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Bioactivity of *Backhousia citriodora*: Antibacterial and Antifungal Activity

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Backhousia citriodora products are used as bushfoods and flavorings and by the aromatherapy industry. The antimicrobial activity of 4 samples of *B. citriodora* oil, leaf paste, commercial tea (0.2 and 0.02 g/mL), and hydrosol (aqueous distillate) were tested against 13 bacteria and 8 fungi. Little or no activity was found to be associated with the leaf tea and hydrosol, respectively. Leaf paste displayed antimicrobial activity against 7 bacteria including *Clostridium perfringens*, *Pseudomonas aeruginosa*, and a hospital isolate of methicillin resistant *Staphylococcus aureus* (MRSA). The 4 essential oils were found to be effective antibacterial and antifungal agents; however, variation was apparent between oils that did not correlate with citral content. The antimicrobial activity of *B. citriodora* essential oils was found to be greater than that of citral alone and often superior to *Melaleuca alternifolia* essential oil. *B. citriodora* has significant antimicrobial activity that has potential as an antiseptic or surface disinfectant or for inclusion in foods as a natural antimicrobial agent.

KEYWORDS: ***Backhousia citriodora*; essential oil; antimicrobial; citral; lemon myrtle**

INTRODUCTION

Backhousia citriodora or lemon myrtle has been identified as one of the plants that are likely to undergo major commercial exploitation over the next 5 years (*1*, *2*). The leaves are used both as a bushfood and to produce an essential oil (*3*). The essential oil is then either incorporated into food products, cosmetics, or toiletries or used as an aromatherapy oil. The leaves contain between 1.1 and 3.2% oil and can be of one of two chemovars, the most common of which is the citral form (*4*, *5*). Citral, an isomeric mix of geranial and neral, is a known antimicrobial agent with activity against both bacteria and fungi (*6*, *7*). For example, citral is thought to be responsible for the resistance to postharvest fungal infection of lemons (*8*). Citral present in essential oils may also control food-borne pathogens and prevent spoilage (*6*). Essential oils with citral as a main constituent include lemon, lemon verbena, and lemongrass. These oils are also known for their antimicrobial activity (*9*−*11*) although their citral content is considerably less than that of *B. citriodora*.

The levels of citral in *B. citriodora* oil can vary between 80 and 98% with each of the other components (e.g. linalool and myrcene) accounting for less than 1% each (*5*). *B. citriodora* oil is reputed to possess antimicrobial activity; however, there has been little scientific investigation of the antibacterial or antifungal activity of this oil. Recent study into the activity of *B. citriodora* oil against a limited number of microorganisms confirmed the antimicrobial activity of the oil; however the number of bacteria tested was limited and only single samples of oil were investigated (*12*−*14*). The antimicrobial actions of *B. citriodora* are believed to be directly related to the high citral content; however, it is known that the antimicrobial activity of essential oils can differ from that of their major constituents, probably due to synergistic or antagonistic effects of minor components (*15*, *16*).

In this study we investigate the antimicrobial activity of *B. citriodora* oil and hydrosol, both of which are used as antimicrobial agents. As this plant is also commonly used as a bush food, for example as a tea or included in cheeses, the antimicrobial activity of leaf paste and leaf tea derived from *B. citriodora* were also included. In addition, as previous unpublished data from this laboratory have indicated that variation in antimicrobial activity occurs in different *B. citriodora* oils, we have included samples from four different sources. This report presents the results of the first phase of this work—determination of the antibacterial and antifungal properties of *B. citriodora* essential oils, leaf paste, tea, and hydrosol (aqueous distillate).

MATERIALS AND METHODS

All test substances were derived from *Backhousia citriodora* and consisted of four essential oils, obtained from independent sources, a commercial brand of *B. citriodora* tea, a hydrosol, and crushed dried leaf material. All assays were repeated a minimum of two times.

