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ORIGINAL ARTICLE

Evaluation of antibacterial activity of Australian basidiomycetous macrofungi using a high-throughput 96-well plate assay

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Abstract

Context: The production of antimicrobial compounds by macrofungi is not unexpected because they have to compete with other organisms for survival in their natural hostile environment. Previous studies have indicated that macrofungi contain secondary metabolites with a range of pharmacological activities including antimicrobial agents.

Objective: To investigate macrofungi for antimicrobial activity due to the increasing need for new antimicrobials as a result of resistance in the bacterial community to existing treatments.

Materials and methods: Forty-seven different specimens of macrofungi were collected across Queensland, Australia. Freeze-dried fruiting bodies were sequentially extracted with three solvents: water, ethanol, and hexane. These extracts were tested against representative Gram+ve, *Staphylococcus aureus* and Gram−ve, *Escherichia coli* bacteria.

Results and discussion: Overall water and ethanol extracts were more effective against *S. aureus* than *E. coli*, whereas a small number of hexane extracts showed better results for their antimicrobial potential against *E. coli* at higher concentrations only. Encouraging results were found for a number of macrofungi in the genera *Agaricus* (Agaricaceae), *Amanita* (Amanitaceae), *Boletus* (Boletaceae), *Cantharellus* (Cantharellaceae), *Fomitopsis* (Fomitopsidaceae), *Hohenbuehelia* (Pleurotaceae), *Lentinus* (Polyporaceae), *Ramaria* (Gomphaceae), and *Strobilomyces* (Boletaceae) showing good growth inhibition of the pathogens tested.

Conclusion: The present study establishes the antimicrobial potential of a sample of Australian macrofungi that can serve as potential candidates for the development of new antibiotics.

Keywords: Fungi, antibacterial, water extracts, 96-well plate assay, *S. aureus*, *E. coli*

Introduction

The accidental discovery of penicillin from fungi (Fleming, 1929) widely attracted scientific attention for the potential role of fungi as antimicrobial agents and lead to the discovery and development of other antibiotics. However, the emergence and subsequent spread of antibiotic resistant bacterial strains is of increasing concern as reviewed by Levy and Marshall (2004). Currently, this problem presents a significant challenge to medicine because of the therapeutic failure of life-saving drugs (Alfonso, 2005) and hence, more and better antibiotics are needed as indicated by the "10 × '20 Initiative" to develop 10 new antibiotics by 2020 by Infectious Diseases Society of America (2010).

Most fungi-derived pharmaceuticals have been sourced from Ascomyceteous fungi where most (but not all) species produce microscopic fruiting bodies; for example, those used in pharmaceuticals include *Penicillium*, *Aspergillus*, and so on, whereas perhaps fewer pharmaceuticals, certainly in an industrial context, have been derived from the higher phyla of fungi,

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the Basidiomycota. The Basidiomycota contains an abundance of species that produce large fruiting bodies including typical mushrooms, coral fungi, puffballs, bracket fungi, and so on. Some species are frequently used as a food source such as the common field mushroom, *Agaricus bisporus* (J. E. Lange) Imbach (Agaricaceae); others have been used chiefly in medicine (Boa, 2004; Molitoris, 1994), whereas some are known for their notoriously toxic properties such as Amanitas.

According to Chang and Buswell (1996), the Romans perceived mushrooms as "Food of the Gods," the Chinese treasured them as a health food, whereas *Lentinula edodes* (Berk.) Pegler (Marasmiaceae), the shiitake mushroom, was highly prized by Japanese emperors as an aphrodisiac and was cultivated at secret and heavily guarded locations. In accordance with their traditional uses, macrofungi have been extensively investigated for their therapeutic significance resulting in the discovery of an antibiotic pleuromutilin from *Pleurotus mutilis* (Fr.) Sacc. and *Pleurotus passeckerianus* Pilat (Pleurotaceae) (Kavanagh et al., 1951). A number of pleuromutilin derivatives have since been developed for veterinary use in the treatment of *Mycoplasma* infections (Drews et al., 1975; Werner et al., 1978; Hannan et al., 1997; Hunt, 2000; Jones et al., 2002; Xu et al., 2009). Furthermore, retapamulin (Altabax) has emerged as an antibiotic for human use for the topical treatment of Gram+ve bacterial skin infections including methicillin-resistant *Staphylococcus aureus* (Jones et al., 2006; Novak & Shlaes, 2010).

