Assessment of the toxicity of selected Australian native plant extracts using the Artemia franciscana nauplii bioassay

I Cock

Citation

I Cock. *Assessment of the toxicity of selected Australian native plant extracts using the Artemia franciscana nauplii bioassay*. The Internet Journal of Toxicology. 2007 Volume 5 Number 2.

Abstract

Thirty nine methanolic extracts from twenty five Australian native plants were investigated for toxicity using the Artemia franciscana nauplii lethality bioassay and compared to the reference toxins potassium dichromate and Mevinphos. 7 extracts (18 %) showed marked lethality towards Artemia franciscana nauplii at 24 h, 11 extracts (28 %) at 48 h and 19 extracts (49 %) at 72 h. Of the positive controls, only Mevinphos displayed significant lethality at 24 h. Potassium dichromate treatment resulted in only approximately 10 % mortality at 24 h but induced 100% mortality by 48 h. Of the non-toxic extracts, A. aulacocarpa leaf, L. bracteata leaf, L. juniperium leaf and flower, S. australe leaf and B. celsissima leaf extracts have previously been shown to be good antibacterial agents, confirming their potential for antibiotic usage.

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

INTRODUCTION

Plants have long been recognised as a valuable source of medicines for treating a variety of different diseases and complaints. Most, if not all civilisations, have used plants as medicines. The use of plant natural therapeutics in Asia is wide spread, being used in the treatment of numerous disorders including eczema, malaria and respiratory disorders [1]. Africa also has a long history of medicinal plant use. For example, Phytolacca dodecandra is used as a moluscicide in the control of schistosomiasis [2]. The antitumour agent's vinblastine and vincristine (derived from Catharanthus roseus) are currently used in the treatment of a variety of tumours [3; 4]. Europe and the Americas also have a history of medicinal plant use. Studies demonstrate the myriad of medicinal plant uses by indigenous North and Central Americans [5] and South Americans [6]. Approximately 1500 medicinal plant species are currently in use in Europe [1].

As a result of its isolation, Australia has a variety of unique and distinctive flora not found elsewhere in the world. Australian Aborigines used a variety of plant medicines to help maintain their health prior to European settlement [7; 8]. More than 150 plants from nearly 60 widely varied botanical families were used by Australian Aborigines as antiseptic agents [8]. Although there is enormous potential for the development of medicinal agents from Australian plants, much of our knowledge of the antimicrobial nature of Australian plants is anecdotal. Recent studies [9; 10; 11] have demonstrated the antibacterial and antifungal activity of extracts from a wide variety of Australian plants.

To be medically useful as antimicrobial agents, plant preparations should be non-toxic or of low toxicity to human cells [12]. Limited information is available on the toxicity of antibacterial preparations from Australian plants. Recent studies have reported on the low toxicity of Backhousia citriodora essential oils [13] and on the toxicity of extracts from a variety of Australian plants [10] towards human cell lines. A study from this laboratory has indicated the ability of several Australian plants to act as antimicrobial agents [11]. To further assess their potential it is necessary to assess their toxicity. The Artemia franciscana nauplii (brine shrimp larvae) lethality bioassay was used in the current study. This assay has been used to examine the toxicity of a wide variety of compounds [14]. It is an efficient, inexpensive and relatively rapid way to detect toxic compounds, requiring only low amounts of sample (<20 mg). This test correlates well with cytotoxic activity of some human tumours and therefore has the potential to detect new antitumour agents

[15].