Preparation of Test Material. All four 100% pure, steam-distilled, *B. citriodora* essential oils were provided by primary producers and labeled A, B, C, or D, respectively, upon arrival. Citral content for

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Table 1. Antibacterial and Antifungal Activity of *Backhousia citriodora* Essential Oils as Determined by the Disk Diffusion Assay[a]

			Backhousia citriodora essential oil conc								
			100%		10 %		1%		0.1%		*Melaleuca alternifolia*
test organism	properties	growth conditions (°C)	oil A	oil B	oil A	oil B	oil A	oil B	oil A	oil B	essential oil (100%)
Alcaligenes faecalis	G –ve	37	40.75	46	14.25	25.5	8.75	8.5	0	0	13
Clostridium perfringens	G +ve	37	11.5	11	0	8.5	0	0	0	0	19
Echerichia coli	G –ve	37	10	11	10.25	11.25	7.5	7.5	0	0	15
Echerichia coli type B$^{\delta}$	G –ve	37	nt	nt	16. 5	18	9.25	8	0	0	20.5
Enterococcus faecalis	G –ve	37	12.75	16	3.5	7.5	0	0	0	0	9
Mycobacterium phlei	acid-fast	37	nt	nt	7.5	12.25	0	0	0	0	20.5
MRS A$^{\delta}$	G +ve	37	11.5	16.5	7.75	0	0	0	0	0	18
Pseudomonas aeruginosa	G –ve	37	0	0	0	0	0	0	0	0	0
Staphylococcus aureus	G +ve	37	18	32	11. 5	13	0	0	0	0	12
Salmonella california	G –ve	37	7.75	8.5	0	9.25	0	8	0	0	20
Salmonella enteritidis	G –ve	37	10	9	7.5	9.75	0	8	0	0	19
Salmonella typhimurium	G –ve	37	8	8	8.5	8.5	7	7	0	0	18.5
Shigella sonnei	G –ve	37	15.75	0	18	33	8.25	8.5	0	0	15
Candida albincans	yeast	37	0	0	0 (11.5)	0 (15)	0	0	0	0	0
Aspergillus niger	fungi	37	>90*	>90*	>90*	>90*	>90*	>90*	0	12	25.5
Fusarium graminearum	fungi	30	>90*	>90*	18	9.5	0	0	0	0	nt
Leptospaeria maculans	fungi	30	>90*	>90*	21	13	0	0	0	0	nt
Microsporum gypsium	fungi	30	>90*	>90*	25	0	0	0	0	0	55
Trichophyton mentagrophytes	fungi	30	>90*	>90*	40	16.5	0	0	0	0	nt
Trichophyton rubrum	fungi	30	>90*	>90*	45	40	0	0	0	0	57.5
Trichophyton tonsurans	fungi	30	>90*	>90*	11	16	8	10	8	10	nt

[a] All figures indicate the mean of at least two assays. Unless otherwise indicated, all tests were performed on nutrient agar plates. Key: >90* indicates no growth on plates containing test substance; *E. coli B*$^{\delta}$ and MRSA$^{\delta}$ are hospital isolates; nt indicates not tested; G –ve = gram negative; G +ve = gram positive.

these oils was between 93 and 98% according to CG-MS data supplied by each producer. All oils were stored in the dark at room temperature, and the date of breaking the seal was recorded. *Melaleuca alternifolia* oil was donated by one of the primary producers.

A commercial brand of lemon myrtle leaf tea was obtained and infused in boiling water for 20 min under foil to prevent vapor loss at two concentrations, 5 g in 25 mL of boiling water and 5 g in 250 mL of boiling water. These represent a 10 and 1 times concentrate of the manufacturers recommended concentration of tea. An additional sample of 5 g in 25 mL of water cooled to 45 °C was also included. Infusions were subsequently passed through a 0.25 μm syringe filter (Sartorius) and used immediately.

Dried leaf material was made into a paste in sterile, room-temperature water (2:1 ratio), briefly (1 min) ground in a mortar and pestle, and used immediately.

Citral (analytical grade, Sigma) was prepared at a concentration of 10% and 1% in sterile water in the presence or absence of 0.5% Tween 80 and used immediately.

Microorganisms and Media. A variety of microorganisms (bacteria and fungi) that differ in cell structure, biochemical properties, and growth conditions were used in antimicrobial assays (listed in **Table 1**). To ensure that the chemical composition of the growth media did not interfere with the diffusion of the oil components, all organisms were grown on nutrient broth (10 g of tryptone, 5 g of yeast extract, 10 g of sodium chloride, water to 1 L) and/or nutrient agar (as for nutrient broth plus 15 g of agar/L of media). All assays were repeated, independently, a minimum of two times. All organisms were originally obtained from the culture collection of the University of New South Wales with the exception of the hospital isolates of methicillin resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* type B (*E. coli* type B).

