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An extract from teak (*Tectona grandis*) bark inhibited *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus*

A. Neamatallah, L. Yan, S.J. Dewar and B. Austin
School of Life Sciences, Heriot-Watt University, Edinburgh, UK

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ABSTRACT

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Aims: The aim of this study was to characterize the inhibitory mechanism in teak (*Tectona grandis*) bark and to determine its effectiveness against *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus* (MRSA).

Methods and Results: Methanol extracts of teak bark were inhibitory to *L. monocytogenes* and MRSA by means of disc diffusion. Gas chromatography–mass spectrometry, and ^{1}H and ^{13}C nuclear mass resonance analyses revealed that the inhibitory compound had a molecular weight of 174, and a structure of 5-hydroxy-1,4-naphthalenedione (Juglone).

Conclusions: 5-hydroxy-1,4-naphthalenedione (Juglone) inhibited *L. monocytogenes* and MRSA.

Significance and Impact of the Study: A compound in an extract of teak bark was inhibitory to *L. monocytogenes* and MRSA.

Keywords: 5-hydroxy-1,4-naphthalenedione, antibacterial activity, Juglone, *Listeria monocytogenes*, *Staphylococcus aureus*, teak (*Tectona grandis*).

INTRODUCTION

Listeria monocytogenes, the causal agent of listeriosis/gastroenteritis (Miettinen *et al.* 1999; Tham *et al.* 2000) has been associated with smoked (Paranjpye *et al.* 1992; Vaz-Velho *et al.* 2001) and nonsmoked fish fillets (Nedoluha *et al.* 2001). Because of the implications for human health, efforts have been made to reduce the population of *Listeria* in fish tissues (Eklund *et al.* 2004) with attention focused on the use of sodium chlorite (Su and Morrissey 2003), nisin and lactoperoxidase (Elotmani and Assobhei 2004), divercin (Richard *et al.* 2003), steam (Bremer *et al.* 2002), liquid smoke (Vitt *et al.* 2001) and microbial antagonists (Duffes *et al.* 2000). During an examination of the effects of wood on the fish smoking process, it was determined that a methanol extract of teak bark demonstrated inhibitory properties against *L. monocytogenes* and an example of a highly antibiotic-resistant strain of a human pathogen, i.e. methicillin-resistant *Staphylococcus aureus* (MRSA). This inhibition has been examined further, and the nature of the inhibitory compound characterized.

Correspondence to: B. Austin, School of Life Sciences, John Muir Building, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK (e-mail: b.austin@hw.ac.uk).

MATERIAL AND METHODS

Plant material

Teak bark, authenticated by the Department of Arid Land Agriculture, King Abdul Aziz University, Jeddah, was collected and air-dried in Saudi Arabia. The bark was maintained at room temperature in polythene bags.

Extraction and purification of the antibacterial compound

Tiny slivers of the bark were cut with a sharp knife, and 2·5 g quantities were soaked overnight in 25 ml volumes of analytical grade methanol (Fisher, Loughborough, UK) at room temperature. The methanol supernatant was filtered

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(0·45 μm pore size membranes; Millex- HA, Carigtwohill, Ireland) and evaporated to dryness (Büchi, Flawil, Switzerland). The solid was reconstituted in 200 ml of a solvent mixture containing water, methanol (Fisher) and dichloromethane (Fisher) (2 : 1 : 1; v/v). The solvent mixture was evaporated at 40°C. Thereafter, the dried material was reconstituted in 1·5 ml of methanol, and further fractionated by solid phase extraction (Sep-Pak Vac 35 cc silica cartridge; Waters, Elstree, UK) using gradient hexane and ethyl acetate mixture as the mobile phase (from 100% hexane to 100% ethyl acetate with 1% steps). Fractions were examined by silica thin layer chromatography (TLC), with hexane and ethyl acetate (70 : 30) as the migration solvent (Wagnam and Weinstein 1973).

Bacterial cultures

Listeria monocytogenes ATCC 19115 (American Type Culture Collection, Manassas, VA, USA), NCTC 10357^T (type culture; National Collection of Type Cultures, Colindale, London, UK) and four fresh isolates obtained from smoked haddock (Neamatallah *et al.* 2003) and MRSA 4551 (supplied by Professor S.G.B. Amyes, University of Edinburgh) were maintained at room temperature on plates of modified Listeria selective medium (MLSM; Neamatallah *et al.* 2003) and tryptone soya agar (TSA; Oxoid, Basingstoke, UK), as appropriate, with subculturing every week. Long-term storage was as suspensions in 15% (v/v) glycerol at −70°C.