The therapeutic potential of mushrooms has been extensively reviewed by Wasser and Weis (1999) and Lindequist et al. (2005). Extracts of various fungal fruiting bodies such as *Pleurotus ostreatus* (Jacq.) P. Kumm. (Iwalokun et al., 2007), *Pholiota adiposa* (Fr.) P. Kumm. (Strophariaceae) (Dulger, 2004), *Coprinus digitalis* (Batsch) Fr. (Agaricaceae) (Efremenkova et al., 2003), *Podaxis pistillaris* (L.) Fr. (Agaricaceae) (Al-Fatimi et al., 2006), *Lycoperdon pusillum* Batsch [now *Bovista pusilla* (Batsch) Pers.], and *Lycoperdon giganteum* Batsch [now *Calvatia gigantea* (Batsch) Lloyd] (Lycoperdaceae) (Jonathan & Fasidi, 2003) have shown activity against a range of different Gram+ve and Gram–ve bacteria and also fungi. Stamets (2006) mentioned that macrofungi produce numerous novel pharmaceuticals. However, only a small proportion (10%) of the total estimated number of macrofungi species on Earth (140,000) has been described (Hawksworth, 2001). It means that there is an enormous inherent scope for the nutritional and medicinal value among macrofungi that still needs to be discovered. The same is true for the Australian macrofungi. Among 10,000 estimated Australian macrofungi, <4000 have been described, thus leaving behind a large proportion of macrofungi yet to be named indicating the scarce taxonomical information available (May, 2003).

Macrofungi have been used as food and medicine by indigenous Australians (Kalotas, 1996) but only limited research has been carried out for the evaluation of their antimicrobial potential (Ovenden et al., 2005; Beattie et al., 2010). Therefore, considering the previous reports on the antimicrobial potential of macrofungi and in view of the continuous need for the development of new antimicrobials, the present study aimed to evaluate a sample of Australian macrofungi for their antibacterial activity against sensitive strains of *Staphylococcus aureus* (Gram+ve) and *Escherichia coli* (Gram–ve). The agar well diffusion and disc diffusion methods are commonly used for antimicrobial activity testing but these methods have some limitations. For example, some compounds may be more diffusible and can produce a greater zone of inhibition despite their lower activity in comparison with less diffusible compounds that might be more active but may produce smaller zone of inhibition (Janes et al., 2007; Lund et al., 2009). Therefore, in the present study, a high-throughput 96-well microplate bioassay procedure was used.

Materials and methods

Sample collection

Macrofungi fruiting bodies (47 different species) were collected from a range of natural environments across Queensland, Australia during May 2008 to October 2009 (Table 1). The fruiting bodies were identified based on sporocarp morphology and macroscopic characters. The collections were freeze-dried and stored at –80°C until extraction.

Preparation of macrofungi extracts

Freeze-dried macrofungi (500 mg) were macerated in 25 mL distilled water and then extracted for 1 h in an ultrasonic water bath. Following centrifugation (15,000 rpm for 15 min), the supernatant was removed. The residue was re-extracted with 25 mL water in the sonicating water bath for 30 min, centrifuged, and the supernatant was pooled with that from the first extraction, collectively forming 50 mL of water extract, which was filtered through 0.45-µm membrane filter and freeze-dried. The remaining insoluble material was extracted sequentially with 100% ethanol and *n*-hexane, respectively, following the same procedure as described for the water extracts. The ethanol extracts were evaporated to dryness under reduced pressure in a rotary evaporator at 40°C, whereas the hexane extracts were evaporated overnight in a fume hood.

Preparation of mushroom extracts concentrations

The water extracts were each dissolved in 2 mL of distilled water. The ethanol and hexane extracts were first dissolved in 400 µL absolute alcohol, sonicated for 10 min, and made up to 2 mL with distilled water. This stock solution was diluted with tryptone soya yeast extract broth (TSYEB) to make a concentration of 50% that was serially diluted three times with TSYEB to obtain the respective concentrations of 25, 12.5 and 6.25% for all the extracts tested in the antibacterial assay.