MATERIALS AND METHODS

PLANT MATERIAL

COLLECTION OF PLANT SAMPLES

Plant samples were as previously described [11]. Briefly, Acacia aulacocarpa (leaves), Acacia complanta (leaves and flowers), Allocasuarina littoralis (leaves), Astrotricha longifolia (leaves and flowers), Banksia colina (leaves), Eucalyptus baileyana (leaves), Eucalyptus major (leaves and flowers), Jacksonia scoparia (leaves), Leptospermum juniperium (leaves and flowers), Melaleuca quinquenervia (leaves) and Mirbelia oxylobiodes (leaves and flowers) were collected from Toohey Forest, Brisbane and were identified with reference to a taxonomic key to Toohey Forest plants [16]. Backhousia citriodora (leaves), Grevillea robusta (leaves and flowers) and Macadamia integriflora (leaves and flowers) were collected from verified trees on Logan campus of Griffith University, Australia. Adansonia gregorii (leaves and flowers), Brachychiton acerifolius (leaves and flowers), Buckinghamia celsissima (leaves), Callistemon citrinus (leaves and flowers), Callistemon salignus (leaves and flowers), Davidsonia pruriens var. jerseyana (fruit), Grevillea juncifolia (leaves and flowers), Leptospermum bracteata (leaves and flowers), Syzygium australe (leaves), Syzygium leuhmannii (leaves) and Westringa fruticosa (leaves and flowers) were collected from verified trees in the suburbs of Brisbane, Australia.

PREPARATION OF CRUDE EXTRACTS

Plant samples were dried in a Sunbeam food dehydrator and the dried material was ground to a coarse powder. 1 g of each of the samples of dried plant material was extracted extensively in 50 ml methanol (Ajax, AR grade) for 24 hours at 4 oC 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. The extract was passed through 0.22 µm filter (Sarstedt) and stored at 4 oC.

REFERENCE TOXINS FOR BIOLOGICAL SCREENING

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in distilled 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 the Artemia franciscana nauplii lethality assay developed by Meyer et al [14] for the screening of active plant constituents with the following modifications. 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 25oC, 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. 400 µl of seawater containing approximately 50 (mean 53, n = 268, SD 12) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted to 2 mg/ml in seawater for toxicity testing, resulting in a 1 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 ± 1oC 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 [17].

RESULTS

Thirty nine samples from twenty five Australian native plant species were extracted in methanol and dried as previously described [11]. The weight of the dried extracted material is recorded in table 1. The weight of dried extractable material varied across samples, ranging from 83 mg (L. juniperium flowers) extracted per 1 g starting plant material up to 567 g

(C. citrinus flowers) from the original 1 g of ground dried plant material. All extracts were resuspended in 15 ml of 20 % methanol, resulting in the crude test extract concentrations reported in table 1.

Figure 1

Table 1: Botanical names of plant species extracted, weight of dried extractable material and the concentrations of each extract.

Plant species	Plant part extracted	Dried extract (mg)	Extract conc. (mg/ml)
Acacia aulacocarpa	leaves	212	14.1
Acacia complanta	leaves	234	15.6
Acacia complanta	flowers	374	24.9
Adansonia gregorii	leaves	99	6.6
Adansonia gregorii	flowers	115	7.7
Allocasuarina littoralis	leaves	376	25.1
Astrotricha longifolia	leaves	223	14.9
Astrotricha longifolia	flowers	384	25.6
Backhousia citriodora	leaves	235	15.7
Banksia collina	leaves	299	19.9
Brachychiton acerifolius	leaves	409	27.3
Brachychiton acerifolius	flowers	105	7.0
Buckinghamia celsissima	leaves	395	26.3
Callistemon citrinus	leaves	561	37.4
Callistemon citrinus	flowers	567	37.8
Callistemon salignus	leaves	539	35.9
Callistemon salignus	flowers	525	35
Davidsonia pruriens var. jerseyana	fruit	362	24.1
Eucalyptus baileyana	leaves	218	14.5
Eucalyptus major	leaves	427	28.5
Eucalyptus major	flowers	533	35.5
Grevillea juncifolia	leaves	164	10.9
Grevillea juncifolia	flowers	334	22.3
Grevillea robusta	leaves	378	25.2
Grevillea robusta	flowers	312	20.8
Jacksonia scoparia	leaves	442	29.5
Leptospermum bracteata	leaves	192	12.8
Leptospermum bracteata	flowers	274	18.3
Leptospermum juniperium	leaves	246	16.4
Leptospermum juniperium	flowers	83	5.5
Macadamia integriflora	leaves	151	10.1
Macadamia integriflora	flowers	183	12.2
Melaleuca quinquenervia	leaves	355	23.7
Mirbelia oxylobioides	leaves	306	20.4
Mirbelia oxylobioides	flowers	314	20.9
Syzygium australe	leaves	402	26.8
Syzygium leuhmannii	leaves	122	8.1
Westringa fruticosa	leaves	418	27.9
Westringa fruticosa	flowers	425	28.3

