

Davidsonia pruriens F. Muell. Fruit and Leaf Extracts Lack Antibacterial and Antifungal Activity

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ABSTRACT

Introduction: High antioxidant levels have been linked with multiple therapeutic properties, including antibacterial activity. Therefore, high antioxidant plant preparations are good targets for antibacterial testing. *D. pruriens* has high antioxidant capacity although it is yet to be tested for the ability to inhibit the growth of bacterial pathogens. **Methods:** The ability of *D. pruriens* fruit and leaf extracts to inhibit the growth of a panel of bacterial and fungal pathogens was investigated by disc diffusion assay. Toxicity was examined using the *Artemia franciscana* nauplii bioassay. **Results:** All *D. pruriens* fruit and leaf extracts were completely ineffective at inhibiting the growth of gram-positive and gram-negative panels of bacteria, as well as fungi. The extracts were non-toxic in the *Artemia* bioassay following 24 h exposure. **Conclusion:** Despite their high antioxidant capacity, *D. pruriens* fruit and leaf extracts were completely ineffective bacterial and fungal

growth inhibitors. However, these extracts may have other therapeutic properties and testing against protozoa, virus and tumour cells is required.

Key words: Cunoniaceae, Davidson's plum, Antibacterial activity, Australian plant, Traditional medicine, Medicinal plants, Toxicity.

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INTRODUCTION

Traditional plant derived medicines have been used in most parts of the world for a variety of therapeutic purposes, including fighting microbial disease. Indeed, the ability of plant extracts to block the growth of pathogenic bacteria has become a focus of substantial recent study.¹⁻⁵ Much of the research into traditional medicinal plant use has focused on Asian,⁶⁻⁸ African,⁹⁻¹¹ Middle Eastern¹²⁻¹⁴ and South American¹⁵ plants. The recent establishment of bacterial pathogens that are either extremely (XDR) or totally resistant (TDR) to common clinically used antibiotics¹⁶ has resulted in the need to develop new and effective 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.¹⁷ For a number of reasons reviewed elsewhere,¹⁶ 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.^{18,19}

Despite the potential of plants to provide us with useful pharmaceutical agents, the field is still relatively poorly studied. Only an estimated 5-10 % of the approximately 300,000-500,000 plant species worldwide have been screened for one or more bioactivities.⁹ 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. Another important selection method is to examine plants based on their phytochemical composition. The study of plants with high antioxidant contents is often particularly fruitful as high antioxidant contents have been associated with a decreased incidence of chronic diseases²⁰ and can prevent the development of some degenerative diseases including

cancer,²¹ cardiovascular diseases,²² neural degeneration,²³ diabetes and obesity.²⁴

Several studies have highlighted the high antioxidant contents of several Australian native fruits^{25,26} and the therapeutic properties for some of these species have been reported. *Terminalia ferdinandiana* Excell. has been particularly well studied and has been reported to have anticancer activity,^{27,28} anti-inflammatory activity²⁹ and anti-giardial activity.^{30,31} It also has potent antibacterial activity,³²⁻³⁵ including a methicillin resistant strain of *Staphylococcus aureus* (MRSA).³⁶ *Tasmannia lanceolata* R. Br. has also been relatively well studied and has been shown to have anti-giardial activity,³⁷ as well as broad spectrum antibacterial activity.^{38,39} Similarly, several Australian *Syzygium* spp. have also been reported to have good anticancer²⁶ and antibacterial activities.⁴⁰⁻⁴³ *Kunzea pomifera* F. Muell., *Acronychia acidula* F.; Muell. and *Citrus glauca* (Lindl.) Burkill have also been reported to have noteworthy antibacterial activity.⁴⁴ Despite these earlier studies, many of the other high antioxidant Australian plants have been relatively neglected.