Test organisms and culture conditions

The sensitive strains of clinically important *S. aureus* strain 6571 [National Collection of Type Cultures (NCTC), Health Protection Agency Centre for Infection, London, UK] and *E. coli* strain 9001 (NCTC) were used for the screening tests. The organisms were grown in TSYEB (CM0129 with the addition of 6 g/L Yeast LP0021, Oxoid, Basingstoke, UK) for 24 h. The overnight growth of the culture was quantified to an absorbance reading of 0.5 at 540 nm using a spectrophotometer (Unicam, HeliosAlpha, UK) by diluting with TSYEB, to obtain a standard inoculum with 10^5 CFU/mL for use in the assay.

Table 1. List of macrofungi collected between May 2008 and October 2009 across Queensland, Australia for evaluation of their antibacterial activity.

Sample name	Location	Latitude, longitude
Agaricus aff. xanthodermus	Collingwood Park, Brisbane	27°36'47″S, 152°51'47″E
Agaricus sp. 1	Goodna, Brisbane	27°36'04″S, 152°53'57″E
Agaricus sp. 2	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Agaricus sp. 3	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Agaricus sp. 4	Redland Bay, Brisbane	27°36'45″S, 153°18'11″E
Agaricus sp. 5	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Amanita ochrophylla	Fraser Island, Central Coast, Qld	19°42'21″S, 145°46'26″E
Amanita flavella	Goodna, Brisbane	27°36'34″S, 152°53'57″E
Amanita sp. 1	Goodna, Brisbane	27°36'34″S, 152°53'57″E
Armillaria mellea	Brisbane Botanical Garden, Mt. Coot-tha	27°28'37″S, 152°58'40″E
Auricularia auricula-judae	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Boletus sp. subsect. *luridi*	Springbrook NP, South Eastern Qld	28°12'52″S, 153°16'08″E
Cantharellus sp.	Cairns, Qld	17°13'06″S, 145°25'54″E
Calvatia sp.	Goodna, Brisbane	27°36'34″S, 152°53'57″E
Chlorophyllum molybdites	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Colus sp.	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Coprinus comatus	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Cortinarius sp.	Goodna, Brisbane	27°36'34″S, 152°53'57″E
Craterellus sp.	Main range NP, Brisbane	27°54'31″S, 152°19'16″E
Cyathus striatus	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Fomitopsis lilacinogilva	Fraser Island, Central Coast, Qld	19°42'21″S, 145°46'26″E
Ganoderma sp. 1	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Ganoderma sp. 2	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Gymnopus luxurians	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Hexagonia sp. 1	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Hexagonia sp. 2	Lamington NP, South Eastern Qld	28°15'28″S, 153°08'39″E
Hohenbuehelia sp.	Fraser Island, Central Coast, Qld	19°42'21″S, 145°46'26″E
Lentinus sp. 1	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Lentinus sp. 2	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Lentinus sp. 3	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Microporus xanthopus	Lamington NP, South Eastern Qld	28°15'28″S, 153°08'39″E
Omphalotus sp.	Main range NP, South Eastern Qld	27°54'31″S, 152°19'16″E
Phallus multicolor	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Phallus rubicundus	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Psathyrella sp.	University campus, St. Lucia, Brisbane	27°29'52″S, 153°00'46″E
Ramaria sp. 1	Springbrook, South Eastern Qld	28°12'52″S, 153°16'08″E
Ramaria sp. 2	Davis creek national park, Cairns	17°13'06″S, 145°25'54″E
Ramaria zippellii	Fraser Island, Central Coast, Qld	19°42'21″S, 145°46'26″E
Russula sp.	Mt. Tamborine, South Eastern Qld	27°55'36″S, 153°11'03″E
Russula erumpens	Collingwood Park, Brisbane	27°36'47″S, 152°51'47″E
Schizophyllum commune	University rainforest, St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Scleroderma sp.	Cairns, Qld	17°13'06″S, 145°25'54″E
Scytinopogon angulispora	Lamington NP, South Eastern Qld	28°15'28″S, 153°08'39″E
Strobilomyces sp.	Cairns, Qld	17°13'06″S, 145°25'54″E
Tylopilus sp. 1	St. Lucia, Brisbane	27°29'37″S, 153°00'21″E
Tylopilus sp. 2	Hardings paddock, South Eastern Qld	27°43'00″S, 152°44'45″E
Tricholoma eucalypticum	Goodna, Brisbane	27°36'34″S, 152°53'57″E

