Pharmacognosy Communications
Volume 3 | Issue 2 | Apr–Jun 2013
www.phcogcommn.org

Research Letter

Antimicrobial activity of *Backhousia citriodora* (lemon myrtle) methanolic extracts

Cock IE[a,b*]

[a]*Biomolecular and Physical Sciences, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland 4111, Australia*
[b]*Environmental Futures Centre, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland 4111, Australia*

ABSTRACT: **Introduction:** *Backhousia citriodora* is a native Australian plant with uses as a bush food component as well as in toiletries and cosmetics. Essential oils produced from leaves of this plant have reputed antiseptic properties. Despite this, solvent extractions of *Backhousia citriodora* leaves have not been rigorously examined for antiseptic properties. **Methods:** The antimicrobial activity of methanolic leaf extracts of *Backhousia citriodora* was investigated by disc diffusion and growth time course assays against a panel of bacteria and fungi. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *B. citriodora* leaf methanolic extract proved to be a versatile antibacterial agent inhibiting the growth of 12 of the 14 bacteria tested (86%). Only *C. fruendii* and *S. aureus* growth were unaffected by *B. citriodora* extract. Gram-positive and Gram-negative bacteria were both affected by *B. citriodora* extract although Gram-negative bacteria appeared more susceptible. The extract also displayed antifungal activity against a nystatin resistant strain of *Aspergillus niger* but did not affect *Candida albicans* or *Saccharomyces cerevisiae* growth. The antibacterial activity of *B. citriodora* extract was further investigated by growth time course assays which showed significant growth inhibition in cultures of *Bacillus cereus*, *Bacillus subtilis*, *Aeromonas hydrophilia* and *Pseudomonas fluorescens* within 1 h. The extract displayed low toxicity in the *Artemia franciscana* nauplii bioassay. **Conclusions:** The low toxicity of the methanolic extract and its inhibitory bioactivity against a panel of bacteria validate Australian Aboriginal usage of *B. citriodora* as an antiseptic agent and confirms its medicinal potential.

KEYWORDS: *Backhousia citriodora*, lemon myrtle, Australian plants, antibacterial activity, medicinal plants, methanol extracts

INTRODUCTION

Plants produce a wide variety of compounds which in addition to giving them characteristic pigment, odour and flavour characteristics, may also have antimicrobial properties.[1] For thousands of years, traditional plant derived medicines have been used in most parts of the world and their use in fighting microbial disease is becoming the focus of intense study.[2,3] Much of the research into traditional medicinal plant use has focused on Asian,[4] African[5] and South American[6] plants. Relatively few studies have focused on the antibacterial activity of Australian native plants, although recently there has been increased study in this field.

*Correspondence
Tel.: +61 7 37357637; fax: +61 7 37355282.
E-mail: I.Cock@griffith.edu.au
DOI: 10.5530/pc.2013.2.12

Backhousia citriodora (lemon myrtle) is an Australian plant, native to subtropical areas of eastern Australia. The leaves of this plant are widely used as a bush food and as a component of toiletries and cosmetics.[7] Studies in this laboratory[8] and elsewhere[9,10] have demonstrated the antibacterial activity of *B. citriodora* leaves. Interestingly, no reports of Aboriginal medicinal use of *B. citriodora* were found in the literature, although the leaves were used in cooking. Most of the studies of *B. citriodora* antibacterial potential focus on the essential oil of the leaves.[9,10] In most plants of this species, more than 90% of the oil is a mixture of neral (α-citral; Figure 1a) and geranial (β-citral; Figure 1b).[11] Both neral and geranial have been previously reported to have potent antibacterial activity against a variety of bacteria.[9,12,13]

The use of essential oils for the testing of antimicrobial activity is not without problems. The relative insolubility of many of the oil components retards their diffusion through agar gels in agar dilution or disc diffusion studies. Many studies have utilised solubilising agents (eg. Tween 80)

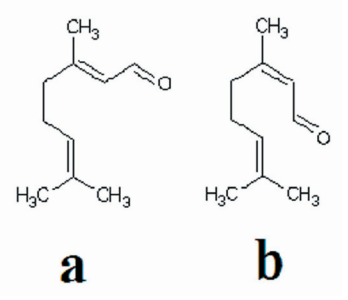

Figure 1. The chemical structures of (a) neral (α-citral) and (b) geranial (β-citral), the major oil components of *B. citriodora* leaf essential oils.

to aid oil component diffusion, resulting in variable results.[14,15] Solubilising agents appear to increase the susceptibility of some bacteria to antimicrobial agents, decrease the susceptibility of others, whilst having no effect on yet other bacteria. A recent study has demonstrated the antibacterial activity of methanolic extracts of *B. citriodora* leaves against a limited panel of bacteria.[8] The current study was undertaken to validate and extend these observations against a wider panel of bacteria and fungi.