Davidsonia pruriens F. Muell. (family Cunoniaceae; commonly known as Davidson's plum or ooray) is a medium sized rainforest tree (Figure 1a) that is endemic to the north-eastern regions of Australia. Its leaves are large and compound (Figure 1b) and it produces dark purple to black fruit in clusters on the trunk and branches (Figures 1c and 1d). The fruit were popular with the first Australians as a highly nutritious food and have high antioxidant contents. Indeed, *D. pruriens* fruit have been reported to have radical scavenging activity and total reducing capacities of 3.2 and 49 $\mu\text{mol/g}$ of fresh fruit respectively.²⁷ This noteworthy antioxidant capacity is attributable to the phytochemical constituents of the fruit. Several triterpenoids, including methyl 3-oxo-bauer-7-en-28-oate (Figure 1e) and 3-oxo-bauer-7-ene-28-oic acid (Figure 1f) have been identified in *D. pruriens* fruit.⁴⁵ Of particular interest, the fruit is rich in the anthocyanidin glycosides delphinidin-3-sambubioside (Figure 1g), cyanidin-3-sambubioside (Figure 1h), 3-peonidin-sambubioside (Figure 1i) and petunidin-3-sambubioside (Figure 1j).²⁷ Many flavonoids,

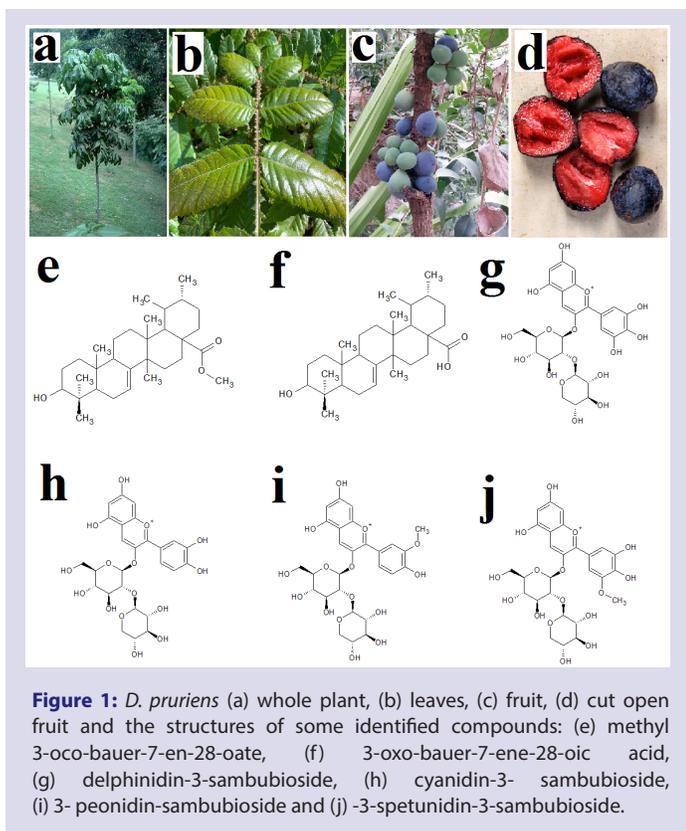


Figure 1: *D. pruriens* (a) whole plant, (b) leaves, (c) fruit, (d) cut open fruit and the structures of some identified compounds: (e) methyl 3-oxo-bauer-7-en-28-oate, (f) 3-oxo-bauer-7-ene-28-oic acid, (g) delphinidin-3-sambubioside, (h) cyanidin-3-sambubioside, (i) 3-peonidin-sambubioside and (j) -3-spetunidin-3-sambubioside.

including anthocyanidin, have bacterial growth inhibitory activity.⁴⁶ Despite this, *D. pruriens* has yet to be rigorously tested for antibacterial activity. The present study was undertaken to test fruit and leaf extracts against a panel of human bacterial pathogens.