Antibacterial activity assay

A high-throughput 96-well microplate bioassay procedure was used according to the method of Sultanbawa et al. (2009), with some modifications. Within each sterile 96-well plate, the first two rows contained 200 µL media only (serving as a sterility check). Test samples were loaded in the next rows with respective concentrations of 50, 25, 12.5 and 6.25%, all comprising 50 µL culture + 150 µL extract. The last row contained 50 µL bacterial culture and 150 µL media serving as negative control. The experiment was replicated three times in separate plates and the same procedure was followed for all three extracts. The standard antibiotics, penicillin G, and oxytetracycline hydrochloride (Sigma-Aldrich, St. Louis, MO) were used as the positive controls against *S. aureus* and *E. coli*.

After loading the samples, the plates were read at 640 nm (Tecan, Sunrise, Austria) to determine the absorbance (t_0). Then the plates were incubated at 37°C for 22 h, after which the solutions in the plates were mixed using a pipette before measuring the absorbance again (t_{22}). The percent inhibition was calculated using the formula (Sultanbawa et al., 2009):

$$\%\ \text{inhibition} = \{1-(t_{22}-t_0)/(C_{22}-C_0)\}\times 100$$

where C_0 is the absorbance value of the corresponding negative control well at t_0 and C_{22} is the absorbance value of the corresponding negative control well at t_{22}.

Results

Overall 141 extracts were prepared, comprising of three replicate samples from each of 47 mushroom species extracted using three solvents (water, ethanol, and hexane). The fruiting bodies used represented fungi from 21 families within nine orders from the three subclasses (Agaricomycetidae, Phallomycetidae, and Agaricomycetes incertae sedis) of the class Agaricomycetes. Due to meager taxonomical information available for Australian macrofungi, many of those collected could be identified to genus level only. Extracts were evaluated at four different dilutions (50%, 25%, 12.5%, and 6.25%) against two contrasting microorganisms, Gram+ve *S. aureus* and Gram−ve *E. coli*.

The water and ethanol extracts showed differential activity against the bacteria (Table 2). Ten macrofungi, namely *Agaricus* sp. 1, *Amanita* sp., *Amanita ochrophylla* (Cooke & Massee) Cleland; Amanitaceae, *Boletus* sp. subsect. *luridi* (Boletaceae), *Cantharellus* sp. (Cantharellaceae), *Fomitopsis lilacinogilva* (Berk.) J. E. Wright & J. R. Deschamps; Fomitopsidaceae, *Hohenbuehelia* sp., *Lentinus* sp. 3, *Ramaria* sp. 1, and *Strobilomyces* sp. showed excellent inhibition against both bacteria tested in the present study with water and/or ethanol extracts. For example, the ethanol extract of *F. lilacinogilva* completely inhibited *S. aureus* and showed good activity against *E. coli* at all the test concentrations (Table 2). On the other hand, 11 water extracts, 17 ethanol extracts, and 21 hexane extracts possessed either no or weak antibacterial activity against both pathogens. Only a small number of hexane extracts exhibited any activity at all; these were *Tylopilus* sp. 2, *Cantharellus* sp., *Psathyrella* sp., *Cyathus striatus* (Huds.) Pers. (Nidulariaceae), and *Chlorophyllum molybdites* (G. Mey.) Massee (Agaricaceae), which were effective against *E. coli* only at higher concentrations (data not shown).

Discussion

Ethanol has previously been noted as the solvent responsible for extracting components with maximum antimicrobial activity (Jonathan & Fasidi, 2003; Dulger et al., 2004). In the present study, ethanol was similar to water in terms of producing extracts with antimicrobial activity. This might be due to the sequential extraction procedure adopted here, which would have allowed components that are soluble in both ethanol and water to have been extracted in water, whereas for other studies the same active components could be contained within two different solvents.

