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Antimicrobial activity of essential oils and other plant extracts

K.A. Hammer[1], C.F. Carson[1] and T.V. Riley[1,2]

[1]Department of Microbiology, The University of Western Australia, and [2]The Western Australian Centre for Pathology and Medical Research, Queen Elizabeth II Medical Centre, Nedlands, Western Australia

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K.A. HAMMER, C.F. CARSON AND T.V. RILEY. 1999. The antimicrobial activity of plant oils and extracts has been recognized for many years. However, few investigations have compared large numbers of oils and extracts using methods that are directly comparable. In the present study, 52 plant oils and extracts were investigated for activity against *Acinetobacter baumanii*, *Aeromonas veronii* biogroup *sobria*, *Candida albicans, Enterococcus faecalis, Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serotype *typhimurium, Serratia marcescens* and *Staphylococcus aureus*, using an agar dilution method. Lemongrass, oregano and bay inhibited all organisms at concentrations of $\leqslant 2{\cdot}0\%$ (v/v). Six oils did not inhibit any organisms at the highest concentration, which was 2·0% (v/v) oil for apricot kernel, evening primrose, macadamia, pumpkin, sage and sweet almond. Variable activity was recorded for the remaining oils. Twenty of the plant oils and extracts were investigated, using a broth microdilution method, for activity against *C. albicans*, *Staph. aureus* and *E. coli*. The lowest minimum inhibitory concentrations were 0·03% (v/v) thyme oil against *C. albicans* and *E. coli* and 0·008% (v/v) vetiver oil against *Staph. aureus*. These results support the notion that plant essential oils and extracts may have a role as pharmaceuticals and preservatives.

INTRODUCTION

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones 1996). These purposes vary from the use of rosewood and cedarwood in perfumery, to flavouring drinks with lime, fennel or juniper berry oil (Lawless 1995), and the application of lemongrass oil for the preservation of stored food crops (Mishra and Dubey 1994). In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds 1996; Lis-Balchin and Deans 1997).

While some of the oils used on the basis of their reputed antimicrobial properties have well documented *in vitro* activity, there are few published data for many others (Morris *et al*. 1979; Ross *et al*. 1980; Yousef and Tawil 1980; Deans and Ritchie 1987; Hili *et al*. 1997). Some studies have concentrated exclusively on one oil or one micro-organism. While these data are useful, the reports are not directly comparable due to methodological differences such as choice of plant extract(s), test micro-organism(s) and antimicrobial test method (Janssen *et al*. 1987).

The aim of this study was to test a large number of essential oils and plant extracts against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and a yeast. The purpose of this was to create directly comparable, quantitative, antimicrobial data and to generate data for oils for which little data exist.

Correspondence to: Dr K.A. Hammer, Department of Microbiology, The University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009 (e-mail: khammer@cyllene.uwa.edu.au).

MATERIALS AND METHODS

Organisms and growth conditions

Micro-organisms were obtained from the culture collections of the Department of Microbiology at The University of Western Australia and the Western Australian Centre for Pathology and Medical Research. Organisms were as follows: *Acinetobacter baumanii* NCTC 7844, *Aeromonas veronii* biogroup *sobria* ATCC 9071 (*Aer. sobria*), *Candida albicans*

Table 1 Minimum inhibitory concentrations (MICs) of selected essential oils (% v/v) against 10 different micro-organisms