All extracts were diluted to 2000 µg/ml in artificial seawater for toxicity testing, resulting in a 1000 µg/ml concentration in the Artemia franciscana lethality bioassay. The results of A. franciscana bioassay screening of the Australian plant methanolic extracts are shown in table 2. Previous reports [18; 19] express LC_{50} values of toxins following 24 h of exposure. Of the 39 extracts tested, 7 (18 %) showed greater than 50 % mortality at 24 h. Of these, A. complanta flowers, A. littoralis leaves, E. baileyana leaves and E. major flowers have been previously shown to have significant antibacterial activity [11]. Of the positive controls, only Mevinphos displayed significant mortality at 24 h. Potassium dichromate treatment resulted in only approximately 10 % mortality at 24 h. Therefore, mortality at later times (48 and 72 h) are also reported here.

An extra four extracts showed greater than 50 % mortality at 48 h compared to 24 h (B. acerifolius leaves, E. major leaves, G. juncifolia flowers and G. robusta leaves). This equates to approximately 28% of the tested extracts showing toxicity at 48 h. Of these, only E. major flowers have been previously shown to have significant antibacterial activity [11]. At 72 h, 8 further extracts (compared to 48 h) induced greater than 50 % mortality. Of these, B. citriodora, C. citrinus leaves, C. salignus leaves and flowers, L. bracteata flowers, and S. leuhmannii leaves have been previously shown to have significant antibacterial activity [11].

Figure 2

Table 2: Toxicity of Australian plant extracts (1 mg/ml) to . Numbers indicate the mean % mortality of at least triplicate experiments ± standard deviation.