Gram−ve bacteria are generally more resistant to antibiotics than Gram+ve, due to the structural complexity of their cell wall being less permeable, so consequently antimicrobials are often less effective against Gram−ve bacteria (Costerton & Cheng, 1975; Walsh, 2003). Likewise, Gram−ve bacteria have been reported to be less sensitive to extracts from macrofungi (Yamac & Bilgili, 2006; Barros et al., 2007; Karaman et al., 2009). Similarly, in the present study, the extracts were generally less effective toward the Gram−ve bacteria (*E. coli*) as compared with Gram+ve bacteria (*S. aureus*). However, this relationship does not hold for every macrofungi. For example, Gbolagade et al. (2007) and Tambekar et al. (2006) concluded a better inhibitory activity against Gram−ve than Gram+ve bacteria from extracts of *A. bisporus*, *Pleurotus sajor-caju* [now *Lentinus sajor-caju* (Fr.) Fr.], *Pleurotus florida* (Mont.) Singer, and *Polyporus giganteus* [now *Meripilus giganteus*] (Pers.) P. Karst. In the same way, the ethanol extracts of three macrofungi in the present study, *Amanita* sp. 1, *Amanita flavella* E. J. Gilbert & Cleland (Amanitaceae), and *Boletus* sp. subsect. *luridi*, showed better activity against Gram−ve *E. coli* rather than Gram+ve *S. aureus*. The better activity of these macrofungi against Gram−ve bacteria suggests promising antibacterial potential.

Antibacterial activity was observed amongst representatives across all the subclasses and nine orders assessed in this study (Table 2). Previous screening of macrofungi in the order Agaricales (subclass Agaricomycetidae) indicated a higher percentage of active isolates (Suay et al., 2000). In the present study, certain macrofungi from the Agaricales namely, *Hohenbuehelia*, *Amanita*, and *Agaricus* revealed strong antibacterial activity. Both the water and ethanol extracts of *Hohenbuehelia* sp. exhibited strong inhibition of *S. aureus* at all four test concentrations, whereas *E. coli* inhibition decreased in a dose-dependent manner with the ethanol extract

Table 2. Growth inhibition (%) of *S. aureus* and *E. coli* with water and ethanol extracts of various macrofungi belonging to the class Agaricomycetes.

		Water extract		Ethanol extract	
Sample	Conc. (%)	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
Subclass: Agaricomycetidae					
Order: Agaricales					
Agaricus aff. xanthodermus	50	+++	+++	+++	–
	25	+++	+++	+	–
	12.5	+++	–	+	+
	6.25	++++	–	+	+
Agaricus sp. 1	50	++++	++++	++++	–
	25	++++	++++	–	+
	12.5	++++	++++	–	+
	6.25	++++	–	–	+
Agaricus sp. 2	50	++	+++	–	–
	25	+	++	–	–
	12.5	–	+	–	+
	6.25	–	+	–	+
Agaricus sp. 3	50	–	+	++++	–
	25	–	–	+++	++
	12.5	–	–	–	++
	6.25	+	–	–	+
Agaricus sp. 4	50	n. d.	n. d.	++++	++++
	25	+	+	+++	++++
	12.5	–	–	+	++
	6.25	–	–	+	+
Amanita sp.	50	++++	++++	++++	++++
	25	++++	++++	++++	++++
	12.5	++	++++	+++	++++
	6.25	–	++	+	++++
Amanita flavella	50	–	–	++++	++++
	25	–	–	+++	++++
	12.5	–	–	+	++
	6.25	–	–	–	++
Amanita ochrophylla	50	++++	+++	+++	++++
	25	++++	+++	+	+
	12.5	++++	+	–	+
Armillaria mellea	50	++++	++++	–	–
	25	n. d.	n. d.	–	–
	12.5	++++	++++	–	–
	6.25	+	–	–	–
Calvatia sp.	50	+	–	++++	++++
	25	–	–	++++	++++
	12.5	–	–	+++	++
	6.25	–	–	+	+
Chlorophyllum molybdites	50	++++	++++	–	++++
	25	++	+	–	++
	12.5	++	+	–	+
	6.25	++	–	–	+
Cortinarius sp.	50	++	–	+++	–
	25	++	–	+++	++
	12.5	++	–	+	++
	6.25	+	–	–	+
Coprinus comatus	50	++	+++	+++	+++
	25	++	++	+	+++
	12.5	+	++	–	+
	6.25	–	+	–	+

Table 2. continued on next page

Table 2. Continued.