Details of plant oils and extracts				Test organism									
Plant species	Common name	Extract type*	Source†	*Acinetobacter baumanii*	*Aeromonas sobria*	*Candida albicans*	*Enterococcus faecalis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Serratia marcescens*	*Staphylococcus aureus*
Aniba rosaeodora	Rosewood	EO	W	0·12	0·12	0·25	0·5	0·12	0·5	>2·0	0·25	0·5	0·25
Apium graveolens	Celery seed	EO	S	>2·0	1·0	1·0	2·0	2·0	>2·0	>2·0	>2·0	>2·0	1·0
Boswellia carterii	Frankincense	EO	R	1·0	1·0	1·0	2·0	1·0	>2·0	>2·0	>2·0	>2·0	1·0
Cananga odorata	Ylang ylang	EO	FL	1·0	0·5	1·0	2·0	2·0	>2·0	>2·0	>2·0	>2·0	1·0
Cedrus atlantica	Cedarwood	EO	W	>2·0	>2·0	>2·0	0·5	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Citrus aurantifolia	Lime	EO	FR	1·0	1·0	2·0	>2·0	1·0	>2·0	>2·0	>2·0	>2·0	2·0
Citrus aurantium	Orange	EO	P	>2·0	1·0	1·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	2·0
Citrus aurantium	Petitgrain	EO	LT	0·5	0·5	0·25	2·0	0·25	>2·0	>2·0	>2·0	>2·0	0·5
Citrus aurantium var. *bergamia*	Bergamot	EO	P	2·0	2·0	1·0	>2·0	1·0	>2·0	>2·0	>2·0	>2·0	>2·0
Citrus limon	Lemon	EO	P	>2·0	1·0	2·0	2·0	>2·0	>2·0	>2·0	>2·0	>2·0	2·0
Citrus x paradisi	Grapefruit	EO	P	>2·0	1·0	1·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Citrus reticulata var. *madurensis*	Mandarin	EO	P	>2·0	>2·0	2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Commiphora myrrha	Myrrh	EO	R	>2·0	>2·0	>2·0	0·25	>2·0	>2·0	>2·0	>2·0	>2·0	0·5
Coriandrum sativum	Coriander	EO	S	0·25	0·25	0·25	1·0	0·25	0·5	>2·0	1·0	0·5	0·25
Cucurbita pepo	Pumpkin	FO	S	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Cupressus sempervirens	Cypress	EO	LT	>2·0	>2·0	>2·0	1·0	>2·0	>2·0	>2·0	>2·0	>2·0	2·0
Cymbopogon citratus	Lemongrass	EO	L	0·03	0·12	0·06	0·12	0·06	0·25	1·0	0·25	0·25	0·06
Cymbopogon martinii	Palmarosa	EO	L	0·12	0·12	0·06	0·25	0·06	0·25	>2·0	0·5	0·25	0·12
Cymbopogon nardus	Citronella	EO	L	0·25	nd	0·12	1·0	0·5	1·0	>2·0	>2·0	>2·0	0·25
Daucus carota	Carrot seed	EO	S	>2·0	>2·0	2·0	2·0	>2·0	>2·0	>2·0	>2·0	>2·0	1·0
Eucalyptus polybractea	Eucalyptus	EO	LT	1·0	0·5	1·0	2·0	1·0	2·0	>2·0	>2·0	1·0	2·0
Foeniculum vulgare	Fennel	EO	S	1·0	0·5	0·5	>2·0	0·5	>2·0	>2·0	1·0	>2·0	0·25
Gaultheria procumbens	Wintergreen	EO	H	0·25	0·25	0·25	>2·0	0·5	1·0	>2·0	0·5	0·5	2·0
Juniperus communis	Juniper	EO	B	>2·0	1·0	2·0	2·0	>2·0	>2·0	>2·0	2·0	>2·0	2·0
Lavandula angustifolia	French lavender	EO	FL	1·0	nd	0·5	>2·0	0·5	2·0	>2·0	>2·0	>2·0	1·0
Lavandula angustifolia	Tasmanian lavender	EO	FL	0·5	0·5	0·25	2·0	0·25	>2·0	>2·0	>2·0	2·0	1·0
Macadamia integrifolia	Macadamia	FO	NT	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Melaleuca alternifolia	Tea tree	EO	LT	0·25	0·5	0·5	2·0	0·25	0·5	>2·0	0·5	0·5	0·5
Melaleuca cajuputi	Cajuput	EO	LT	1·0	1·0	1·0	2·0	1·0	>2·0	>2·0	>2·0	2·0	1·0
Melaleuca quinquenervia	*Niaouli*	*EO*	*LT*	*0·25*	*0·25*	*0·25*	*1·0*	*0·25*	*0·5*	*>2·0*	*0·5*	*0·5*	*0·5*
Mentha x piperita	Peppermint	EO	H	0·5	nd	0·5	2·0	0·5	1·0	>2·0	1·0	2·0	1·0
Mentha spicata	Spearmint	EO	H	0·25	0·25	0·12	2·0	0·25	0·5	>2·0	0·5	0·25	0·25
Ocimum basilicum	Basil	EO	H	0·5	0·5	0·5	>2·0	0·5	2·0	>2·0	2·0	>2·0	2·0
Oenothera biennis	Evening primrose	FO	S	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Origanum majorana	Marjoram	EO	H	0·25	0·25	0·25	2·0	0·25	0·5	>2·0	0·5	0·5	0·5
Origanum vulgare	Oregano	EO	H	0·12	0·12	0·12	0·25	0·12	0·12	2·0	0·12	0·25	0·12
Pelargonium graveolens	Geranium	EO	H	0·25	0·25	0·12	0·5	0·25	>2·0	>2·0	>2·0	>2·0	0·25
Pimpinella anisum	Aniseed	EO	S	0·5	0·25	0·5	2·0	0·5	>2·0	>2·0	2·0	1·0	0·25
Pimenta racemosa	Bay	EO	L	0·12	0·12	0·12	0·5	0·12	0·25	1·0	0·25	0·25	0·25