Normal growth of all microorganisms was evaluated by growth on nutrient agar at appropriate conditions (37 or 30 °C as shown in **Table 1**) in the absence of any test substance. Where possible, organisms were grown preferentially at 37 °C to avoid the effects of temperature variation on loss of volatile components from the oils. Anaerobic conditions were achieved by placing agar plates in a "GasPak" anaerobic jar (Sigma). Antimicrobial assays were carried out using standard agar dilution and disk diffusion methods.

Agar Dilution Assays. Dilutions of each test substance were prepared in cooled (42 °C) molten nutrient agar (1.5 mL of test substance plus 13.5 mL of agar; 10^{-1} dilution) or prediluted in sterile deionized water (10^{-1}−10^{-4}) before adding 1.5 mL of each dilution to a further 13.5 mL of molten agar, giving final concentrations of 10^{-2}−10^{-5}. A 15 mL volume of agar containing the diluted test substance was subsequently placed in a sterile Petri dish and allowed to set. A loopful of each microorganism (1×10^6 organisms/mL), taken from a fresh 24 h broth culture, was streaked onto a plate of each test substance at each concentration after the method of Mitscher et al. (*17*). The plates were then incubated at appropriate growth conditions and checked after 24 and 48 h. Microbial growth was scored on a scale of 1−4, where 0 equals no growth and 4 equals full growth as determined by growth of organism on control plates. Control plates consisted of agar containing either 15 mL of nutrient agar alone, 10% sterile deionized water (produces normal growth of all microorganisms), or commercial antibiotics. Antibiotic controls consisted of either streptomycin sulfate (final concentration 10 μg/mL) or amphotericin B (final concentration 10 μg/mL). All organisms used in this study were susceptible to at least one of theses antibiotics with the exception of the hospital isolate MRSA. All organisms within this study displayed expected antibiotic sensitivity profiles (data not shown).

Disk Diffusion Assay. The 24 h broth cultures of bacteria were freshly prepared for each assay. Nutrient agar plates (15 mL) were prepared, allowed to set, and then surface dried (37 °C, 30 min). A 1 mL volume of bacterial broth culture (1×10^6 organisms/mL) was subsequently poured evenly over the surface of the dried agar plates, and the plates were placed at 37 °C for approximately 20 min until bacterial overlay had dried on the surface. A 6 mm sterile paper disk (Wattman) was placed onto the dried surface, in the center of the plate, and 12 μL of test substance was gently placed onto the disk. Plates were subsequently incubated at the appropriate temperature for 24 h. Zones of inhibition were calculated by measuring diameter in mm (including disk). Control plates consisted of nonimpregnated disks and disks impregnated with 12 μL of sterile distilled water, both of which resulted in full growth of all organisms. Disks impregnated with 12 μL of 100% tea tree oil (*Melaleuca alternifolia*) were also included for comparison. This assay was repeated for a limited number of organisms (**Table 3**) with addition of 0.5% Tween 80 into the test substance. All assays were repeated, independently, at least twice.

RESULTS

B. citriodora hydrosol, leaf paste, and leaf tea were tested against 15 microorganisms, *Alcaligenes faecalis*, *Clostridium*

perfringens, *Echerichia coli*, *Echerichia coli* type B, *Enterococcus faecalis*, *Mycobacterium phlei*, MRSA, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella california*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Shigella sonnei*, *Candida albincans*, and *Aspergillus niger*. With the exception of the tea prepared at 10 times recommended strength (0.2 g/mL), no antimicrobial activity was detected at any concentration for either the hydrosol or the tea infusions irrespective of assay system used (data not shown). The tea (0.2 g/mL) showed a slight inhibition of *Clostridium perfringens* with a 9 mm zone of inhibition (including disk). The leaf paste, however, demonstrated antimicrobial activity against 7 bacteria; *A. faecalis* (13 mm), *C. perfringens* (29.5 mm), *M. phlei* (15 mm), MRSA (16 mm), *P. aeruginosa* (13 mm), *S. aureus* (16 mm), and *C. albicans* (8 mm). *M. alternifolia* oil (100%) demonstrated activity against all organisms tested except *P. aeruginosa* (**Table 1**).