MATERIALS AND METHODS

Plant material

Collection of plant material and extraction

Davidsonia pruriens F.Muell. fruit and leaves were harvested from a confirmed plant on Nathan campus of Griffith University, Australia. 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 fruit (GU2019DPFa) and leaves (GU2019DPLa) are stored in the School of Environment and Science, Griffith University. The harvested leaves and flowers were washed in deionised water and processed within 4 hours of collection. The 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, AR grade) or deionised water for 24 hours 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. The extract was passed through 0.22µm filter (Sarstedt) and stored at 4°C.

Qualitative phytochemical studies

Phytochemical analysis of the *D. pruriens* fruit and leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by standard assays.^{47,48}

Antibacterial screening

Test micro-organisms

All media were purchased from Oxoid Ltd., Australia. Reference strains of *Escherichia 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 faecalis*, *Aspergillus niger*, *Bacillus cereus*, *Candida albicans*, *Citrobacter freundii*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Salmonella newport*, *Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus* and *Staphylococcus epidermidis* strains were obtained from Ms. Michelle Mendell and Ms. Jane Gifkins, Griffith University. All bacterial stock cultures were subcultured and maintained in nutrient broth at 4°C. The fungal strains were cultured in Sabourand broth (Oxoid, Australia).

Evaluation of antimicrobial activity

Antimicrobial activity of the *D. pruriens* fruit and leaf extracts was determined using a modified disc diffusion assay.⁴⁹⁻⁵¹ Briefly, 100µL of each microbial suspension in log phase was spread onto individual nutrient agar plates (or Sabourand agar for the fungal strains) and the extracts were tested for antimicrobial 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 2h before incubation at 37°C for 24h. 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 (\pm SEM) are reported in this study. Standard discs of ampicillin (10µg), chloramphenicol (10µg) and nystatin (100µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial and antifungal activity. Filter discs infused with 10µL of distilled water were used as a negative control.

Artemia franciscana nauplii lethality assay

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay (ALA).^{52,53} 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 24h, 48h and 72h. At the completion of the 72h 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. LC₅₀ values were calculated for each treatment using probit analysis.

Statistical analysis

Data are expressed as the mean \pm 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 *D. pruriens* fruit and leaves with yields between 144 and 283mg of extracted material (Table 1). In general, methanol was a more effective extractant than water and the fruit extracts contained greater masses of extracted material than the corresponding leaf extracts. The extracts were resuspended in 10mL of deionised water (containing 1% DMSO), resulting in the extract

concentrations shown in Table 1. Qualitative phytochemical studies showed that both methanolic and aqueous extracts of both the fruit and leaves had similar phytochemical profiles. All contained high levels of phenolic compounds and flavonoids, as well as moderate levels of tannins. Lower levels of saponins and triterpenoids were also detected in all extracts. All other classes of compounds were either absent or below the threshold of detection in these assays.

Antimicrobial activity

To determine the growth inhibitory activity of the *D. pruriens* fruit and leaf extracts, aliquots (10 μ L) of each extract were screened in the disc diffusion assay. The *D. pruriens* fruit and 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 (ampicillin against *A. faecalis*). We were therefore unable to determine the MIC values for any extract as they were completely ineffective at all concentrations tested. Similarly, all of the extracts were ineffective at inhibiting the growth of the three fungal species screened in this study (Figure 4).

Quantification of toxicity

The toxicity of the *D. pruriens* fruit and leaf extracts was initially tested at 2mg/mL in the *A. franciscana* nauplii bioassay (Figure 5). The mortality in the presence of all extracts was not significantly different to that of the untreated control at 24h and thus were deemed to be non-toxic. Extracts with 24h LC₅₀ values >1000 μ g/mL have previously been defined as non-toxic.⁵² In contrast, the potassium dichromate positive control induced substantial mortality within 4h (results not shown), with 100% mortality induction seen by 24h. The mortality increased following exposure to the *D. pruriens* fruit and leaf extracts at 48h and was further increased following 72h exposure.