Antibacterial bioassay

A disc diffusion method was used to determine bacterial inhibition by teak bark extracts. Thus, the *Listeria* and MRSA cultures were grown overnight at room temperature in Fraser broth (Oxoid) and tryptone soya broth (TSB; Oxoid), respectively. Lawns of *L. monocytogenes* and MRSA were prepared using 0·1 ml volumes of overnight broth cultures on MLSM and TSA respectively. These broth cultures contained *c.* 10^8 cells ml^{-1}. Then, 1, 2, 3 and 5 μl volumes of purified extract, which corresponded with 1·7, 3·4, 5·1 and 8·5 mg of bark, were pipetted onto 6 mm diameter Whatman (Maidstone, UK) filter paper discs, air-dried, and placed on the bacterial lawns before incubation at 37°C overnight. Comparisons were made with identical quantities of Juglone (Sigma, Poole, UK). Antibacterial activity was recorded when zones of clearing were observed. Purified material from silica TLC Bio-assay plates (Nunc, Hereford, UK) was examined to determine the presence of bioactive compounds (after Austin and Billaud 1990). Thus, the TLC sheet was placed on top of MLSM or TSA, as appropriate, and incubated at 4°C for 3 h to allow antimicrobial compounds to diffuse into the medium. These were overpoured with 100 ml of molten cooled MLSM or TSA seeded with 1 ml of an overnight broth culture, which cultures contained *c.* 10^8 cells ml^{-1} of *L. monocytogenes* or MRSA. After incubation at 37°C for 18 h, 5·0 ml of 10% (w/v) tetrazolium (sodium salt; Sigma) was added, and the presence or absence and precise location of zones of clearing were recorded. Spots in the TLC, considered to contain antimicrobial compounds, were scraped into solvent, i.e. ethyl acetate, and inhibition re-affirmed by antibiogrammes (Austin and Billaud 1990).

Chemical characterization

The purified bioactive material was accumulated from silica using Sep-Pak Vac 35 cc cartridges. The extracts in chloroform (Fisher) were examined by ^{1}H and ^{13}C nuclear magnetic resonance [NMR; Bruker (Coventry, UK), DPX400MH] for proton and carbon spectra, respectively, gas chromatography (GC), GC-mass spectrometry (QP-7000; Shimadzu, Duisburg, Germany) using fused silica capillary columns (30 m × 0·25 mm ID) and 0·25 μm thick films of 5% phenyl and 95% methylsilicon, and infrared spectra (RXIFT-IR; Perkin Elmer, Boston, MA, USA).

RESULTS AND DISCUSSION

From the teak bark, a compound was obtained with inhibitory activity against *L. monocytogenes* and MRSA (Table 1). By GC-mass spectrometry, this compound was deduced to have a molecular weight of 174. ^{1}H and ^{13}C NMR suggested that aromatic rings/phenolic groups, 6 hydrogens, 2 double bonds, and hydrogen were contained in the molecule. The evidence suggested that there was a double benzene ring. The most likely structure was 5-hydroxy-1,4-naphthalenedione (Fig. 1) =$C_{10}H_6O_3$= Juglone, a commercial preparation of

Table 1 Inhibition of *Listeria monocytogenes* and MRSA by an extract of teak bark

	Volume of purified extract			
Culture reference no.	1 μl	2 μl	3 μl	5 μl
L. monocytogenes 1*	8 (7)†	9 (8)	11 (9)	11 (10)
L. monocytogenes 2	7 (7)	9 (8)	11 (9)	11 (10)
L. monocytogenes 3	8 (7)	10 (9)	12 (9)	12 (10)
L. monocytogenes 4	7 (7)	9 (8)	12 (9)	12 (10)
L. monocytogenes ATCC 19115	7 (7)	9 (8)	12 (9)	11 (10)
L. monocytogenes NCTC 10357	7 (7)	8 (8)	9 (8)	10 (10)
MRSA 4551	0 (0)	7 (7)	8 (8)	9 (8)

Data are for zones of clearing as measured from the edge of the discs (mm).

*Recovered from smoked haddock.

†Data in parentheses refer to the zones of clearing obtained with the commercial preparation of Juglone.

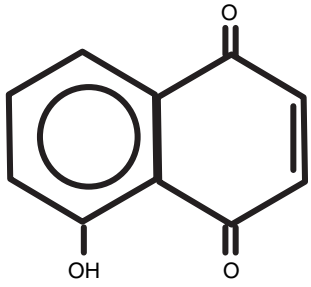

Fig. 1 Structure of 5-hydroxy-1,4-naphthalenedione

which was also inhibitory to *L. monocytogenes* and MRSA (Table 1).

5-hydroxy-1,4-naphthalenedione has been previously isolated from a few plants, i.e. walnut, butternut (Funt and Martin 1999) and muthala (Cai *et al.* 2000). There are unconfirmed reports of activity and widespread use against a wide range of bacterial, viral and parasitic diseases (Blumenthal 1998). However, the mode of action is really unknown, although there has been some indication of cytotoxicity (Inbaraj and Chignell 2004). Juglone has been found to be inhibitory to oral pathogens, notably *Streptococcus mutans*, *Streptococcus sanguis*, *Porphyromonas gingivalis* and *Prevotella intermedia* (Didry *et al.* 1994; Cai *et al.* 2000), and may explain the value of twigs/sticks used for oral hygiene in Africa and the Middle East (Cai *et al.* 2000; Wu *et al.* 2001). However, this is the first indication of more widespread inhibition against other Gram-positive bacterial pathogens, notably *L. monocytogenes* and MRSA. Certainly, there is increasing evidence for the medicinal benefit of natural plant compounds, which may be a source of compounds for combating antibiotic-resistant pathogens, such as MRSA.

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