Initially, two *B. citriodora* essential oils, A and B, were evaluated using the disk diffusion assay. With the exception of *P. aeruginosa*, both oils demonstrated varying degrees of antimicrobial activity against all 21 organisms tested, with the fungi being the most susceptible (**Table 1**). Both oils demonstrated activity against six of the organisms (*A. faecalis, E. coli, E. coli type B, S. typhimurium, S. sonnei, A. niger*, and *T. tonsurans*) at a concentration of 1%. Oil B also demonstrated antimicrobial activity against *S. enteritidis* and *S. california* at a 1% concentration. Oil B continued to demonstrate activity against *A. niger*, and both oils inhibited growth of *T. tonsurans* at a concentration of 0.1%. Variable results were, however, apparent with selected organisms. For example, *E. faecalis* repeatedly displayed no susceptibility to either oil at concentrations of 100% yet demonstrated susceptibility at both 10% and 1% concentrations. Results *for C. albicans* were also variable. *C. albicans* results showed that, of the three assays carried out, no inhibition at oil concentrations of 100% or 1% was observed, yet one of three assays at a 10% concentration indicated an 11.5 mm (oil A) and 15 mm (oil B) zone of inhibition while the remaining two assays again indicated no activity.

Although both oils demonstrated activity against the majority of the organisms, there was considerable variation in the activity of oil A compared to oil B. For example, *C. perfringens* was apparently more susceptible to oil B than oil A while MRSA was more susceptible to oil A than oil B at concentrations of 10%. *M. altenifolia* oil (100%) displayed varying degrees of activity against all organisms tested, with the exception of *P. aeruginosa*.

A limited number of organisms, chosen on the basis of their varying susceptibilities to *B. citriodora* oil, were utilized to assay antimicrobial activity of *B. citriodora* oil A via agar dilution, *S. aureus, E. coli, S. typhimurium, M. phlei, C. perfringens, C. albicans*, and *A. niger*. Antimicrobial activity was demonstrated against all organisms at dilutions up to 1% with some inhibition of all bacteria other than *E. coli* still evident at 0.1% (**Table 2**). *A. niger* and *Cl. perfringens* growth was completely inhibited at 1%. Growth of all organisms was normal at dilutions greater than 0.1% with the exception of the fungi *A. niger* which was fully inhibited by dilutions of 0.01% and demonstrated approximately 50% inhibition at a concentration of 0.001% *B. citriodora* oil.

The effect of adding a solubilizing agent, Tween 80, into the disk diffusion assay was also undertaken as part of a comparison to the antibacterial activity of *B. citriodora* oil and commercial citral. The disk diffusion assay was repeated, with a limited number of organisms, in the presence and absence of 0.5% Tween 80. The addition of 0.5% Tween 80 into the disk diffusion assay had variable effects (**Table 3**). The presence of Tween 80 significantly reduced the susceptibility of both *E. coli* and *S. aureus* to 10% citral and *S. aureus* to 1% citral while *A. faecalis* became more susceptible to 1% citral in the presence of Tween 80. Similarly, the apparent susceptibility of *E. coli* and *S. typhimurium* to *B. citriodora* oil B (10%) and oil D (10%), respectively, was also reduced. Conversely the presence of 0.5% Tween 80 apparently increased the susceptibility of *S. aureus* to *B. citriodora* oils C (10% and 1%) and D (10%), *S. typhimurium* susceptibility to oil B (10%), and *A. faecalis* to oil B (1%). No organism tested displayed any growth, irrespective of oil source, when 10% *B. citriodora* essential oil was incorporated into the agar (**Table 3**). No organism displayed any growth inhibition in the presence of 0.5% Tween 80 alone (data not shown). Further repeats of the agar dilution assay for 10% concentrations of all oils and commercial citral resulted in no growth of any organism tested (**Table 3**).

