Research Journal of Microbiology 3 (7): 535-538, 2008
ISSN 1816-4935

Anti-Mycobacterial Activity of Extracts Derived from Australian Medicinal Plants

Michelle Meilak and Enzo A. Palombo
Environment and Biotechnology Centre, Faculty of Life and Social Sciences,
Swinburne University of Technology, P.O. Box 218, Hawthorn, Victoria 3122, Australia

Abstract: Extracts of seventeen traditional Australian medicinal plants used to treat infections and respiratory conditions were tested for anti-mycobacterial activity against the fast-growing strains, *Mycobacterium fortuitum* and *M. smegmatis*. Four extracts, the aerial parts of *Pterocaulon sphacelatum* (Asteraceae), the bark and leaves of *Acacia ligulata* (Mimosaceae), the leaves and stems of *Eremophila alternifolia* (Myoporaceae) and the leaves of *Eremophila longifolia*, showed activity against *M. smegmatis* only, while the two *Eremophila* extracts were also active against *M. fortuitum*. The minimum inhibitory concentrations ranged from 20-66 mg mL^{-1}. The identification of the anti-mycobacterial compounds from these extracts may yield new and effective agents to combat diseases caused by *Mycobacterium* species.

Key words: *Mycobacterium*, ethanol extracts, minimum inhibitory concentration, antibacterial

INTRODUCTION

Tuberculosis (TB) is a serious respiratory infection and one of the most important diseases worldwide. Epidemiological evidence indicates that one third of the world's population is infected with the causative bacterial agent, *Mycobacterium tuberculosis*, eight million cases emerge annually and about 2 million people die from the disease (Gautam *et al*., 2007). Recent data from the World Health Organization support these estimates with 8.8 million new TB cases and 1.6 million people deaths, including 195,000 patients infected with HIV, in 2005 (WHO, 2007). By 2020, it has been estimated that nearly 1 billion people will become infected, 200 million will develop disease and 35 million will die from TB, although recent figures suggest that the global epidemic is on the threshold of decline (WHO, 2007). However, the emergence and spread of drug-resistant *M. tuberculosis*, especially multidrug-resistant (MDR) strains resistant to standard TB drugs such as isoniazid and rifampin, reduces the efficacy of current treatment regimes and threatens programs aimed at controlling TB (Sacchettini *et al*., 2008). Drug-resistant TB is caused by a number of factors including slow-acting drugs and inadequate health-care systems, leading to poor compliance, incomplete treatment and relapse (Sacchettini *et al*., 2008). Treatment of MDR-TB requires the use of at least three drugs not prescribed previously to the patient and in some cases four or more anti-TB agents are necessary. Nevertheless, not all cases are cured. As a result, there is an urgent need for potent new drugs to deal properly with cases of MDR-TB. Moreover, the increasing occurrence of infections caused by other species of *Mycobacterium* is of additional concern (Bodle *et al*., 2008).

The increased incidence of new and reemerging infectious diseases, such as TB, has led to the need for new antimicrobial compounds that have diverse chemical structures and different mechanisms of

Corresponding Author: Enzo A. Palombo, Environment and Biotechnology Centre,
Faculty of Life and Social Sciences, Swinburne University of Technology,
P.O. Box 218, Hawthorn, Victoria 3122, Australia
Tel: +61 3 9214 8571 Fax: +61 3 9819 0834

action. Natural products, such as those found in plants, or their semi-synthetic derivatives, may provide novel compounds that can be developed into antimicrobial drugs (Bisht *et al.*, 2006; McRae *et al.*, 2007). Numerous plant species have been used as traditional medicines by the Aboriginal peoples of Australia (Lassak and McCarthy, 2001). However, few have been systematically investigated for their therapeutic, pharmacological and chemical properties. Previously, we found that compounds isolated from the leaves of the Australian tropical medicinal plant, *Planchonia careya*, were active against fast-growing strains of mycobacteria (McRae *et al.*, 2008). In the present study, we have broadened our search in the hope of discovering new potential anti-mycobacterial phytochemicals. Given the unique nature of Australia's native flora, it is likely that new or alternative anti-TB agents will be discovered among plants known to have medicinal properties. To increase the probability of finding appropriate drugs, the plants chosen for investigation have been used as traditional medicines to treat respiratory infections.

MATERIALS AND METHODS

The ethanol extracts used in this study are part of a larger collection that has been described previously (Palombo and Semple, 2001). The extracts studied here were derived from 17 plants belonging to 10 families and were selected because of their traditional use in the treatment of infections, particularly respiratory illness (Table 1). Extracts were prepared as follows. Plant material was freeze-dried and milled to a coarse powder. Powdered material was placed in a glass column plugged with

Table 1: Botanical names of plant species screened, parts extracted and traditional medicinal uses