MATERIALS AND METHODS

Plant collection and extraction

The extracts investigated in this study have been described previously.[8] Briefly, *Backhousia citriodora* leaves were collected from a verified tree on Logan campus of Griffith University, Australia. Leaf samples were dried in a Sunbeam food dehydrator and the dried material was ground to a coarse powder. 1 g of each of the powdered leaves was extracted extensively in 50 ml methanol (Ajax, AR grade) 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 in an Eppendorf concentrator 5301. The resultant pellet was dissolved in 15 ml 20% methanol to give a 15 mg/ml sample. The extract was passed through 0.22 μm filter (Sarstedt) and stored at 4°C until use.

Test microorganisms

All media was supplied by Oxoid Ltd. All microbial strains were obtained from Tarita Morais, Griffith University. Stock cultures of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Bacillus cereus*, *Bacillus subtilis*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeuroginosa*, *Pseudomonas fluorescens*, *Salmonella salford*, *Serratia marcescens*, *Staphylococcus aureus* and *Yersinia enterocolitia* were subcultured and maintained in nutrient broth at 4°C. *Aspergillus niger*, *Candida albicans*, and *Saccharomyces cerevisiae* were maintained in Sabouraud media at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of *B. citriodora* leaf extract was determined using a modified Kirby-Bauer disc diffusion method.[16,17] Briefly, 100 μl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria, or 10^5 cells/ml for fungi. 100 μl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

The extract was tested using 6 mm sterilised filter paper discs. Discs were impregnated with 10 μl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Alcaligenes feacalis*, *Aeromonas hydrophilia*, *Bacillus cereus*, *Bacillus subtilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Pseudomonas aeuroginosa, Pseudomonas fluorescens*, *Serratia marcescens*, *Yersinia enterocolitia*, *Candida albicans* and *Saccharomyces cerevisiae* were incubated at 30°C for 24 hours, and then the diameters of the inhibition zones were measured in millimetres. Plates inoculated with *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella Salford and Staphylococcus aureus* were incubated at 37°C for 24 hours, and then the diameters of the inhibition zones were measured. *Aspergillus niger* inoculated plates were incubated at 25°C for 48 hours then the zones of inhibition were measured. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate. Mean values are reported in this report. Standard discs of ampicillin (2 μg), chloramphenicol (10 μg) or ciprofloxacin (2.5 μg) were obtained from Oxoid Ltd. and served as positive controls for antimicrobial activity. For fungi, nystatin discs (100 μg, Oxoid Ltd.) were used as a positive control. Filter discs impregnated with 10 μl of distilled water were used as a negative control.

Bacterial growth time course assay

Bacterial growth time course studies were performed as previously described.[18–20] Briefly, 3 ml of bacterial cultures (*Bacillus cereus*, *Bacillus subtilis, Pseudomonas fluorescens, Aeromonas hydrophilia*) in nutrient broth were added to 27 ml nutrient broth containing 3 ml *B. citriodora* extract (diluted 1 in 100 in sterile deionised water). The tubes were incubated at 30°C with gentle shaking. The optical density was measured at 550 nm after 0, 1, 2, 4 and 6 h incubations. Control tubes were incubated under the same conditions but without the extract. All assays were performed in triplicate.

Toxicity screening

Reference toxins for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in deionised water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay. Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) was obtained from Sigma-Aldrich as a mixture of cis (76.6%) and trans (23.0%) isomers and prepared as a 4 mg/ml stock in distilled water. The stock was serially diluted in artificial seawater for use in the bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.[21–23] *Artemia franciscana* Kellogg cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/l distilled water were prepared prior to use. 2 g of *A. franciscana* cysts were incubated in 1 L synthetic seawater under artificial light at 25°C, 2000 Lux with continuous aeration. Hatching commenced within 16–18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. Seawater (400 µl) containing approximately 39 (mean 39.2, n = 124, SD 18.6) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted to 5 mg/ml in seawater for toxicity testing, resulting in a 2.5 mg/ml concentration in the bioassay. 400 µl of diluted plant extracts and the reference toxins were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µl seawater) was run in at least triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 72 h all nauplii were sacrificed and counted to determine the total number per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.