Table 2. Determination of the Antimicrobial Activity of *Backhousia Citriodora* Oil by Agar Dilution[a]

	dilution of oil in agar				
test organism	10%	1%	0.1%	0.01%	0.001%
Staphylococcus aureus	0	1	3	4	4
Echerichia coli	0	1	4	4	4
Salmonella typhimurium	0	2	3	4	4
Mycobacterium phlei	0	1	3	4	4
Clostridium perfringens	0	0	1	4	4
Candida albicans	0	1	2	4	4
Aspergillus niger	0	0	0	2	4

[a] Key: 0 = no growth of organism; 1 = 25% of normal growth; 2 = 50% of normal growth; 4 = 100% of normal growth as compared to water controls. All figures are based on the mean of at least two independent assays. All assays were carried out on nutrient agar. Results shown are for *B. citriodora* oil A.

In general, the antimicrobial activity of *B. citriodora* oil was greater than that of citral against all organisms, although variation in the efficacy of the different oils was apparent. Oils A and B were more effective than citral against all five organisms tested. Citral was more effective against *E. coli* than oils C and D and also against *S. aureus* than oil C. All four oils were more effective than citral against *S. typhimurium, C. albicans*, and *A. faecalis* (**Table 3**).

The antimicrobial activity of *B. citriodora* oil was greater than that of *Melaleuca alternifolia* oil against *A. faecalis, M. phlei, S. aureus, F. graminearum, M. gypsium, T. mentagrophytes*, and *T. rubrum*. The activity of *M. alternifolia* oil (100%) was significantly greater than that of *B. citriodora* against *C. perfringens* and the *Salmonella* spp. tested. All other organisms were equally susceptible to the two types of essential oil.

DISCUSSION

The antimicrobial activity of *B. citriodora* tea was investigated on the basis of three tea preparations: tea prepared as per manufacturers instructions in boiling water, tea prepared as per manufacturers instruction but in water at 45 °C to prevent loss of any antimicrobial activity through evaporation, and finally tea prepared at 10 times the manufacturers recommended concentration. Only the 10× concentrate tea displayed any antimicrobial activity against the 15 organisms tested. A small zone of inhibition (9 mm) was consistently detected when this tea preparation was tested against *Clostridium perfringens*. Like the 10× concentrate tea, *C. perfringens* was also susceptible to the leaf paste but consistently produced a much greater zone of

Table 3. Antimicrobial Activity of *Backhousia Citriodora* Oil and Citral in the Presence and Absence of 0.5% Tween 80[a]

sample	assay method	conc of oil (%)	0.5% Tween 80 incorporated?	test organism				
				E. coli	*S. aureus*	*S. typhimurium*	*C. albicans*	*A. faecalis*
B. citriodora oil A	agar incorporation	10	–	ng	ng	ng	ng	ng
	disk diffusion	10	–	10.25	11.5	8.5	22	14.5
	disk diffusion	10	+	8	13	0	22	19
	disk diffusion	1	–	7.5	7	7	0	8.75
	disk diffusion	1	+	0	7	0	8	7
B. citriodora oil B	agar incorporation	10	–	ng	ng	ng	ng	ng
	disk diffusion	10	–	11.25	13	8.5	17	25.5
	disk diffusion	10	+	0	11	9	20	14
	disk diffusion	1	–	7.5	7	7	7	8.5
	disk diffusion	1	+	0	7	0	8	8
B. citriodora oil C	agar incorporation	10	–	ng	ng	ng	ng	ng
	disk diffusion	10	–	0	4.5	6.5	22	25
	disk diffusion	10	+	0	8.75	8	20	28
	disk diffusion	1	–	0	0.5	0	7	9
	disk diffusion	1	+	0	2.5	0	8	9
B. citriodora oil D	agar incorporation	10	–	ng	ng	ng	ng	ng
	disk diffusion	10	–	0	8	7	16	27
	disk diffusion	10	+	0	15	0	18	32
	disk diffusion	1	–	0	7	0	8	8
	disk diffusion	1	+	0	7	0	10	9
citral (Sigma)	agar incorporation	10	–	ng	ng	ng	ng	ng
	disk diffusion	10	–	17	11	0	0	15
	disk diffusion	10	+	7.5	4.25	0	0	15
	disk diffusion	1	–	0	6.5	0	0	0
	disk diffusion	1	+	0	0.25	0	0	0