Botanical name (Family[a], Genus, Species[b])	Plant part(s) extracted[c]	Traditional medicinal use(s)
Asteraceae		
Pterocaulon sphacelatum (Labill.) Benth. and Hook. f. ex F. Muell.	AP	Colds, respiratory infections, skin sores, eye complaints
Campanulaceae		
Isotoma petraea F. Muell.	WP	Respiratory difficulty
Cyperaceae		
Lepidosperma viscidum R. Br.	SB	Colds
Gyrostemonaceae		
Codonocarpus cotinifolius (Desf.) F. Muell.	S, L	Skin sores, chest pain, respiratory infection
Lamiaceae		
Prostanthera striatiflora F. Muell.	AP	Respiratory infection, malaise, infected sores
Loranthaceae		
Amyema quandang (Lindley) Tieghem var. *quandang*	L	Fever
Mimosaceae		
Acacia kempeana F. Muell.	B, RB, L	Chest infection, severe colds, general sickness
Acacia ligulata Cunn. ex Benth.	B, L	Coughs, colds, chest infection, general illness
Acacia tetragonophylla F. Muell.	S, L	Cough, treatment of circumcision wounds, dysentery
Myoporaceae		
Eremophila alternifolia R. Br.	S, L	General sickness, pain respiratory tract infections
Eremophila duttonii F. Muell.	S, L	Respiratory tract infection, sore throat, skin cuts, painful ears, inflamed eyes
Eremophila latrobei F. Muell. subsp. *glabra* (L.S. Smith) Chinn.	S, L	Malaise, cough, respiratory tract infection, sore throat
Eremophila longifolia (R. Br.) F. Muell.	S	Skin sores and boils, respiratory tract infection, eye wash
Eremophila sturtii R. Br.	S, L	Cough, general sickness, skin cuts, sore eyes, respiratory infection
Poaceae		
Cymbopogon ambiguus (Steudel) A. Camus	L	Respiratory tract infection, headache, fever, skin complaints, eye wash
Cymbopogon obtectus S.T. Blake	AP	Respiratory tract infection
Thymelaeaceae		
Pimelea microcephala R. Br. subsp. *microcephala*	S, L	Throat and chest complaints

[a]Family names follow Cronquist (1981). [b]Genus and species names follow Jessop (1993). [c]Plant parts extracted: AP: Aerial Parts; B: Bark; L: Leaves; RB: Root Bark; S: Stem; SB: Stem Base; WP: Whole Plant

cotton wool and extracted with ethanol at room temperature for 24 h. A second extraction with ethanol was carried out and the two portions combined and concentrated in vacuo. The concentrated extract was centrifuged at 10,000 x g for 20 min at 20°C. The supernatant was recovered, filter sterilised and stored at -70°C.

Isolates of the fast-growing mycobacterial strains, *Mycobacterium fortuitum* and *M. smegmatis*, were obtained from the Department of Microbiology, Monash University, Victoria, Australia. *M. fortuitum* and *M. smegmatis* are commonly used as alternatives to the virulent species, *M. tuberculosis*, to indicate the anti-mycobacterial potential of plant extracts and other natural products (Gautam *et al*., 2007). Bacteria were cultivated in Brain Heart Infusion (BHI) broth and on BHI agar (Oxoid, Ltd.) at 37°C for 5 days. To determine anti-mycobacterial activity of extracts, plate-hole diffusion assays were used. Briefly, molten BHI agar inoculated with bacterial culture was poured into a petri dish and allowed to solidify. A sterile cork-borer (5 mm diameter) was used to make holes in the agar. Plant extracts (10 μL) were added to the holes and the plates left for 30 min to allow the extracts to absorb into the agar, after which the plates were incubated for 5 days at 37°C. Ethanol was used as a negative control. To aid in the visualization of zones of growth inhibition around the holes, a 2 mg mL^{-1} solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was sprayed over the agar. The plates were further incubated at 37°C for 30 min, after which the zones of inhibition (clear areas amongst a background of purple) were measured. For the extracts which showed activity, the Minimum Inhibitory Concentrations (MIC) were determined using two-fold serial dilutions of the extracts in plate-hole diffusion assays (McRae *et al*., 2008).

RESULTS AND DISCUSSION

Screening of all extracts using plate-hole diffusion assays revealed that four displayed anti-mycobacterial activity (zones of inhibition ranged from 10-18 mm; data not shown). The results of MIC assays are presented in Table 2. As indicated from the plate-hole diffusion assays, four of the extracts showed activity against *M. smegmatis* and two were also active against *M. fortuitum*. The greater level of activity towards *M. smegmatis* was also reflected in the MIC results which showed that, for the extracts which were active against both species, the MIC levels were greater for *M. fortuitum*. Of particular interest is that the *Eremophila* extracts exhibited broader activity compared to the other plants. These and other members of the *Eremophila* genus have previously been shown to have considerable broad-spectrum activity against Gram positive bacteria, including antibiotic-resistant strains (Palombo and Semple, 2001, 2002; Ndi *et al*., 2007a). However, this is the first study to demonstrate activity against species of *Mycobacterium*. The antibacterial activity of an extract of *Eremophila duttonii* against *Staphylococcus aureus* was shown to be the result of increased cell permeability due to cell membrane damage (Tomlinson and Palombo, 2005) while the antimicrobial constituents of some *Eremophila* species have recently been identified as *o*-naphthoquinones and serrulatane diterpenoids (Ndi *et al*., 2007b, c; Smith *et al*., 2007). Since terpenoids exert their antimicrobial activity by destabilization and permeabilization of bacterial membranes (Smith *et al*., 2007), it would be of interest to determine if the active extracts identified in this study exert similar damage to the mycobacterial cell membrane. There are limited reports of traditional uses of Australian plants in the treatment of TB (Lassak and McCarthy, 2001), although specific activity against bacteria has not been determined. The present study is the first extensive screening of Australian medicinal