RESULTS AND DISCUSSION

B. citriodora leaf extract was diluted to a 15 mg/ml concentration and 10 µl was tested in the disc diffusion assay against 17 microorganisms (Table 1). The extract had broad antibacterial activity, being capable of inhibiting the growth of 12 of the 14 bacteria tested (86%). Only *C. fruendii* and *S. aureus* were unaffected by *B. citriodora* extract. However, the antibacterial activity was not particularly strong against any of the bacteria tested (as determined by the diameter of the zone of inhibition) compared to the inhibition of the antibiotic controls.

Table 1: Antibacterial activity of *B. citriodora* extract

Microbial species	Mean zone of inhibition ± SD (mm)	
	Antibiotic	*B. citriodora* extract
Gram negative rods		
Aeromonas hydrophilia	17.3 ± 0.6 (Chl)	8.3 ± 0.6
Alcaligenes faecalis	13.3 ± 0.6 (Amp)	7.7 ± 1.2
Citrobacter fruendii	23.0 ± 1.0 (Chl)	–
Enterobacter aerogenes	17.3 ± 0.3 (Chl)	7.0 ± 0
Escherichia coli	16.7 ± 0.6 (Amp)	6.0 ± 0
Klebsiella pneumoniae	18.3 ± 0.6 (Amp)	7.0 ± 0
Pseudomonas aeuroginosa	31.6 ± 0.3 (Cip)	7.6 ± 0.3
Pseudomonas fluorescens	21.0 ± 0 (Chl)	7.6 ± 0.3
Salmonella salford	25.3 ± 0.3 (Amp)	7.6 ± 0.3
Serratia marcescens	25.7 ± 0.6 (Chl)	6.3 ± 0.3
Yersinia enterocolitia	16.3 ± 0.3 (Amp)	7.3 ± 0.3
Gram positive rods		
Bacillus cereus	25.3 ± 0.6 (Chl)	7.6 ± 0.3
Bacillus subtilis	22.7 ± 0.6 (Amp)	7.6 ± 0.3
Gram positive cocci		
Staphylococcus aureus	16.3 ± 0.3 (Amp)	–
Fungi		
Aspergillus niger	18.0 ± 0 (Cip)	8.6 ± 0.3
Candida albicans	25.7 ± 0.6 (Nys)	–
Yeast		
Saccharomyces cerevisiae	21.3 ± 0.6 (Nys)	–

Numbers indicate the mean diameters of inhibition of triplicate experiments ± standard deviation. – indicates no growth inhibition. Amp indicates ampicillin (2 µg). Chl indicates chloramphenicol (10 µg). Cip indicates ciprofloxacin (2.5 µg). Nys indicates nystatin (100 µg).

Both Gram-positive and Gram-negative bacteria were affected by *B. citriodora* extract although Gram-negative bacteria appeared to be more susceptible. Of the 11 Gram-negative bacteria tested, 10 (91%) were inhibited by *B. citriodora* leaf extract. The leaf extract inhibited the growth of 2 of the 3 Gram-positive bacteria tested (67%). This is in agreement with a previous report of the antibacterial activity of essential oil from *B. citriodora* which also reported the susceptibility of both Gram-positive and Gram-negative bacteria to an essential oil from *B. citriodora*.[9] Other studies have shown Gram-negative bacteria to be more susceptible to other Australian plant

extracts.[24–26] However, this is in contrast to previous inhibition results reported for other plant extracts. A greater susceptibility of Gram-positive bacteria has been previously reported for South American,[27] African[28,29] and Australian[30] plant extracts. Results within this laboratory have also confirmed the greater susceptibility of Gram-positive bacteria towards other Australian plant extracts.[8,31–35] The Gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including antibiotics.[36] Due to the susceptibility of Gram-negative bacteria to *B. Citriodora* leaf extract, it appears that the uptake of the *B. citriodora* extract antibiotic compounds are presumably not affected by the cell wall outer membrane.