[a] Key: growth of all organisms in disk diffusion assay was measured in diameter of zone of including the disk (6 mm). All figures are based on the mean of at least two independent assays. Growth on agar dilution was recorded as no growth (ng) for each of the organisms in this table. All assays were carried out on nutrient agar.

inhibition (29.5 mm). This is probably due to the much greater concentration of leaf material present in the leaf paste and consequent higher concentration of oil. Interestingly, of the seven organisms inhibited by the leaf paste, only *M. phlei* was more sensitive to 100% *M. alternifolia* oil. In addition, *P. aeruginosa* growth was inhibited by the *B. citriodora* leaf paste but not the teas, hydrosols, or any of the essential oils used in this study including *M. alternifolia*. These findings are consistent with previous reports which show an innate resistance of *P. aeruginosa* to *M. alternifolia* oil, although it has been suggested that some strain variation in susceptibility may occur as reports of *P. aeruginosa* being susceptible to *M. alternifolia* oil have appeared in the literature (*14*, *18*). No antimicrobial activity was detected in the *B. citriodora* hydrosol tested in this study.

Two methods were trialed to determine the antimicrobial activity of *B. citriodora* essential oil, the agar dilution assay and the disk diffusion assay. The agar dilution assay was initially used to evaluate antimicrobial activity against a limited number of microorganisms. Using this assay, some degree of antimicrobial activity was demonstrated by *B. citriodora* oil against all organisms to a 1% concentration. Growth of all the bacteria was normal at dilutions greater than 0.1%, except that growth of *Aspergillus niger* was prevented up to dilutions of 0.01% with growth at 0.001% approximately half that on the control plate. The results for the agar dilution assay were consistent and reproducible, and the oil was found to form a stable mix with the agar.

In the disk diffusion assay, the oil was found to prevent the growth of all organisms tested at 100% concentration other than *P. aeruginosa*, *E. coli B*, and *C. albicans*. At this concentration *B. citriodora* oil was consistently more effective than 100% *M. alternifolia* oil against the bacteria *A. faecalis*, *E. faecalis*, *S. aureus*, and the fungi *Aspergillus*, *Microsporum*, and *Trichophyton*. *B. citridora* was found to be particularly effective against the fungi with the growth of some being inhibited to at all concentrations higher than 0.01%. The results for the disk diffusion assay did however show some degree of inconsistency with certain microorganisms, most notably *E. coli B*, *Salmonella* sp., and *C. albicans*. *C. albicans* has previously been reported to be susceptible to *B. citriodora* oil with a minimum inhibitory concentration (MIC) of 0.03% (*14*). This inconsistency may be explained by the use of oils from different sources (and hence slight variation in the chemical composition of the oils) or different growth conditions for the organisms or by the use of different isolates of *C. albicans*. Variation in strain susceptibility of microorganisms to essential oils has previously been reported (*18*). This phenomenon warrants further investigation.

While the standard methods of disk diffusion and agar dilution utilized in this study are suitable for the aqueous teas and hydrosols, the insoluble nature of the majority of constituents of essential oils often limits their diffusion through agar, leading to incorrect or inconsistent results (*18*). Many studies now use solubilizing agents, such as Tween 80, to overcome this problem (*11*, *18*). The introduction of 0.5% Tween 80 into the disk diffusion assay produced variable effects. With some organisms the presence of Tween 80 significantly reduced the susceptibility; with others the susceptibility was increased, while others were unaffected. No organism displayed any growth inhibition in the presence of 0.5% Tween 80 alone. While some authors continue to publish data relating to the antimicrobial activity of essential oils on the basis of the disk diffusion assay (with or without solubilizing agents), from this study we would conclude that this assay did not yield consistent results and is not recommended for future testing of essential oils. Conversely, the agar dilution method gave consistent reproducible results for both the oils and citral.