Plant species	Plant part extracted	24 h	48 h	72 h
Acacia aulacocarpa	leaves	0 ± 0	1.3 ± 2.2	23.4 ± 7.6
Acacia complanta	leaves	1.0 ± 1.7	3.2 ± 3.0	6.5 ± 5.6
Acacia complanta	flowers	89.2 ± 10.7	87.4 ± 15.8	89.9 ±17.5
Adansonia gregorii	leaves	0 ± 0	6.5 ± 5.7	26.9 ± 8.3
Adansonia gregorii	flowers	0 ± 0	0 ± 0	1.6 ± 2.7
Allocasuarina littoralis	leaves	68.4 ±12.3	97.8 ± 2.2	100.0 ± 0
Astrotricha longifolia	leaves	0 ± 0	2.2 ± 1.9	7.7 ± 6.8
Astrotricha longifolia	flowers	0 ± 0	0.8 ± 1.4	6.1 ± 3.3
Backhousia citriodora	leaves	0 ± 0	0 ± 0	85.8 ± 6.4
Banksia collina	leaves	0 ± 0	0 ± 0	5.5 ± 4.9
Brachychiton acerifolius	leaves	0 ± 0	5.6 ± 6.3	89.3 ± 2.4
Brachychiton acerifolius	flowers	29.8 ± 13.1	90.7 ± 1.1	100.0 ± 0
Buckinghamia celsissima	leaves	0 ± 0	4.0 ± 3.9	12.1 ± 6.2
Callistemon citrinus	leaves	0 ± 0	0 ± 0	59.4 ± 5.5
Callistemon citrinus	flowers	0 ± 0	4.2 ± 4.6	26.7 ± 5.0
Callistemon salignus	leaves	0 ± 0	3.6 ± 3.7	84.8 ± 7.8
Callistemon salignus	flowers	0 ± 0	0 ± 0	98.8 ± 3.3
Davidsonia pruriens var. jerseyana	fruit	8.2 ± 4.3	45.3 ± 6.4	92.5 ± 6.7
Eucalyptus baileyana	leaves	100.0 ± 0	100.0 ± 0	100.0 ± 0
Eucalyptus major	leaves	13.9 ± 6.5	78.5 ± 8.6	98.9 ± 1.9
Eucalyptus major	flowers	86.9 ± 10.5	100.0 ± 0	100.0 ± 0
Grevillea juncifolia	leaves	75.2 ± 12.4	100.0 ± 0	100.0 ± 0
Grevillea juncifolia	flowers	12.1 ± 5.6	98.1 ± 3.2	100.0 ± 0
Grevillea robusta	leaves	13.6 ± 7.7	98.4 ± 2.7	100.0 ± 0
Grevillea robusta	flowers	1.9 ± 3.2	1.9 ± 3.2	1.9 ± 3.2
Jacksonia scoparia	leaves	0 ± 0	0 ± 0	9.6 ± 4.2
Leptospermum bracteata	leaves	0 ± 0	0 ± 0	14.0 ± 2.8
Leptospermum bracteata	flowers	0 ± 0	1.8 ±3.0	97.0 ± 2.6
Leptospermum juniperium	leaves	0 ± 0	3.5 ± 3.1	17.6 ± 5.1
Leptospermum juniperium	flowers	0 ± 0	1.0 ± 1.8	4.4 ± 4.7
Macadamia integriflora	leaves	0 ± 0	0 ± 0	0 ± 0
Macadamia integriflora	flowers	83.3 ± 4.4	98.6 ± 2.5	100.0 ± 0
Melaleuca quinquenervia	leaves	57.9 ± 4.8	90.2 ± 4.1	95.3 ± 1.8
Mirbelia oxylobioides	leaves	3.0 ± 2.6	6.1 ± 10.5	31.3 ± 16.3
Mirbelia oxylobioides	flowers	3.6 ± 3.5	9.1 ± 6.1	34.2 ± 12.4
Syzygium australe	leaves	2.0 ± 3.4	8.5 ± 3.9	13.0 ± 5.5
Syzygium leuhmannii	leaves	0 ± 0	11.0 ± 5.1	56.8 ± 8.2
Westringa fruticosa	leaves	0 ± 0	6.4 ± 5.7	9.1 ± 4.3
Westringa fruticosa	flowers	0 ± 0	0 ± 0	2.7 ± 2.4
Potassium dichromate		10.2 ± 1.3	100.0 ± 0	100.0 ± 0
Mevinphos		90.1 ± 4.8	98.3 ± 0.1	100.0 ± 0
seawater control		0 ± 0	0 ± 0	0 ± 0

Ten plant extracts induced greater than 50% mortality by 48 h (A. complanta leaves, A. littoralis leaves, Brachychiton acerifolius flowers, Eucalyptus baileyana leaves, Eucalyptus

major leaves and flowers, Grevillea juncifolia leaves and flowers, Macadamia integriflora flowers and Melaleuca quinquenervia leaves). These were considered sufficiently toxic to warrant further investigation to determine the dependence of toxicity on the concentration of the extract the A. franciscana is exposed to. Table 3 shows the LC_{50} values of these extracts towards A. franciscana. E. baileyana leaf extract was the most toxic of the plant extracts tested with 24, 48 and 72 h LC_{50} values of 216 µg/ml (± 22). Macadamia integriflora, whilst taking longer to induce mortality, proved to be similarly toxic with 48 h and 72 h LC_{50} values almost identical to those of E. baileyana leaf extract.

Figure 3

Table 3: LC (95% confidence interval) for brine shrimp nauplii exposed to Australian plant extracts and the reference toxins Mevinphos and potassium dichromate.