The *B. citriodora* extract also demonstrated limited antifungal activity. The extract inhibited the growth of a nystatin resistant strain of *A. niger* but was unable to inhibit *C. albicans* growth. This is an important result as this strain of *A. niger* was resistant to all other antimicrobial agents tested except ciprofloxacin. The only yeast tested in these studies (*S. cerevisiae*), was not inhibited by the *B. citriodora* extract.

The antibacterial activity of the *B. citriodora* extract was further investigated by bacterial growth time course assays in the presence and absence of the extract. The concentration of the extract used in these assays was 13.6 µg/ml. *B. citriodora* extract was able to significantly inhibit *Bacillus cereus* (Figure 2a), *Bacillus subtilis* (Figure 2b), *Pseudomonas fluorescens* (Figure 2c) and *Aeromonas hydrophilia* (Figure 2d) growth within 1 h for all bacteria tested indicating a rapid antimicrobial action.

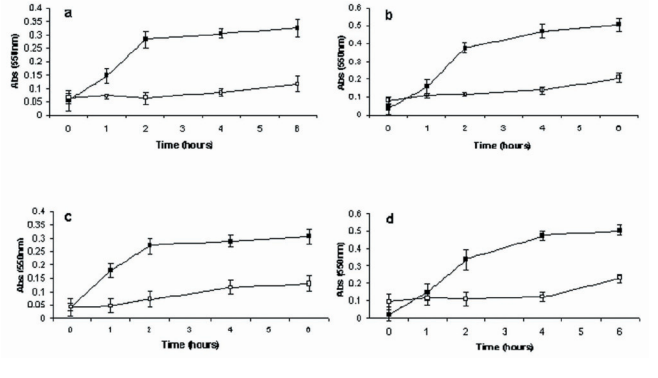

Figure 2. Inhibition of bacterial growth by *B. citriodora* leaf methanolic extract against (a) *B. cereus*, (b) *B. subtilis*, (c) *P. fluorescens*, (d) *A. hydrophilia*. For all graphs, □ represent measured bacterial growth values for test cultures (with extract) and ■ represent control bacterial growth values (no extract). All bioassays were performed in at least triplicate and are expressed as mean ± standard deviation.

The toxicity of the *B. citriodora* extract was tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000 µg/ml (Figure 3). The *B. citriodora* leaf extract induced low levels of mortality at 24 and 48 h (Figure 3a), similar to the % mortality seen for the seawater control (Figure 3d). However, exposure to *B. citriodora* leaf extract induced elevated mortality by 72 h compared to that of the seawater control, although even these results indicate a low level of toxicity. In contrast, both positive controls induced mortality within 24 h, with 100% mortality induction seen by 36 h.

To quantify the toxicity of the extract, LC_{50} values were determined by testing across the concentration range 2000 µg/ml to 10 µg/ml in the *Artemia franciscana* nauplii bioassay (Table 2). For comparison, serial dilutions of potassium dichromate and Mevinphos were also tested. No LC_{50} values are reported for the *B. citriodora* leaf

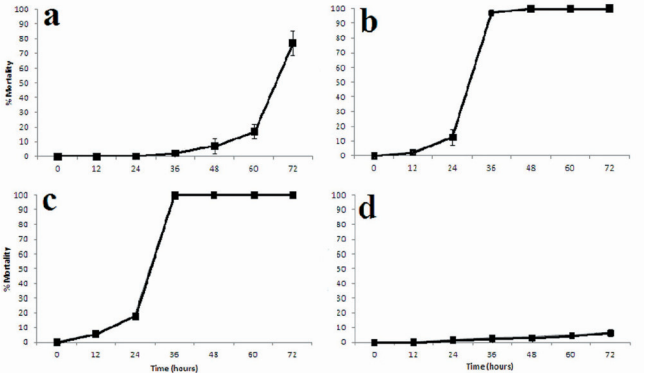

Figure 3. Brine shrimp lethality of (a) *B. citriodora* leaf methanolic extract (2000 µg/ml), (b) potassium dichromate (800 µg/ml), (c) Mevinphos (2000 µg/ml) and (d) seawater control. All bioassays were performed in at least triplicate and are expressed as mean ± standard error.

