani Test	-	-
	Triterpenoids	Salkowski Test	+	+
	Phytosterols	Acetic Anhydride Test	-	-
	Alkaloids	Meyer's Test	-	-
		Wagner's Test	-	-
		Draggendoff's Test	-	-
	Flavonoids	Kumar Test	+++	+++
	Tannins	Ferric Chloride Test	+	+
		Lead Acetate Test	+	+
	Anthraquinones	Free	-	-
		Combined	-	-

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

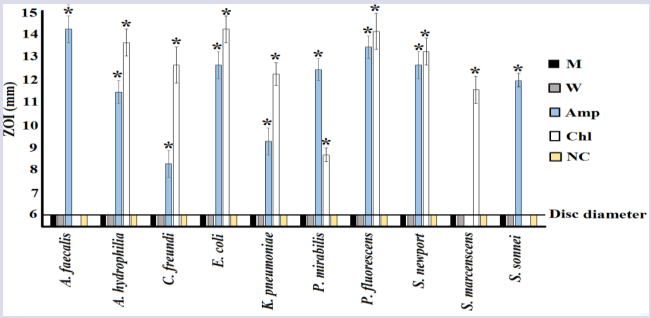

Figure 2: Growth inhibitory activity of *C. australasica* leaf extracts and reference antibiotics against gram-negative bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. M = methanolic extract; W = aqueous extract; NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM.

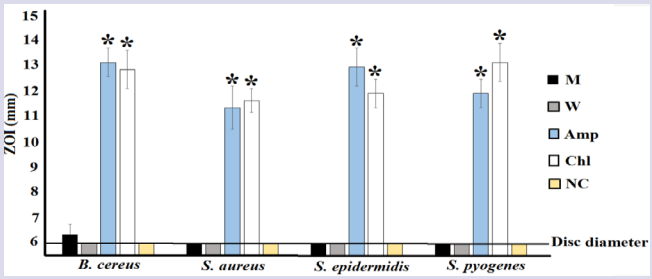

Figure 3: Growth inhibitory activity of *C. australasica* leaf extracts and reference antibiotics against gram-positive bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. M = methanolic extract; W = aqueous extract; NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM.

deionised water (containing 1% DMSO), resulting in an extract concentrations shown in Table 1. Qualitative phytochemical studies showed that both extracts had similar phytochemical profiles. Both contained high levels of phenolic compounds and flavonoids. Lower levels of saponins, triterpenoids and tannins were also detected. Cardiac glycosides, phytosterols, alkaloids and anthraquinones were completely absent or below the detection thresholds for these assays.

Antibacterial Activity

To determine the growth inhibitory activity of the *C. australasica* leaf extracts, aliquots (10µL) of each extract were screened in the disc diffusion assay. The *C. australasica* leaf extracts were ineffective at inhibiting the growth of all gram-negative (Figure 2) and gram positive (Figure 3) bacterial species tested. In contrast, both positive control antibiotics (ampicillin and chloramphenicol) were effective growth inhibitors, with ZOI's of up to 14.3mm (chloramphenicol against *E. coli*). We were therefore unable to determine the MIC values for any extract as they were completely ineffective at all concentrations tested.

Quantification of Toxicity

The toxicity of the *C. australasica* leaf extracts was initially tested at 2mg/mL in the *A. franciscana* nauplii bioassay (Figure 4). The mortality in the presence of all extracts was not significantly different to that of the untreated control at 24hr and thus were deemed to be non-toxic. Extracts with 24hr LC_{50} values >1000µg/mL have previously been defined as non-