Table 1 — *continued*

Details of plant oils and extracts				Test organism									
Plant species	Common name	Extract type*	Source†	*Acinetobacter baumanii*	*Aeromonas sobria*	*Candida albicans*	*Enterococcus faecalis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Serratia marcescens*	*Staphylococcus aureus*
Pinus sylvestris	Pine	EO	N	2·0	2·0	2·0	>2·0	2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Piper nigrum	Black pepper	EO	B	>2·0	>2·0	>2·0	1·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Pogostemon patchouli	Patchouli	EO	L	>2·0	>2·0	0·5	0·12	>2·0	>2·0	>2·0	>2·0	>2·0	0·25
Prunus armeniaca	Apricot kernel	FO	S	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Prunus dulcis	Sweet almond	FO	S	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Rosmarinus officinalis	Rosemary	EO	H	1·0	0·5	1·0	>2·0	1·0	2·0	>2·0	>2·0	>2·0	1·0
Salvia officinalis	Sage	EO	H	0·5	0·5	0·5	2·0	0·5	2·0	>2·0	2·0	1·0	1·0
Salvia sclarea	Clary sage	EO	H	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0
Santalum album	Sandalwood	EO	W	>2·0	>2·0	0·06	0·25	>2·0	>2·0	>2·0	>2·0	>2·0	0·12
Syzygium aromaticum	Clove	EO	BD	0·25	nd	0·12	0·5	0·25	0·25	>2·0	>2·0	0·25	0·25
Thymus vulgaris	Thyme	EO	H	0·12	0·12	0·12	0·5	0·12	0·25	>2·0	>2·0	0·25	0·25
Vetiveria zizanioides	Vetiver	EO	L	>2·0	>2·0	0·12	0·12	>2·0	>2·0	>2·0	>2·0	>2·0	0·06
Zingiber officinale	Ginger	EO	RH	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	>2·0	2·0

* EO, essential oil; FO, fixed oil; R, resin.
† B, berry; BD, bud; FL, flower; FR, fruit; H, herb; L, leaf; LT, leaves and twigs; N, needles; NT, nut; P, peel; RH, rhizome; S, seed; W, wood.

ATCC 10231, *Enterococcus faecalis* NCTC 8213, *Escherichia coli* NCTC 10418, *Klebsiella pneumoniae* NCTC 11228, *Pseudomonas aeruginosa* NCTC 10662, *Salmonella enterica* subsp. *enterica* serotype *typhimurium* ATCC 13311 (*Salm. typhimurium*), *Serratia marcescens* NCTC 1377 and *Staphylococcus aureus* NCTC 6571. Organisms were maintained on blood agar (BA) (Unipath). Overnight cultures were prepared by inoculating approximately 2 ml Mueller Hinton broth (MHB) (Unipath) with 2–3 colonies of each organism taken from BA. Broths were incubated overnight at 35 °C with shaking. Inocula were prepared by diluting overnight cultures in saline to approximately 10^8 cfu ml^{-1} for bacteria and 10^7 cfu ml^{-1} for *C. albicans*. These suspensions were further diluted with saline as required.

Essential oils

Macadamia oil was provided by Australian Plantations Pty Ltd, Wyrallah, NSW, Australia. All other plant oils and extracts were obtained from Sunspirit Oils Pty Ltd, Byron Bay, NSW, Australia. Details of the sources of extracts, as provided by Sunspirit Oils Pty Ltd, are given in Table 1. Plant oils and extracts were derived from a total of 37 genera. All oils were diluted v/v in both agar and broth dilution methods.

Agar dilution method

The agar dilution method followed that approved by the NCCLS with the following modification: a final concentration of 0·5% (v/v) Tween-20 (Sigma) was incorporated into the agar after autoclaving to enhance oil solubility. Briefly, a series of twofold dilutions of each oil, ranging from 2% (v/v) to 0·03% (v/v), was prepared in Mueller Hinton agar with 0·5% (v/v) Tween-20. Plates were dried at 35 °C for 30 min prior to inoculation with 1–2 μl spots containing approximately 10^4 cfu of each organism, using a multipoint replicator (Mast Laboratories Ltd, Liverpool, UK). Mueller Hinton agar, with 0·5% (v/v) Tween-20 but no oil, was used as a positive growth control. Inoculated plates were incubated at 35 °C for 48 h. Minimum inhibitory concentrations (MICs) were determined after 24 h for the bacteria and after 48 h for *C. albicans*. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded.