Table 2: Anti-mycobacterial activity of Australian medicinal plant extracts

	Minimum inhibitory concentration (mg mL^{-1}) against	
Plant extract (parts extracted)	*M. fortuitum*	*M. smegmatis*
Acacia ligulata (bark and leaves)	-	51
Eremophila alternifolia (stems and leaves)	66	33
Eremophila longifolia (leaves)	40	20
Pterocaulon sphacelatum (aerial parts)	-	60

-: No activity

plants for anti-mycobacterial activity and it provides scientific evidence of specific activity that supports the traditional use of plants to treat respiratory illness. However, additional testing against the respiratory pathogen, *M. tuberculosis*, is needed to confirm that the observed activity extends to this species. Future research will also aim to determine the active compounds in these extracts.

ACKNOWLEDGMENTS

We are grateful to Dr. Susan Semple, University of South Australia, for providing plant extracts and Dr. Tim Stinear, Monash University, for providing strains of *Mycobacterium*.

REFERENCES

Bisht, D., M. Owais and K. Venkatesan, 2006. Potential of Plant-Derived Products in the Treatment of Mycobacterial Infections. In: Modern Phytomedicine, Ahmed, I., F. Aqil and M. Owais (Eds.) Wiley-VCH, Weinheim, pp: 293-311.

Bodle, E.E., J.A. Cunningham, P. Della-Latta, N.W. Schluger and L. Saiman, 2008. Epidemiology of nontuberculosis mycobacteria in patients without HIV infection, New York City. Emerg. Infect. Dis., 14: 390-396.

Cronquist, A.J., 1981. An Integrated System of Classification of Flowering Plants. 2nd Edn. Columbia University Press, New York, pp: 1262.

Gautam, R., A. Saklani and S.M. Jachak, 2007. Indian medicinal plants as a source of antimycobacterial agents. J. Ethnopharmacol., 110: 200-234.

Jessop, J.P., 1993. A list of the vascular plants of South Australia. Botanic gardens of adelaide and state herbarium, Adelaide, South Australia.

Lassak, E.V. and T. McCarthy, 2001. Australian Medicinal Plants. 1st Edn. Methuen Australia, New Holland .

McRae, J., Q. Yang, R. Crawford and E. Palombo, 2007. Review of the methods used for isolating pharmaceutical lead compounds from traditional medicinal plants. Environmentalist, 27: 165-174.

McRae, J.M., Q. Yang, R.F. Crawford and E.A. Palombo, 2008. Antibacterial compounds from *Planchonia careya* leaf extracts. J. Ethnopharmacol., 116: 554-560.

Ndi, C.P., S.J. Semple, H.J. Griesser and M.D. Barton, 2007a. Antimicrobial activity of some Australian plant species from the genus *Eremophila*. J. Basic Microbiol., 47: 158-164.

Ndi, C.P., S.J. Semple, H.J. Griesser, S.M. Pyke and M.D. Barton, 2007b. Antimicrobial compounds from the Australian desert plant *Eremophila neglecta*. J. Nat. Prod., 70: 1439-1443.

Ndi, C.P., S.J. Semple, H.J. Griesser, S.M. Pyke and M.D. Barton, 2007c. Antimicrobial compounds from *Eremophila serrulata*. Phytochemistry, 68: 2684-2690.

Palombo, E.A. and S.J. Semple, 2001. Antibacterial activity of traditional Australian medicinal plants. J. Ethnopharmacol., 77: 151-157.

Palombo, E.A. and S.J. Semple, 2002. Antibacterial activity of Australian plant extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). J. Basic Microbiol., 42: 444-448.

Sacchettini, J.C., E.J. Rubin and J.S. Freundlich, 2008. Drugs versus bugs: In pursuit of the persistent predator *Mycobacterium tuberculosis*. Nature Rev. Microbiol., 6: 41-52.

Smith, J.E., D. Tucker, K. Watson and G.L. Jones, 2007. Identification of antibacterial constituents from the indigenous Australian medicinal plant *Eremophila duttonii* F. Muell. (Myoporaceae). J. Ethnopharmacol., 112: 386-393.

Tomlinson, S. and E.A. Palombo, 2005. Characterisation of antibacterial Australian medicinal plant extracts by investigation of the mechanism of action and the effect of interfering substances. J. Basic Microbiol., 45: 363-370.

World Health Organization, 2007. Global tuberculosis control: Surveillance, planning, financing. WHO Report. Geneva, Switzerland (WHO/HTM/TB/2007.376).