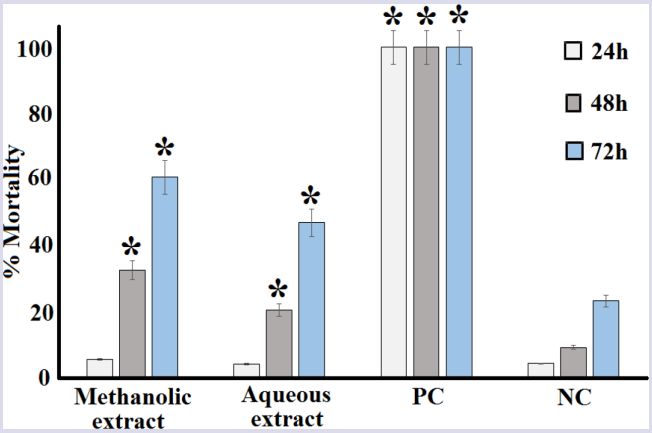

Figure 4: The lethality of the *C. australasica* leaf extracts, potassium dichromate positive control (PC) (1000µg/mL) and seawater (negative control; NC) following 24, 48 and 72 hr of exposure. All bioassays were performed three times in triplicate (n=9) and are expressed as mean ± SEM. * indicates results that are significantly different to the untreated (seawater) control at the equivalent exposure time (p<0.01).

toxic.[39] In contrast, the potassium dichromate positive control induced substantial mortality within 4h (results not shown), with 100% mortality induction seen by 24hr. The mortality increased following exposure to the *C. australasica* leaf extracts at 48h and was further increased following 72hr exposure.

DISCUSSION

Due to recent increases in bacterial resistance to many antibiotics, the development of new antibiotic chemotherapies is a high priority for medical science.[20,21] A concurrent decrease in the discovery of new antibiotic medicines by conventional strategies has increased interest in re-evaluating medicinal plants for new antibiotic chemotherapies.[20] As *C. australasica* is taxonomically related to multiple *Citrus* spp. that have antibacterial activity,[28] it was deemed a viable target for antibacterial screening. Interestingly, the *C. australasica* extracts were completely ineffective growth inhibitors against all gram-positive and gram-negative bacteria tested.

It is noteworthy that a single assay technique was used to screen for antibacterial activity in this study. We chose to use the disc diffusion assay as it is a rapid methodology and it has previously been widely utilised in other studies. Therefore, comparisons between studies are relatively simple. However, as the disc diffusion method is reliant on the diffusion of a molecule through the aqueous environment of an agar gel, this assay may be affected by the solubility of the extract compounds in the aqueous environment. Polar compounds that are highly soluble in water would be expected to diffuse easily in the gel, whereas less soluble compounds would not diffuse as readily and thus be concentrated around the disc. For this reason, whilst this is a handy assay for screening aqueous extracts, this technique may not be ideal for nonpolar compounds (e.g. when screening essential oil and their components). For examining nonpolar mixtures, other techniques such as liquid dilution assays may be preferred. Interestingly, the qualitative phytochemical screening studies presented herein showed that the *C. australasica* leaf extracts contained nonpolar terpenoid components (albeit at a low to moderate level). Therefore, the activity of the extracts may have been significantly under estimated as the movement of these nonpolar components through the agar gel would be hindered. Liquid dilution studies may have been better suited to screen the *C. australasica* for activity and future studies will use these techniques to re-examine the extracts for antibacterial activity.

Diffusion of molecules within agar gel is also affected by the size of the molecules. The movement of large, complex phytochemicals (e.g. complex tannins) through agar gels by diffusion would also be retarded and may provide a false idea of the efficacy of an extract. As many tannins have well described antibiotic properties, screening for growth inhibition using agar diffusion techniques may give a distorted view of its inhibitory potential.

The findings reported here also indicate that the extracts examined were non-toxic (24 hr LC_{50} >1000 µg/mL) in the *Artemia* nauplii bioassay. 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.[35] However, further studies are required to determine whether this is also true for the *C. australasica* leaf extracts examined in these studies.

CONCLUSION

Methanolic and aqueous *C. australasica* leaf extracts lacked antibacterial activity in the disc diffusion assay against a panel of human pathogenic bacteria, despite their close taxonomic relationship with *Citrus* spp. with established antibacterial properties. The extracts were nontoxic towards *Artemia* nauplii.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