Broth microdilution method

The broth microdilution assay was performed as described previously (Hammer *et al.* 1996) with the following modifications: MHB was used instead of heart infusion broth and, in tests with *C. albicans*, sub-cultures were performed after 48 h incubation. For most oils, the highest concentration tested was 4·0% (v/v), although for some this was 8·0% (v/v). The lowest concentration tested was 0·008% (v/v).

RESULTS

The MICs of 52 plant oils and extracts obtained by the agar dilution method are shown in Table 1. Lemongrass, oregano and bay inhibited all organisms at $\leqslant$2·0% (v/v). Rosewood, coriander, palmarosa, tea tree, niaouli, peppermint, spearmint, sage and marjoram inhibited all organisms except *Ps. aeruginosa* at $\leqslant$2·0% (v/v). Six oils, comprising the five fixed oils (pumpkin, macadamia, evening primrose, apricot kernel and sweet almond) and the essential oil clary sage, failed to inhibit any organisms at the highest concentration, which was 2·0% (v/v). Myrrh and cypress inhibited Gram-positive organisms only, while carrot, patchouli, sandalwood and vetiver inhibited Gram-positive bacteria and *C. albicans* only. Mandarin oil inhibited *C. albicans* at 2·0% (v/v), while bacteria were not inhibited at $\leqslant$2·0% (v/v). None of the oils inhibited Gram-negative bacteria only.

Pseudomonas aeruginosa was inhibited by the lowest number of extracts (three), significantly less susceptible than *Salm. typhimurium* (17). *Candida albicans* and *Staph. aureus* were the most susceptible organisms, inhibited at $\leqslant$2·0% (v/v) by 41 and 40 extracts, respectively.

Table 2 shows MICs and minimum cidal concentrations (MCCs) of 20 plant oils and extracts obtained by the broth microdilution method. Thyme had the lowest MIC of 0·03% (v/v) against *C. albicans* and *E. coli*, and vetiver had the lowest MIC of 0·008% (v/v) against *Staph. aureus*. Comparison of MICs obtained by agar and broth methods showed that differences exceeding two serial dilutions were seen with peppermint, patchouli, sandalwood, thyme and vetiver. The greatest difference was for *C. albicans* and sandalwood, where the MIC obtained by agar dilution was 0·06% (v/v) compared with the MIC by broth microdilution of $>$8·0% (v/v).

DISCUSSION

Anecdotal evidence and the traditional use of plants as medicines provide the basis for indicating which essential oils and plant extracts may be useful for specific medical conditions. Historically, many plant oils and extracts, such as tea tree, myrrh and clove, have been used as topical antiseptics, or have been reported to have antimicrobial properties (Hoffman 1987; Lawless 1995). It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Mitscher *et al.* 1987). Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required.

Various publications have documented the antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, bay, basil, tea tree, celery seed and fennel (Morris *et al.* 1979; Ross *et al.* 1980; Yousef and Tawil 1980; Hili *et al.* 1997; Lis-Balchin and Deans 1997). Oils such as sweet almond, carrot and mandarin were shown to possess little or no antimicrobial activity (Morris *et al.* 1979; Deans and Ritchie 1987; Smith-Palmer *et al.* 1998). These findings were confirmed in the present investigation. Some of the oils tested here, including pumpkin, evening primrose and rosewood, have not been investigated previously. Of these, only rosewood oil showed any significant antimicrobial activity. Not surprisingly, the fixed oils, which are used largely as diluents for essential oils or as sources of dietary fatty acids (Newall *et al.* 1996; Reynolds 1996), did not show significant antimicrobial activity.

When comparing data obtained in different studies, most publications provide generalizations about whether or not a plant oil or extract possesses activity against Gram-positive and Gram-negative bacteria and fungi. However, not all provide details about the extent or spectrum of this activity. Some publications also show the relative activity of plant oils and extracts by comparing results from different oils tested against the same organism(s).

Comparison of the data obtained in this study with previously published results is problematic. First, the composition of plant oils and extracts is known to vary according to local climatic and environmental conditions (Janssen *et al.* 1987; Sivropoulou *et al.* 1995). Furthermore, some oils with the same common name may be derived from different plant species (Windholz *et al.* 1983; Reynolds 1996).