DISCUSSION

The development of new antibiotic chemotherapies is a high priority for medical science due to recent increases in bacterial resistance to many antibiotics.^{16,17} 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.¹⁶ *D. pruriens* was deemed to be a good candidate for antibacterial screening due to its high antioxidant capacity,²⁵ as other high antioxidant plants have been reported to have noteworthy antibacterial activity.³²⁻⁴⁴ Phenolic phytochemicals are generally strong antioxidants that protect cell constituents against oxidative damage by scavenging of free radicals, thereby averting their deleterious effects on nucleic acids, proteins and lipids in cells.⁴⁶ Interestingly, studies into the antioxidant/prooxidant effects of extracts from various plant species have demonstrated that the ability of a plant extract to exert antioxidant activity depends on multiple factors. *Aloe vera* antioxidant components for example may function as either an antioxidant or an oxidant, with their action being dependent upon their concentration, with some compounds having both antioxidant and pro-oxidant properties at different concentrations.⁵⁴ These differential pro-oxidant/antioxidant effects at different concentrations relate to the balance between the free radical scavenging activities and reducing power of the individual components.

Notably, antibacterial activity was only tested in this study using the disc diffusion assay as it is a rapid method and it has previously been widely utilised in other studies, allowing for easy comparisons between studies. 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. Diffusion of molecules within an agar gel is also affected by the size of the molecules. The movement of large, complex phytochemicals through agar gels by diffusion would also be retarded and may provide a false idea of the efficacy of an extract. For this reason, whilst this is a handy assay for

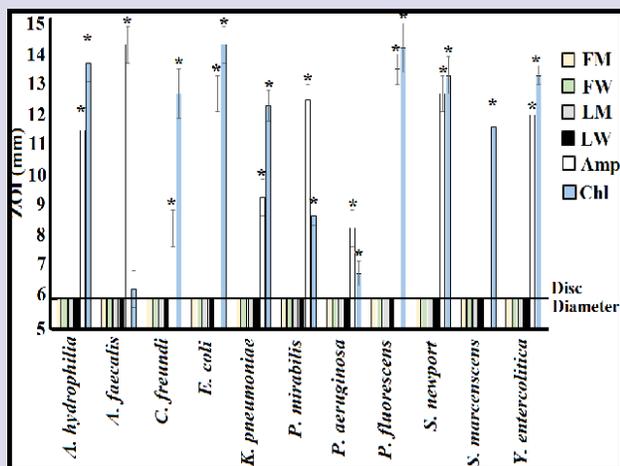


Figure 2: Growth inhibitory activity of *D. pruriens* fruit and leaf extracts and reference antibiotics against gram-negative bacterial species measured as ZOIs (mm) \pm SEM. F = fruit; L = leaf; M = methanolic extract; W = aqueous extract; Amp = ampicillin (10 μ g); Chl = chloramphenicol (10 μ g); NC = 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) \pm SEM.

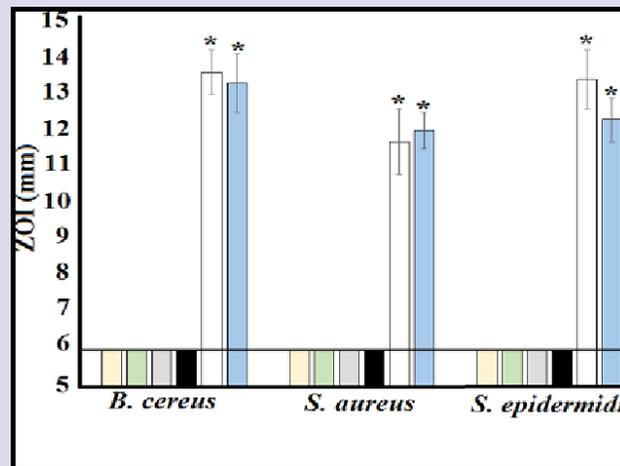


Figure 3: Growth inhibitory activity of *D. pruriens* fruit and leaf extracts and reference antibiotics against gram-positive bacterial species measured as ZOIs (mm) \pm SEM. F = fruit; L = leaf; M = methanolic extract; W = aqueous extract; Amp = ampicillin (10 μ g); Chl = chloramphenicol (10 μ g); NC = 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) \pm SEM.