Table 2: LC_{50} (95% confidence interval) for brine shrimp nauplii exposed to *B. citriodora* leaf methanolic extract, the reference toxins potassium dichromate and Mevinphos

Plant species	Plant part tested	LC_{50} (µg/ml)		
		24 h	48 h	72 h
B. citriodora	leaves	NA	NA	1428 ± 92
Mevinphos		1418 ± 172	546 ± 45	123 ± 18
Potassium Dichromate		–	82 ± 4	79 ± 5

NA indicates that LC_{50} values were not obtained as ≥ 50% mortality was not reached for this time point. Results represent the mean ± standard deviation of triplicate determinations.

extract at 24 or 48 h as no significant increase in mortality above the seawater controls was seen for these extracts at any time tested, indicating that the extract is non-toxic. The extract does display low toxicity at 72 h with an LC_{50} value of 1428 ± 92. As LC_{50} values ≥ 1000 µg/ml are defined as non-toxic,[37] the *B. citriodora* leaf extract was classed as non-toxic.

In conclusion, the findings of this study have established the susceptibilities of a broad range of microbes to *B. citriodora* leaf methanolic extract. Both Gram-positive and Gram-negative were equally susceptible. The broad range of microbial susceptibilities indicates the potential of *B. citriodora* leaf extract as a surface disinfectant as well as for medicinal purposes and as food additives to inhibit spoilage. However, further studies are needed before these extracts can be applied to these purposes. In particular, further toxicity studies using human cells are needed to determine the suitability of these extracts for use as antiseptic agents and as a food additive. Currently there are conflicting reports on the toxicity of *B. citriodora* components towards mammalian cells. One study reported no toxicity of low concentrations of *B. citriodora* essential oil towards HeLa and Hep2 cell lines.[9] However, a different study found a toxic effect towards F1-73 and HepG2 cells as well as skin fibroblasts.[38] Further studies are needed to determine the toxicity of *B. citriodora* leaf components.

ACKNOWLEDGEMENTS

Financial support for this work was provided by the School of Biomolecular and Physical Sciences, Griffith University, Australia.