Secondly, the method used to assess antimicrobial activity, and the choice of test organism(s), varies between publications (Janssen *et al.* 1987). A method frequently used to screen plant extracts for antimicrobial activity is the agar disc diffusion technique (Morris *et al.* 1979; Smith-Palmer *et al.* 1998). The usefulness of this method is limited to the generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium (Janssen *et al.* 1987; Rios *et al.* 1988). Agar and broth dilution methods are also commonly used. The results obtained by each of these methods may differ as many factors vary between assays (Janssen *et al.* 1987; Hili *et al.* 1997). These include differences in microbial growth, exposure of micro-organisms to plant oil, the solubility of oil or oil components, and the use and quantity of an emulsifier.

Table 2 Minimum inhibitory concentration and minimum cidal concentration data (% v/v) obtained by the broth microdilution method

	Staphylococcus aureus		*Escherichia coli*		*Candida albicans*	
Plant species	MIC	MCC	MIC	MCC	MIC	MCC
Aniba rosaeodora	0·12	0·25	0·12	0·12	0·12	0·25
Boswellia carterii	0·5	4·0	1·0	1·0	0·5	1·0
Cananga odorata	>4·0	>4·0	>4·0	>4·0	2·0	4·0
Commiphora myrrha	0·5	0·5	>4·0	>4·0	4·0	>4·0
Cymbopogon citratus	0·06	0·06	0·12	0·12	0·06	0·06
Cymbopogon martinii	0·12	0·12	0·12	0·12	0·12	0·12
Cymbopogon nardus	0·12	0·25	0·25	0·25	0·12	0·12
Juniperus communis	2·0	4·0	4·0	4·0	2·0	4·0
Lavandula angustifolia (Tasmanian)	0·5	1·0	0·25	0·25	0·5	1·0
Macadamia integrifolia	>8·0	>8·0	>8·0	>8·0	>8·0	>8·0
Mentha x *piperita*	0·12	0·25	0·12	0·12	0·12	0·25
Oenothera biennis	>4·0	>4·0	>4·0	>4·0	>4·0	>4·0
Pimenta racemosa	0·12	0·12	0·12	0·12	0·06	0·12
Pogostemon patchouli	0·03	0·03	>2·0	>2·0	>2·0	>2·0
Prunus dulcis	>8·0	>8·0	>8·0	>8·0	>8·0	>8·0
Santalum album	0·03	0·03	>8·0	>8·0	>8·0	>8·0
Syzygium aromaticum	0·12	0·25	0·12	0·12	0·12	0·12
Thymus vulgaris	0·03	0·06	0·03	0·03	0·03	0·06
Vetiveria zizanioides	0·008	0·015	>4·0	>4·0	>4·0	>4·0
Zingiber officinale	>4·0	>4·0	>4·0	>4·0	>4·0	>4·0

These and other elements may account for the large differences in MICs obtained by the agar and broth dilution methods in this study. *In vivo* studies may be required to confirm the validity of some of the results obtained.

The need for a standard, reproducible method for assessing oils has been stressed by several authors (Carson *et al*. 1995; Mann and Markham 1998). In view of this, many methods have been developed specifically for determining the antimicrobial activity of essential oils (Remmal *et al*. 1993; Carson *et al*. 1995; Smith and Navilliat 1997; Mann and Markham 1998). The benefits of basing new methods on pre-existing, conventional assays such as the NCCLS methods are that these assays tend to be more readily accepted by regulatory bodies (Carson *et al*. 1995; Smith and Navilliat 1997). Also, these methods have been designed specifically for assessing the activity of antimicrobial compounds, and factors affecting reproducibility have been sufficiently investigated. Although NCCLS methods have been developed for assessing conventional antimicrobial agents such as antibiotics, with minor modifications these methods can be made suitable for the testing of essential oils and plant extracts (Carson *et al*. 1995).

For some plant oils, such as wintergreen, eucalyptus, clove and sage, there has been much research and reporting of toxic and irritant properties (Lawless 1995; Newall *et al*. 1996; Reynolds 1996). In spite of this, most of these oils are available for purchase as whole oils or as part of pharmaceutical or cosmetic products, indicating that toxic properties do not prohibit their use. However, the on-going investigation of toxic or irritant properties is imperative, especially when considering any new products for human use, be they medicinal or otherwise.

In summary, this study confirms that many essential oils and plant extracts possess *in vitro* antibacterial and antifungal activity. However, if plant oils and extracts are to be used for food preservation or medicinal purposes, issues of safety and toxicity will need to be addressed.

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