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

	Fruit		Leaf		
	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	
Mass of extracted material (mg)	283	28.3	228	144	
Concentration of resuspended extract (mg/mL)	22.6	22.6	22.8	14.4	
Total phenols	+++	+++	++	++	
Phenols	Water soluble phenols	+++	+++	++	++
		Insoluble phenols	+	+	++
Saponins	Froth persistence	+	+	+	+
	Emulsion test	-	-	+	+
Cardiac glycosides	Keller-Kiliani Test	-	-	-	-
Triterpenoids	Salkowski Test	+	+	+	+
Phytosterols	Acetic Anhydride Test	-	-	-	-
	Meyer's Test	-	-	-	-
Alkaloids	Wagner's Test	-	-	-	-
	Draggendorff'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.

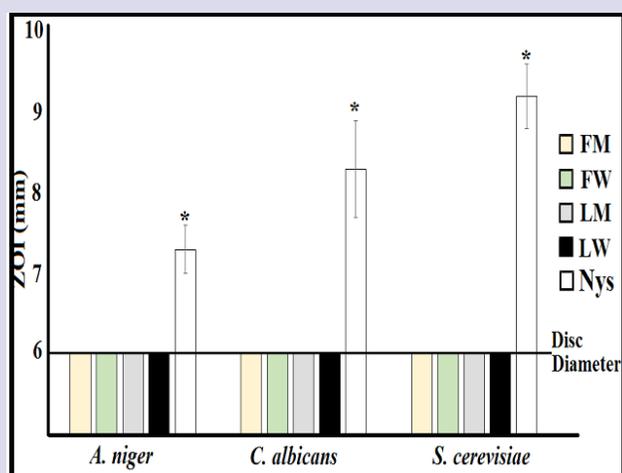


Figure 4: Growth inhibitory activity of *D. pruriens* fruit and leaf extracts and reference antibiotics against fungal species measured as ZOIs (mm) \pm SEM. F = fruit; L = leaf; M = methanolic extract; W = aqueous extract; Nys = Nystatin (100 μ g); NC = negative control. All assays were completed three times, each with internal triplicates (n=9) and the results are expressed as mean zones of inhibition (mm) \pm SEM.

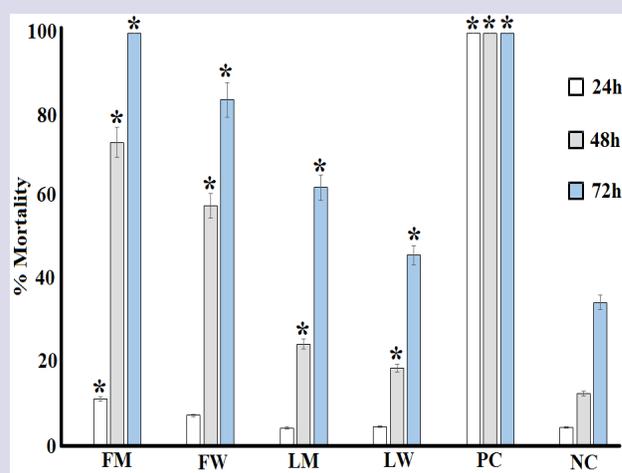


Figure 5: The lethality of the *D. pruriens* fruit and leaf extracts, potassium dichromate control (PC; 1000 μ g/mL) and seawater negative control (NC) following 24, 48 and 72 hr of exposure. F = fruit; L = leaf; M = methanolic extract; W = aqueous extract. All bioassays were performed three times in triplicate (n=9) and are expressed as mean \pm SEM. * indicates results that are significantly different to the untreated (seawater) control at the equivalent exposure time (P<0.01)

screening aqueous extracts, this technique may not be ideal for nonpolar compounds. For examining nonpolar mixtures, other techniques such as liquid dilution assays may be preferred. Liquid dilution studies may have been better suited to screen *D. pruriens* fruit and leaf extracts for activity and future studies will use these techniques to re-examine the extracts for antibacterial activity.