While this study found that the antimicrobial activity of *B. citriodora* was, in general, greater than that of citral alone, Hayes and Markovic (*14*) reported little difference between the antimicrobial activity of citral and *B. citriodora* oil. The different

oils used in this present study did show surprising degrees of variation with oils A and B being consistently more effective than oils C and D despite similar citral content. The fact that the antimicrobial activity of *B. citriodora* oil was greater than that of citral against all organisms tested other than *E. coli* raises interesting questions and suggests that other compounds within the oil effect the susceptibility of the microorganisms.

Hayes and Markovic (*14*) also report that *B. citriodora* oil is more effective than *M. alternifolia* oil against *S. aureus, E. coli, P. aeruginosa, C. albicans, A. niger,* and *Klebsiella pneumoniae.* The *B. citriodora* oils utilized in this study also displayed greater activity than *M. alternifolia* oil against a wide range of organisms, although not against *P. aeruginosa* or *E. coli*. Inhibition of *P. aeruginosa* was only found with the *B. citriodora* leaf paste in this study. Again, strain related or growth related differences might be responsible for these differences. *P. aeruginosa* was grown on NA in this study while Iso-sensitest broth (Oxoid) was used by Hayes and Markovic (*14*). Various degrees of *P. aeruginosa* susceptibility to *M. alternifolia* oil have been reported (*14*, *19*). We have also found that while the type strain *E. coli* described in the present investigation was not more susceptible to *B. citriodora* oil than *M. alternifolia* oil, other strains of *E. coli*, such as the hospital isolate of *E. coli* type B, are more susceptible. *K. pneumoniae* was not investigated in this study.

Few other studies on *B. citriodora* oil have been reported. *B. citriodora* oil is reported to be nonmutagenic; however, only concentrations of 10% and below were tested (*12*). Its use as a bushfood would support the likelihood that the oil is nonmutagenic at lower concentrations. There is some conflicting evidence relating to the mammalian cell toxicity, in vitro, of *B. citriodora* oil, however, with one report stating no toxicity was detected against HeLa and Hep2 cell lines at concentrations less than 1% (*12*). In contrast, a second report states an inhibitory concentration of 50% of between 0.04% and 0.01%, although, again, this may reflect the different cell lines used in each study (Hep2 and HeLa vs F1-73, skin fibroblasts, and HepG2) (*14*). Certain cell lines, like HepG2, are known to be more sensitive to essential oils (*20*). From the limited data available to date, no anticancer activity has been associated with *B. citriodora* oil in that no growth inhibition was detected against colon, breast, leukemia, and salivary gland (cell line) carcinomas in vitro (*12*). On the basis of case studies, the oil is reported to have antiherpesvirus activity (*21*).

The results of this study show that *B. citrodora* oil is an effective antibacterial and excellent antifungal agent that displays no apparent difference in the susceptibility of gram negative and gram positive organisms. Slight differences were found in the antimicrobial activity of the different *B. citriodora* essential oils utilized in this study, all of which were obtained from difference sources (A−D), and only the leaf paste was found to have any activity against *P. aeruginosa*. The differences between oils may be due to different levels of citral or other components, growth conditions, or distillation procedures. Not only is the composition of essential oils and plant extracts known to vary according to local climatic and environmental conditions but distillation times and temperatures can also significantly affect the oil constituents (*22*).

The data generated, however, would indicate that *B. citriodora* essential oil has potential as an antiseptic or surface disinfectant, although further research is needed to determine whether there are other biological activities of this oil that may be of benefit. For example, *B. citriodora* oil is also a powerful antifungal agent and its antimicrobial activity may have role in the food industry for preservation and prevention of microbial spoilage. Further studies are currently underway to investigate the biocidal versus the biostatic effect of this oil, as determination of the biocidal activity will play a vital role in the potential use of this oil as a therapeutic agent. Evaluation of the effect of *B. citriodora* oil on other bacteria and fungi of importance to the medical, food, agricultural, and horticultural industries in addition to determination of any antiviral, antiparasitic, or antioxidant activity is currently under investigation.