Sample	Conc. (%)	Water extract		Ethanol extract	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
Hohenbuehelia sp.	50	++++	++++	++++	++++
	25	++++	++++	++++	+++
	12.5	++++	++++	++++	++
	6.25	++++	++++	++++	+
Psathyrella sp.	50	+++	++	++++	++++
	25	+++	+	++++	++++
	12.5	++	–	++++	++++
	6.25	+	–	+++	+++
Order: Boletales					
Boletus subsect. *luridi*	50	++++	++++	++++	++++
	25	+	+	++++	++++
	12.5	–	–	+++	++++
	6.25	–	+	+	+++
Strobilomyces sp.	50	++++	++++	–	–
	25	++++	++++	–	–
	12.5	+++	++++	–	–
	6.25	+	+	–	–
Tylopilus sp. 1	50	++++	++++	++++	–
	25	++++	++	++	+
	12.5	++++	+	+	++
	6.25	++++	–	+	++
Tylopilus sp. 2	50	++	–	++++	++++
	25	++	–	++++	++++
	12.5	+	+	+	+++
	6.25	++	+	–	++
Subclass: Phallomycetidae					
Order: Phallales					
Phallus multicolor	50	+++	+	++++	++++
	25	+++	+	+	++++
	12.5	+++	–	–	+++
	6.25	+	–	–	++
Order: Gomphales					
Ramaria sp. 1	50	++++	+++	++++	++++
	25	++++	++	++++	++++
	12.5	++++	–	++	+++
	6.25	++++	–	+++	+++
Ramaria sp. 2	50	+++	+	++++	++++
	25	++	–	+++	++++
	12.5	+	–	–	+
	6.25	+	–	–	–
Ramaria zippellii	50	++	+++	++++	++++
	25	+++	+++	++	++++
	12.5	++	++	–	++
	6.25	+	+	–	+
Subclass: Agaricomycetes incertae sedis					
Order: Polyporales					
Ganoderma sp. 1	50	+++	–	+++	++++
	25	–	–	–	+++
	12.5	–	–	–	++
	6.25	–	–	–	+
Ganoderma sp. 2	50	++++	–	++++	+
	25	++	–	++++	++
	12.5	–	–	–	+
	6.25	–	–	–	+

Table 2. continued on next page

Table 2. Continued.

Sample	Conc. (%)	Water extract		Ethanol extract	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
Lentinus sp. 3	50	++++	++	++++	++++
	25	++++	+	++++	+++
	12.5	++++	–	+++	+++
	6.25	+++	–	+	++
Fomitopsis lilacinogilva	50	++++	–	++++	++++
	25	++++	–	++++	++++
	12.5	++	–	++++	+++
	6.25	+	–	++++	++
Order: Auriculariales					
Auricularia auricula-judae	50	–	+	++	–
	25	–	–	++	+
	12.5	–	–	+	++
	6.25	–	–	+	++
Order: Cantharellales					
Craterellus sp.	50	++++	+	–	–
	25	+++	–	–	–
	12.5	+++	–	–	+
	6.25	++	–	–	+
Cantharellus sp.	50	++++	+	++++	++++
	25	++++	–	++++	++++
	12.5	+	–	++++	++++
	6.25	+	–	++++	+++
Order: Russulales					
Russula erumpens	50	++++	–	–	–
	25	++	–	–	–
	12.5	++	–	–	+
	6.25	–	–	+	++

++++ 75–100% inhibition; +++ 50–75% inhibition; ++ 25–50% inhibition; + <25% inhibition; – no activity; n. d. no data; macrofungi exhibiting no activity are not shown.