Plant species	Plant part extracted	LC50 value in µg/ml		
		24 h	48 h	72 h
Acacia complanta	leaves	795 ± 80	785 ± 71	375 ± 41
Allocasuarina littoralis	leaves	892 ± 112	591 ± 71	580 ± 76
Brachychiton acerifolius	flowers	-	757 ± 85	716 ± 81
Eucalyptus baileyana	leaves	216 ± 22	216 ± 22	216 ± 22
Eucalyptus major	leaves	-	858 ± 124	724 ± 106
Eucalyptus major	flowers	762 ± 98	386 ± 49	375 ± 52
Grevillea juncifolia	leaves	845 ± 87	739 ± 88	716 ± 93
Grevillea juncifolia	flowers	-	716 ± 98	671 ± 83
Macadamia integriflora	flowers	809 ± 72	219 ± 33	218 ± 23
Melaleuca quinquenervia	leaves	940 ± 141	739 ± 93	705 ± 97
Mevinphos		1418 ± 172	546 ± 45	123 ± 18
Potassium Dichromate		-	82 ± 4	79 ± 5

- indicates that LC_{50} values were unable to be obtained as no increase in mortality above seawater controls was evident.

DISCUSSION

A previous study from this laboratory [11] has reported on the antibacterial activity of methanolic extracts from some Australian native plants. That study indicated that A. aulacocarpa, B. citriodora, B. celsissima, A. littoralis, as well as members of the Callistemon, Eucalyptus, Leptospermum and Syzygium genuses are particularly promising as antimicrobial agents. The current study reports on the toxicity of these and other Australian plant extracts. A. aulacocarpa leaf extract was previously reported to be a good antimicrobial agent [11], being capable of inhibiting the growth of both Gram-positive and Gram-negative bacteria. The current studies indicate this extract has low toxicity towards A. franciscana. The Artemia franciscana bioassay has been reported to be a good indication of toxicity towards human cells [14]. These results indicate that A. aulacocarpa leaf extract has potential for medicinal use as an antibiotic agent.

Likewise, studies within this laboratory [11] have also shown L. bracteata leaves, L. juniperium leaves and flowers and S. australe leaves to be versatile antibacterial agents towards Gram-positive and Gram-negative bacteria. B. celsissima leaves were also good antibacterial agents, preferentially inhibiting Gram-positive bacteria. All of these extracts showed low toxicity in the Artemia bioassay, indicating their potential as antibiotic agents.

B. citriodora, A. littoralis, C. citrinus, C. salignus, E. baileyana and E. major extracts have all also been reported to have good antibacterial activity [11]. The current study shows that all of these extracts were toxic towards A. franciscana. The B. citriodora toxicity results contrast with the low toxicity previously reported [10]. However, it is worth noting that the current study examined the toxicity of higher extract concentrations (1000 µg/ml) compared to the Setzer et al [10] report (250 µg/ml), which studied the toxicity of B. citriodora extracts towards human cell lines as well as towards brine shrimp. Similarly, it is worth noting that toxicity was only seen in the current studies following 72 h of exposure of A. franciscana to B. citriodora extract. No mention of Artemia exposure time is made in the previous study of B. citriodora toxicity [10]. However, 24 h and 48 h LC_{50}'s have been reported for other plant extracts in many previous studies [14; 15]. It is therefore likely that these authors recorded mortality following a shorter exposure time than reported here. These differences highlight the necessity to further evaluate the toxicity of these extracts against human cell lines. Extracts that show toxicity towards A. franciscana at the concentrations tested, may have low toxicity towards human cell lines and therefore may have potential as antibiotic agents. Even if this is not the case, these extracts still have potential value as antiseptic and cleaning agents.