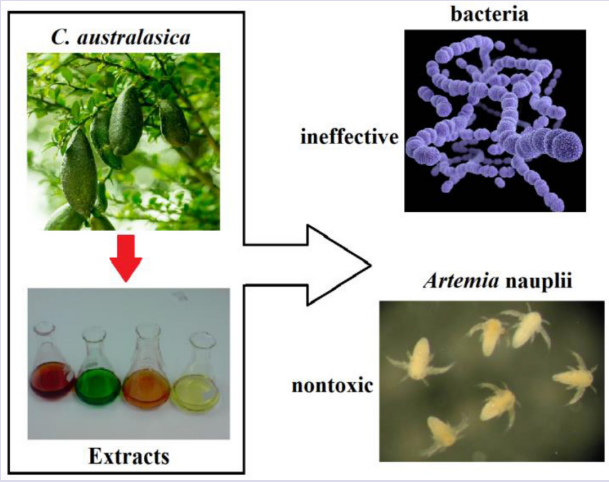
DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50 % mortality; **MIC:** minimum inhibitory concentration; **ZOI:** zone of inhibition.

REFERENCES

1. Kamboj VP. Herbal medicine. Curr Sci. 2000;78:35-9.
2. Hostettmann K, Hamburger M. Search for new lead compounds of natural origin. In: Testa B, Kyburz E, Fuhrer W, Giger R, editors, Helvitica Chimica Acta. Perspectives in medical chemistry. Basel; 1993.
3. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Rep. 2000;17(3):215-34. doi: 10.1039/a902202c, PMID 10888010.
4. Biopharmaceuticals WG. Biochemistry and biotechnology. 2003. 3rd ed. Wiley, Chinchester.
5. Gilani AH, Rahman AU. Trends in ethnopharmocology. J Ethnopharmacol. 2005;100(1-2):43-9. doi: 10.1016/j.jep.2005.06.001, PMID 16127805.
6. Shalom J, Cock IE. *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. Nutr Cancer. 2018;70(4):579-93. doi: 10.1080/01635581.2018.1460680, PMID 29641917.
7. Cock IE, Cheesman MJ. Plants of the Genus *Terminalia*: phytochemistry and antioxidant profiles, proliferation and cancer. In: Cancer. 2nd ed Oxidative Stress and Dietary Antioxidants in Neurological Diseases 2021. p. 495-505. doi: 10.1016/B978-0-12-819547-5.00044-4.
8. Gu B, Shalom J, Cock IE. Anti-Proliferative properties of *Terminalia sericea* Burch. Ex Dc leaf extracts against Caco2 and HeLa cancer cell lines. Pharmacogn J. 2018;10(3):408-15. doi: 10.5530/pj.2018.3.67.
9. Jamieson N, Sirdaarta J, Cock IE. The anti-proliferative properties of Australian plants with high antioxidant capacities against cancer cell lines. Pharmacogn