REFERENCES

1. Cowan MM. Plant products as antibacterial agents. Clinical Microbiology Reviews. 1999; 12:564–82.
2. Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria. Current Opinion in Microbiology. 2000; 3:528–34.
3. Chiariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BP. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. Journal of Ethnopharmacology. 1999; 64: 265–70.
4. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: a comparative overview. Evidence-based Complimentary and Alternative Medicine. 2005; 2:465–73.
5. Hostettmann K, Marston A, Ndjoko K, Wolfender J. The potential of African plants as a source of drugs. Current Organic Chemistry. 2000; 4: 973–1010.
6. Paz EA, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, Vazquez A, Vero S, Zunino L. Screening of Uruguayan medicinal plants for antimicrobial activity. Journal of Ethnopharmacology. 1995; 45:67–70.
7. Hegarty MP, Hegarty EE, Wills RBH. Food safety of Australian bush foods, Rural Industries Research and Development Corp. 2001; Kingston, ACT, Australia.
8. Cock IE. Antibacterial activity of selected Australian plant species. The Internet Journal of Microbiology. 2008; 6:2.
9. Wilkinson JM, Hipwell M, Ryan T, Cavanagh HMA. Bioactivity of *Backhousia citriodora*: antibacterial and antifungal activity, Journal of Agriculture and Food Chemistry. 2003; 51(1):76–81.
10. Ryan T, Cavanagh HMA, Wilkinson JM. Antimicrobial activity of *Backhousia citriodora* oil. Simply Essential. 2000; 38:6–8.
11. Opdyke DLJ. Citral. Monographs on fragrance raw materials. Food and Cosmetics Technology. 1976; 14:615.
12. Inouye S, Takizawa T, Yamaguchi H. Antimicrobial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. Journal of Antimicrobial Chemotherapy. 2001; 47:565–73.
13. Kim JM, Marshall MR, Cornell JA, Preston JF, Wei CI. Antibacterial activity of carvacrol, citral and geraniol against *Salmonella typhimurium* in culture media and in fish cubes. Journal of Food Science. 1995; 60(6):1364–8.
14. Griffin SG, Markham JL, Leach DN. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. Journal of Essential Oil Research. 2000; 12:249–55.
15. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 1999; 86:985–90.
16. Cock IE. Antibacterial activity of *Allocasuarina littoralis* methanolic leaf extracts. The Internet Journal of Microbiology. 2008; 5:2.
17. Cock IE. Antibacterial activity of *Aloe barbadensis* Miller leaf gel components. The Internet Journal of Microbiology. 2008; 4:2.
18. Cock IE. Antimicrobial activity of *Acacia aulacocarpa* and *Acacia complanta* methanolic extracts. Pharmacognosy Communications. 2012; 2(1):66–71.
19. Cock IE. Antimicrobial activity of *Syzygium australe* and *Syzygium leuhmannii* methanolic extracts. Pharmacognosy Communications. 2012; 2(2):71–7.
20. Cock IE. Antimicrobial activity of *Callistemon citrinus* and *Callistemon salignus* methanolic extracts. Pharmacognosy Communications. 2012; 2(3):50–7.
21. Mpala L, Chikowe G, Cock IE. No evidence of antiseptic properties and low toxicity of selected Aloe species, Journal of Pharmaceutical Negative Results. 2010; 1(1):10–6.
22. Cock IE, Ruebhart DR. Comparison of the brine shrimp nauplii bioassay and the ToxScreen-II test for the detection of toxicity associated with Aloe vera (*Aloe barbadensis* Miller) leaf extract. Pharmacognosy Research. 2009; 1(2):102–8.
23. Cock IE, Ruebhart, DR. Assessment of the toxicity of selected Australian native plant extracts using the *Artemia franciscana* nauplii bioassay. The Internet Journal of Toxicology. 2008; 5, 2.
24. Vesoul J, Cock IE. The potential of Bunya nut as an antibacterial food agent. Pharmacognosy Communications. 2012; 2(1):74–82.
25. Vesoul J, Cock IE. An examination of the medicinal potential of *Pittosporum phylloraeoides*: Toxicity, antibacterial and antifungal activities. Pharmacognosy Communications. 2011; 1(2):8–17.
26. Cock IE, Kukkonen L. An examination of the medicinal potential of *Scaevola spinescens*: Toxicity, antibacterial, and antiviral activities. Pharmacognosy Research. 2011; 3(2):85–94.
27. Paz EA, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, Vazquez A, Vero S, Zunino L. Screening of Uruguayan medicinal plants for antimicrobial activity. Journal of Ethnopharmacology. 1995; 45:67–70.
28. Kudi AC, Umoh JU, Eduvie LO, Gefu J. Screening of some Nigerian medicinal plants for antibacterial activity. Journal of Ethnopharmacology. 1999; 67:225–8.
29. Vlietinck AJ, van Hoof L, Totte J, Lasure A, Vanden Berghe D, Rwangabo PC, Mvukiyumwani J. Screening of a hundred Rwandese medicinal plants for antimicrobial and antiviral properties. Journal of Ethnopharmacology. 1995; 46:31–47.
30. Palombo EA, Semple SJ. Antibacterial activity of traditional Australian medicinal plants. Journal of Ethnopharmacology. 2001; 77:151–7.
31. Kalt FR, Cock IE. The medicinal potential of Australian native plants from Toohey Forest, Australia. South Pacific Journal of Natural Sciences. 2011; 28:41–7.
32. Cock IE, Mohanty S. Evaluation of the antibacterial activity and toxicity or *Terminalia ferdinandia* fruit extracts. Pharmacognosy Journal. 2011; 3(20):72–9.

33. Cock IE. Antibacterial and Antifungal Activity of *Buckinghamia celsissima* leaf extracts. The Internet Journal of Microbiology. 2009; 6, 2.
34. Cock IE. Antibacterial and antifungal activity of *Eucalyptus major and Eucalyptus baileyana* methanolic extracts. The Internet Journal of Microbiology. 2009; 6, 1.
35. Mohanty S, Cock IE. Bioactivity of *Syzygium jambos* methanolic extracts: Antibacterial activity and toxicity. Pharmacognosy Research. 2009; 2 (1):4–9.
36. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction 2001; Benjamin Cummings, San Francisco.
37. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica. 1982; 45:31–4.
38. Hayes AJ, Markovic B. Toxicity of Australian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. Food Chemistry Toxicology. 2002; 40:535–43.