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LITERATURE CITED

(1) Dyer, K. *Eat Well Conference, Adelaide*; http://www.chdf.org.au/eatwellsa/ conference/15.html (accessed 8/04/00), pp 1−29.
(2) Fergus, J. *What will be the next big oil from Australia?* International Conference on Essential Oils and Aromas, Hong Kong.
(3) Hegarty, M. P.; Hegarty, E. E.; Wills, R. B. H. *Food safety of Australian plant bushfoods*; Rural Industries Research & Development Corp.: Kingston, ACT, Australia, 2001.
(4) Brophy, J.; Goldsack, R.; Fookes, C.; Forester, P. Leaf oils of the genus *Backhousia* (Myrtaceae). *J. Essent. Oil Res.* **1995**, *7*, 237−254.
(5) Southwell, I. A.; Russell, M.; Smith, R. L.; Archer, D. W. *Backhousia citriodora* F. Muell. (Myrtaceae), a superior source of citral. *J. Essent. Oil Res.* **2000**, *12*, 735−741.
(6) Kim, J.; Marshall, M. R.; Wei, C. Antibacterial activity of some essential oil components against five foodborne pathogens. *J. Agric. Food Chem.* **1995**, *43*, 2839−2845.
(7) Inouye, S.; Tsuruoka, T.; Uchida, K.; Yamaguchi, H. Effect of sealing and Tween 80 on the antifungal susceptibility testing of essential oils. *Microbiol. Immunol.* **2001**, *43*, 201−208.
(8) Rodov, V.; Ben-Yehoshua, S.; Fang, D. Q.; Kim, J. J.; Ashkenazi Preformed antifungal compounds of lemon fruit: citral and its relation to disease resistance. *J. Agric. Food Chem.* **1995**, *43*, 1057−1061.
(9) Inouye, S.; Takizawa, T.; Yamaguchi, H. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *J. Antimicrob. Chemother.* **2001**, *47*, 565−573.
(10) Wannissorn, B.; Jarikasem, S.; Soontorntanasart, T. Antifungal activity of lemon grass oil and lemon grass cream. *Phytother. Res.* **1996**, *10*, 551−554.
(11) Hammer, K. A.; Carson, C. F.; Riley, T. V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985−990.
(12) Ryan, T. *Antimicrobial activity of natural medicines*; Summer scholarship report; Charles Sturt University: Wagga Wagga, Australia, 2000.
(13) Ryan, T.; Cavanagh, H. M. A.; Wilkinson, J. M. Antimicrobial activity of Backhousia citriodora oil. *Simply Essent.* **2000**, *38*, 6−8.
(14) Hayes, A. J.; Markovic, B. Toxicity of australian essential oil Backhousia citriodora (Lemon myrtle). Part 1. Antimicrobial activity and in vitro cytotoxicity. *Food Chem. Toxicol.* **2002**, *40*, 535−543.
(15) Lis-Balchin, M.; Deans, S. G.; Eaglesham, E. Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Fragrance J.* **1998**, *13*, 98−104.
(16) Lis-Balchin, M.; Deans, S. G. Studies on the potential usage of mixtures of plant essential oils as synergistic antibacterial agents in foods. *Phytother. Res.* **1998**, *12*, 472−475.

(17) Mitscher, L. A.; Leu, R.; Bathala, M. S.; Wu, W.; Beal, J. L. Antimicrobial agents from higher plants. I. Introduction, rationale and methodology. *Llloydia* **1972**, *35*, 157−166.
(18) Griffin, S. G.; Markham, J. L.; Leach, D. N. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. *J. Essent. Oil Res.* **2000**, *12*, 249−255.
(19) Carson, C. F.; Riley, T. V. Susceptibility of *Propionibacterium acnes* to the essential oil of *Melaleuca alternifolia*. *Lett. Appl. Microbiol.* **1994**, *19*, 24−25.
(20) Hayes, A. J.; Leach, D. N.; Markham, J. L.; Markovic, B. In vitro cytotoxicity of Australian tea tree oil using human cell lines. *J. Essent. Oil Res.* **1997**, *9*, 575−582.
(21) Hirobe, C.; Furukawa, M.; Fukuhara, N.; Shimotsuura, Y. Anti-herpes viral effect of lemon myrtle. *Acupunct. Electrother. Res.* **2001**, *26*, 111.
(22) Janssen, A. M.; Scheffer, J. J. C.; Baerheim Svendsen, A. Antimicrobial activity of essential oils: a 1976−86 literature review. Aspects of test methods. *Planta Med.* **1987**, *53*, 395−398.

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