Commun. 2014;4(4):71-82.
10. Rabadeaux C, Vallette L, Sirdaarta J, Davis C, Cock IE. An examination of the antimicrobial and anticancer properties of *Khaya senegalensis* (Desr.) A.Juss. bark extracts. Pharmacogn J. 2017;9(2):175-84.
11. Vita JA. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr. 2005;81(1);Suppl:292S-7S. doi: 10.1093/ajcn/81.1.292S, PMID 15640493.
12. Youdim KA, Spencer JPE, Schroeter H, Rice-Evans CA. Dietary flavonoids as potential neuroprotectants. Biol Chem. 2002;383(3-4):503-19. doi: 10.1515/BC.2002.052, PMID 12033439.
13. Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. J Nutr. 2003;133(7):2125-30. doi: 10.1093/jn/133.7.2125, PMID 12840166.
14. 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. J Funct Foods. 2015;17:610-20. doi: 10.1016/j.jff.2015.06.019.
15. Wright MH, Matthews B, Arnold MSJ, Greene AC, Cock IE. The prevention of fish spoilage by high antioxidant Australian culinary plants: *Shewanella putrefaciens* growth inhibition. Int J Food Sci Technol. 2016;51(3):801-13. doi: 10.1111/ijfs.13026.
16. McManus K, Wood A, Wright MH, Matthews B, Greene AC, Cock IE. *Terminalia ferdinandiana* Exell. Extracts inhibit the growth of body odour-forming bacteria. Int J Cosmet Sci. 2017;39(5):500-10. doi: 10.1111/ics.12403, PMID 28488331.
17. Mandeville A, Cock IE. *Terminalia chebula* Retz. fruit extracts inhibit bacterial triggers of some autoimmune diseases and potentiate the activity of tetracycline. Indian J Microbiol. 2018;58(4):496-506. doi: 10.1007/s12088-018-0754-9, PMID 30262960.
18. Ilanko P, McDonnell PA, Van Vuuren SF, Cock IE. Interactive antibacterial profile of *Moringa oleifera* Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases. S Afr J Bot. 2019;124:420-35. doi: 10.1016/j.sajb.2019.04.008.
19. Wright MH, Shalom J, Matthews B, Greene AC, Cock IE. *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, PMID 30497593.
20. Courtney R, Sirdaarta J, Matthews B, Cock IE. Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. Pharmacogn J. 2015;07(1):18-31. doi: 10.5530/pj.2015.1.2.
21. Cheesman MJ, Ilanko A, Blonk B, Cock IE. 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, PMID 28989242.
22. WHO. Antimicrobial resistance. World Health Organization; 2016 [cited May 10 2017]. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/.
23. 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.
24. Hart C, Ilanko P, Sirdaarta J, Rayan P, McDonnell PA, Cock IE. *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.
25. Cock IE, Winnett V, Sirdaarta J, Matthews B. 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);Suppl 1:S190-208. doi: 10.4103/0973-1296.157734, PMID 26109767.
26. Winnett V, Sirdaarta J, White A, Clarke FM, Cock IE. Inhibition of *Klebsiella pneumoniae* growth by selected Australian plants: natural approaches for the prevention and management of ankylosing spondylitis. Inflammopharmacology. 2017;25(2):223-35. doi: 10.1007/s10787-017-0328-1, PMID 28239782.
27. Luro F, Curk F, Froelicher Y, Ollitrault P. Recent insights on Citrus diversity and phylogeny. Agrumed. Archaeology and history of citrus fruit in the Mediterranean: acclimatization, diversifications, uses. [Publications du centre Jean Bérard]. Naples; 2017. p. 16-28.
28. Klimek-Szczykutowicz M, Szopa A, Ekiert H. *Citrus limon* (lemon) phenomenon-A review of the chemistry, pharmacological properties, applications in the modern pharmaceutical, food, and cosmetics industries, and biotechnological studies. Plants 2020;9(1):119. doi: 10.3390/plants9010119, PMID 31963590.
29. Netzel M, Netzel G, Tian Q, Schwartz S, Konczak I. Native Australian fruits—a novel source of antioxidants for food. Innov Food Sci Emerg Technol. 2007;8(3):339-46. doi: 10.1016/j.ifset.2007.03.007.
30. Hart C, Ilanko P, Sirdaarta J, Rayan P, McDonnell PA, Cock IE. *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.
31. Cock I, Mpala L, Chikowe G. No evidence of antiseptic properties and low toxicity of selected *Aloe* species. J Pharm Negative Results. 2010;1(1):10-6. doi: 10.4103/0976-9234.68869.
32. Lee CJ, Wright MH, Arnold MSJ, Greene AC, Cock IE. Inhibition of *Streptococcus pyogenes* growth by native Australian plants: new approaches towards the management of impetigo, pharyngitis and rheumatic heart disease. Pharmacogn Commun. 2016;6(3):164-73. doi: 10.5530/pc.2016.3.6.
33. Wright MH, Arnold MSJ, Lee CJ, Courtney R, Greene AC, Cock IE. 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.
34. Ganesh PR. Iatrogenic Trauma to Gingiva – A Case Series. BEMS Reports. 2016;2(1):23-6. doi: 10.5530/BEMS.2016.1.5.
35. 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.

PICTORIAL ABSTRACT



SUMMARY

- *Citrus australasica* leaf extracts were screened for the ability to block the growth of a panel of human bacterial pathogens.
- No inhibitory activity was evident against any of the bacterial species tested
- Toxicity of the *C. australasica* extracts was determined using the *Artemia* nauplii toxicity bioassay.
- Both the methanolic and aqueous extracts were nontoxic.

About Authors

Ms. Getmore Chikowe completed at B.Sc 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 250 publications in a variety of peer reviewed journals.

Ms. Lindiwe Mpala completed at B.Sc 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.

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