from this fungus. Indeed, activity against *S. aureus* has previously been noted for methanol extracts of the fermentation broths of a different species (not yet reported in Australia), *Hohenbuehelia mastrucata* (Fr.) Singer; Pleurotaceae collected in Spain (Suay et al., 2000). The water extracts of the three *Amanita* spp. in the present study were variable in activity: *A. ochrophylla* was more inhibitory against *S. aureus* than *E. coli*, whereas *Amanita* sp. 1 was more effective against *E. coli*, but *A. flavella* was ineffective against both bacterial species. The ethanol extracts from all three *Amanita* spp. caused greater inhibition of *E. coli* than *S. aureus*. In contrast, the methanol extract of another *Amanita* species, *Amanita virosa* (Fr.) Bertill. was ineffective against these same bacterial species (Janes et al., 2007). Previous studies have indicated that *Agaricus* may be a lucrative genus to investigate with regards to antibiotic activity; water extract of *A. bisporus* show activity against Gram–ve bacteria and the ethanol extract of *Agaricus brasiliensis* Wasser, M. Didukh, Amazonas & Stamets has been shown to be effective against Gram+ve bacteria (Tambekar et al., 2006; Lund, 2009). The water extract of *Agaricus* sp. 1 in this study exhibited good activity against both Gram+ve and Gram–ve bacteria but the water and ethanol extracts of the other four *Agaricus* spp. showed either weak or zero activity against the test pathogens. These examples from the Agaricales highlight the large variation in activity between species within each genus but point to the potential for using taxonomic relationships as a lead in the hunt for antimicrobial activity.

The genus *Ramaria* (subclass Phallomycetidae) has previously indicated potential antimicrobial activity, with the methanol extract of *Ramaria largentii* Marr & D. E. Stuntz from Slovenia and ethanol extract of *Ramaria flava* (Schaeff.) Quél. (Gomphaceae) from Turkey showing weak activity against *S. aureus* while being ineffective against *E. coli* (Gezer et al., 2006; Janes et al., 2007). In the present study, the ethanol extracts of all three Australian *Ramaria* sp. tested inhibited the growth of *E. coli* and the water extract of *Ramaria* sp. 1 revealed strong activity against *S. aureus*. Some interesting activity was also recorded for macrofungi within subclass Agaricomycetes incertae sedis. Complete inhibition of *S. aureus* was achieved with the ethanol extract of *Cantharellus* sp., which also showed strong inhibition of *E. coli* at higher concentrations. While Dulger et al. (2004) concluded that the ethanol extract of *Cantharellus cibarius* was more effective against *E. coli* than *S. aureus*, our results

are in accordance with Santoyo et al. (2009) and Barros et al. (2008). Based on previous tests, *Russula* may not be expected to provide antibacterial compounds, as Keller et al. (2002) found that the methanol extracts from five different species of European *Russula* did not possess any activity against either Gram+ve nor Gram–ve bacteria. Indeed, in our tests extracts of *Russula erumpens* Cleland & Cheel showed only weak activity. Using taxonomic relationships as a guide toward bioactivity is only useful where records exist. The ethanol extract of *F. lilacinogilva* exhibited complete inhibition of *S. aureus* and moderate inhibition of *E. coli* even at the lowest concentration. Likewise, Popova et al. (2009) and Liu et al. (2010) reported that the chloroform extract of *Fomitopsis rosea* (Alb. et Schw. Fr.) Karst and triterpene isolated from dichloromethane extract of *Fomitopsis pinicola* (Swartz ex Fr.) Karst inhibited *S. aureus* and *Bacillus cereus*. The antimicrobial potential of different species in this genus suggests that it may be worthwhile further investigating closely related species.

In conclusion, this is the first report on the screening evaluation of different Australian macrofungi species. From the results of this study, a small number of Australian macrofungi have been identified with promising antibacterial activity against *S. aureus* and *E. coli* that can serve as potential candidates for much needed new antibiotics. Further work is needed toward the evaluation of their antimicrobial potential against a wider range of microorganisms and finally the identification and isolation of the active compounds responsible for this activity could provide new starting material for the development of novel antibiotics.

Declaration of interest

The authors report no conflicts of interest.

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