The findings reported here also indicate that the extracts examined were non-toxic (24 hr LC₅₀ >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.⁵² However, further studies are required to determine whether this is also true for the *D. pruriens* fruit and leaf extracts examined in these studies.

CONCLUSION

Methanolic and aqueous *D. pruriens* fruit and leaf extracts displayed no antibacterial activity in the disc diffusion assay against panels of human pathogenic bacteria and fungi. The extracts were also non-toxic towards *Artemia nauplii*.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; **LC₅₀:** The concentration required to achieve 50 % mortality; **MIC:** minimum inhibitory concentration; **ZOI:** zone of inhibition.

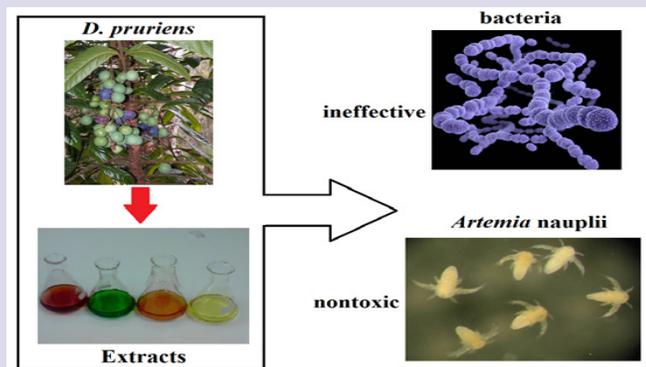
REFERENCES

- Kamboj VP. Herbal medicine. *Curr Sci.* 2000;78(1):35-9.
- Hostettmann K, Hamburger M. Search for new lead compounds of natural origin. In *Perspectives in Medical Chemistry*. Verlag Helvetica Acta, Basel. 1993.
- Wright MH, Sirdaarta J, White A, et al. GC-MS headspace analysis of *Terminalia ferdinandiana* fruit and leaf extracts which inhibit *Bacillus anthracis* growth. *Pharmacogn J.* 2017;9(1):73-82. DOI: 10.5530/pj.2017.1.14
- Mpala LN, Chikowe GR, Cock IE. Growth inhibitory properties of extracts prepared from selected *Leptospermum* and *Melaleuca* species against a panel of pathogenic bacteria. *Pharmacogn Commun.* 2016;6(4):215-24. DOI: 10.5530/pc.2016.4.4
- Omer E, Elshamy AI, Nassar M, et al. *Plantago squarrosa* Murray extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. *Inflammopharmacol.* 2019;27(2):373-85. DOI: 10.1007/s10787-018-0547-0
- Newman DJ, Cragg GM, Snader KM. The influence of natural products on drug discovery. *Nat Prod Rep.* 2000;17(3):215-34.
- Gaillot C, Sirdaarta J, Cock IE. Examination of the antimicrobial and anticancer properties of mangosteen. *Acta Horticulturae.* 2016;1106:231-8.
- Wright MH, Greene AC, Cock IE. Investigating the pharmacognostic potential of Indian *Terminalia* spp. in the treatment and prevention of yersiniosis. *Pharmacogn Commun.* 2017;7(3):108-13. DOI: 10.5530/pc.2017.3.16
- Gilani AH, Atta-ur R. Trends in ethnopharmacology. *J Ethnopharmacol.* 2005;100(1-2):43-9.
- Hübsch Z, Zyl RLV, Cock IE, et al. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. *South African Journal of Botany.* 2014;93:185-97. DOI: 10.1016/j.sajb.2014.04.005
- Cock IE, Vuuren SFV. 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.
- Omer E, Elshamy A, ElGendy AN, et al. *Cakile maritima* Scop. Extracts inhibit the

- growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. *Pharmacogn J.* 2016;8(4):361-74. DOI: 10.5530/pj.2016.4.9
- Biggs I, Sirdaarta J, White A, et al. GC-MS analysis of *Commiphora molmol* oleo-resin extracts which inhibit the growth of bacterial triggers of selected autoimmune diseases. *Pharmacogn J.* 2016;8(3):191-202. DOI: 10.5530/pj.2016.3.4
- Biggs I, Sirdaarta J, White A, Cock IE. GC-MS analysis of frankincense extracts which inhibit the growth of bacterial triggers of selected autoimmune diseases. *Pharmacogn Commun.* 2016;6(1):10-22. DOI: 10.5530/pc.2016.1.3
- 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).
- Cheesman MJ, Ilanko A, Blonk B, et al. Developing new antimicrobial therapies: Are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?. *Pharmacog Rev.* 2017;11(22):57-72. DOI: 10.4103/phrev.phrev_21_17
- WHO. Antimicrobial Resistance. World Health Organization. 2016. Available from: <http://www.who.int/mediacentre/factsheets/fs194/en/>. [Cited on 2017 May 10].
- Sirdaarta J, Matthews B, Cock IE. *Kakadu plum* fruit extracts inhibit the growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. *J Funct Food.* 2015;17:610-20. DOI: 10.1016/j.jff.2015.06.019
- 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.
- Potter JD. Cancer prevention: Epidemiology and experiment. *Cancer Lett.* 1997;114(1-2):7-9.
- Hertog MG, Bueno-de-Mesquita HB, Fehily AM, et al. Fruit and vegetable consumption and cancer mortality in the caerphilly study. *Cancer Epidemiol Biomarkers Prev.* 1996;5(9):673-7.
- Vita JA. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. *Am J Clin Nutr.* 2005;81(1):292S-7S.
- Youdim KA, Spencer JPE, Schroeter H, et al. Dietary flavonoids as potential neuroprotectants. *Biol Chem.* 2002;383(3-4):503-19.
- Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O-b-D-glucoside-rich purple corn colour prevents obesity and ameliorates hyperglycemia in mice. *J Nutr.* 2003;133:2125-30.
- Netzel M, Netzel G, Tian Q, et al. Sources of antioxidant activity in Australian native fruits. Identification and quantification of anthocyanins. *J Agric Food Chem.* 2006;54(26):9820-6.
- 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.
- Tan AC, Konczak I, Ramzan I, et al. Native Australian fruit polyphenols inhibit cell viability and induce apoptosis in human cancer cell lines. *Nutrit Cancer.* 2011;63(3):444-55.
- Shalom J, Cock IE. *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. *Nutrit Cancer.* 2018;70(4):579-93.
- Tan AC, Konczak I, Ramzan I, et al. Potential antioxidant, antiinflammatory, and proapoptotic anticancer activities of Kakadu plum and *Illawara* plum polyphenolic fractions. *Nutrit Cancer.* 2011;63(7):1074-84.
- Cock IE, Rayan P. Ascorbic acid potentiates the *Giardia duodenalis* growth inhibitory activity of pure *Terminalia ferdinandiana* Exell compounds. *Parasitol Res.* 2010. DOI: 10.1007/s00436-019-06579-1
- Rayan P, Matthews B, McDonnell A, et al. *Terminalia ferdinandiana* extracts as inhibitors of *Giardia duodenalis* proliferation: A new treatment for giardiasis. *Parasitol Res.* 2015;114(7):2611-20. DOI: 10.1007/s00436-015-4465-4
- McManus K, Wood A, Wright M, et al. *Terminalia ferdinandiana* Exell. extracts inhibit the growth of body odour-forming bacteria. *Int J Cosmet Sci.* 2017;39(5):500-10.
- Sirdaarta J, Matthews B, Cock I. *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.
- 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. *Pharmacogn J.* 2015;7(1):18-31.
- 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.
- Cheesman MJ, White AR, Matthews B, et al. *Terminalia ferdinandiana* fruit and leaf extracts inhibit methicillin-resistant *Staphylococcus aureus* growth. *Planta Med.* 2019;85(16):1253-62. DOI: 10.1055/a-1013-0434
- Rayan P, Matthews B, McDonnell P, et al. Phytochemical analysis of *Tasmannia lanceolata* extracts and inhibition of *Giardia duodenalis* proliferation. *Pharmacogn J.* 2016;8(3):291-9.
- Vallette L, Rabadeaux C, Sirdaarta J, et al. An upscaled extraction protocol for *Tasmannia lanceolata* (Poir.) A.C. Sm.: Antibacterial, anti-Giardial and anticancer activity. *Pharmacogn Commun.* 2016;6(4):238-54.