Toxicity towards A. franciscana could also indicate other potential medicinal uses for these plant extracts. The A. franciscana bioassay has previously been shown to be a good indicator of antitumour activity [15]. Therefore, extracts toxic towards A. franciscana should also be tested for toxicity towards human tumour cell lines. Whilst the extracts examined in this report appear promising as antimicrobial agents and possibly as anti-tumour agents, further studies using human cell lines are needed to determine the suitability of these extracts for these purposes.

ACKNOWLEDGEMENTS

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

References

1. Hoareau L, DaSilva EJ, 1999, Medicinal plants: a re-emerging health aid, Electronic Journal of Biotechnology, 2, 2, 56-70.
2. Lemma A, 1991, The potentials and challenges of endod, the Ethiopian soapberry plant for the control of schistosomiasis, Science in Africa: Achievements and Prospects, American Association for the Advancement of Sciences (AAAS), Washington DC, USA.
3. Sersa G, Krzic M, Sentjurc M, Ivanusa T, Beravs K, Cemazar M, Auersperg M, Swartz HM, 2001, Reduced tumor oxygenation by treatment with vinblastine, Cancer Research, 61, 10, 4266-4271.
4. Reich S, Overberg-Schmidt US, Bührer C, Henze G, 1999, Low-dose chemotherapy with vinblastine and methotrexate in childhood desmoid tumors, Journal of Clinical Oncology, 17, 3, 1086-1090.
5. Moerman DE, 1998, Native American Ethnobotany, Timber Press, Portland, OR, USA.
6. Roth I, Lindorf H, 2002, South American Medicinal Plants - Botany, Remedial Properties and General Use, Springer, Berlin, Germany.
7. Barr A, Chapman J, Smith N, Wightman G, Knight T, Mills L, Andrews M, Alexander V, 1993, Traditional Medicines in the Northern Territory of Australia by Aboriginal communities of the Northern Territory, Conservation Commission of the Northern Territory, Darwin.
8. Lassak EV, McCarthy T, 2006, Australian Medicinal Plants, Reed New Holland publishers, Australia.
9. Palombo EA, Semple SJ, 2001, Antibacterial activity of traditional Australian medicinal plants, Journal of Ethnopharmacology, 77, 151-157.
10. Setzer MC, Setzer WN, Jackes BR, Gentry GA, Moriarity DM, 2001, The medicinal value of tropical rainforest plants from Paluma, North Queensland, Australia, Pharmaceutical Biology, 39, 1, 67-78.
11. Cock IE, 2008, Antibacterial Activity of Selected Australian Native Plant Extracts, the Internet Journal of Microbiology, 4, 2.
12. WHO, 1978, The promotion and development of traditional medicine, Technical Report Series, no. 615, WHO, Geneva, 1-15.
13. Wilkinson JM, Hipwell M, Ryan T, Cavanagh HMA, 2003, Bioactivity of Backhousia citriodora: antibacterial and antifungal activity, Journal of Agriculture and Food Chemistry, 51, 1, 76-81.
14. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL, 1982, Brine shrimp: a convenient general bioassay for active plant constituents, Planta Medica, 45, 31-34.
15. McLaughlin JL, Rogers LL, Anderson JE, 1998, The use of biological assays to evaluate botanicals, Drug Information Journal, 32, 513-524.
16. Coutts RH, Catterall CP, 1980, Identifying the plants of Toohey forest, Ecos Educational Publications, Nambour, Australia.
17. Finney DJ, 1971, Probit Analysis, 3rd ed., Cambridge University Press, Cambridge.
18. Santos Pimenta LP, Pinto G, Takahashi JA, e Silva LGF, Boaventura MAD, 2003, Biological screening of annonaceous Brazilian medicinal plants using Artemia salina (brine shrimp test), Phytomedicine, 10, 209-212.
19. Wickens K, Pennacchio M, 2002, A search for novel biologically active compounds in the phyllodes of Acacia species, Conservation Science of Western Australia, 4, 3, 139-144.

Author Information

I.E. Cock
Biomolecular and Physical Sciences, Nathan Campus, Griffith University