39. 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.
40. Murhekar S, Wright MH, Greene AC, et al. Inhibition of *Shewanella* spp. growth by *Syzygium australe* and *Syzygium leuhmannii* extracts: Natural methods for the prevention of fish spoilage. *Int J Food Sci Technol*. 2017;54(10):3314-26.
41. Woods A, McManus K, Wright MH, et al. Growth inhibitory activity of selected Australian *Syzygium* species against malodour forming bacteria. *Pharmacogn Commun*. 2017;7(3):129-36.
42. Wright MH, Lee CJ, Pollock CE, et al. Growth inhibitory activity of selected high antioxidant Australian *Syzygium* species against the food poisoning and tissue necrotic pathogen *Clostridium perfringens*. *Pharmacogn Commun*. 2016;6(2):93-9.
43. Cock IE. Antimicrobial activity of *Syzygium australe* and *Syzygium leuhmannii* leaf methanolic extracts. *Pharmacogn Commun*. 2012;2(2):71-7.
44. 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 Technol*. 2016;51(3):801-13.
45. Meksuriyen D, Nanayakkara ND, JrPhoebe CH, et al. Two triterpenes from *Davidsonia pruriens*. *Phytochem*. 1986;25(7):1685-9.
46. Cock IE. The phytochemistry and chemotherapeutic potential of *Tasmannia lanceolata* (*Tasmanian pepper*): A review. *Pharmacogn Commun*. 2013;3(4):13-25.
47. 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
48. Winnett V, Sirdaarta J, White A, et al. Inhibition of *Klebsiella pneumoniae* growth by selected Australian plants: Natural approaches for the prevention and management of ankylosing spondylitis. *Inflammopharmacol*. 2017;25(2):223-35.
49. Lee CJ, Wright MH, Arnold MSJ, et al. 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.
50. 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.
51. Cock IE. Antimicrobial activity of *Callistemon citrinus* and *Callistemon salignus* methanolic extracts. *Pharmacogn Commun*. 2012;2(3):50-7.
52. 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.
53. Sirdaarta J, Cock IE. Vitamin E and Trolox™ reduce toxicity of *Aloe barbadensis* Miller juice in *Artemia franciscana* nauplii but individually are toxic at high concentrations. *Int J Toxicol*. 2008;5(1).
54. Cock IE. Problems of reproducibility and efficacy of bioassays using crude extracts, with reference to *Aloe vera*. *Pharmacogn Commun*. 2011;1(1):52-62.

PICTORIAL ABSTRACT



SUMMARY

- *D. pruriens* fruit and 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 *D. pruriens* extracts was determined using the *Artemia nauplii* toxicity bioassay.
- Both the methanolic and aqueous extracts were non-toxic.

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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 phylliraoides*, *Terminalia ferdinandiana* (Kakadu plum), Australian *Acacias*, *Syzygiums*, *Petalostigmas* and *Xanthorrhoea johnsonii* (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.



Ms. 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.

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