Antimicrobial activity of essential oils and other plant extracts

K.A. Hammer¹, C.F. Carson¹ and T.V. Riley^{1,2}

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

6959/11/98: received 9 November 1998, revised 19 February 1999 and accepted 23 February 1999

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 $\leq 2.0\%$ (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

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). 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.

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 | d extracts | | | Test or | ganism | | | | | | | | |
|--------------------------------------|-----------------------|---------------|--------------|------------------------|------------------|------------------|-----------------------|------------------|-----------------------|------------------------|------------------------|---------------------|-----------------------|
| 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 | $> 2 \cdot 0$ | 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 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | > 2.0 | 1.0 |
| Cananga odorata | Ylang ylang | EO | FL | 1.0 | 0.5 | 1.0 | 2.0 | 2.0 | > 2.0 | $> 2 \cdot 0$ | > 2.0 | > 2.0 | 1.0 |
| Cedrus atlantica | Cedarwood | EO | W | $> 2 \cdot 0$ | > 2.0 | > 2.0 | 0.5 | > 2.0 | > 2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Citrus aurantifolia | Lime | EO | FR | 1.0 | 1.0 | 2.0 | $> 2 \cdot 0$ | 1.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 2.0 |
| Citrus aurantium | Orange | EO | P | $> 2 \cdot 0$ | 1.0 | 1.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 2.0 |
| Citrus aurantium | Petitgrain | EO | LT | 0.5 | 0.5 | 0.25 | 2.0 | 0.25 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.5 |
| Citrus aurantium var. | Bergamot | EO | P | 2.0 | 2.0 | 1.0 | $> 2 \cdot 0$ | 1.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| bergamia | · · | | | | | | | | | | | | |
| Citrus limon | Lemon | EO | P | $> 2 \cdot 0$ | 1.0 | 2.0 | 2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 2.0 |
| Citrus x paradisi | Grapefruit | EO | P | $> 2 \cdot 0$ | 1.0 | 1.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| 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 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.25 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.5 |
| Coriandrum sativum | Coriander | EO | S | 0.25 | 0.25 | 0.25 | 1.0 | 0.25 | 0.5 | $> 2 \cdot 0$ | 1.0 | 0.5 | 0.25 |
| Cucurbita pepo | Pumpkin | FO | \mathbf{S} | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Cupressus sempervirens | Cypress | EO | LT | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 1.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 2.0 |
| Cymbopogon citratus | Lemongrass | EO | L | 0.03 | 0.12 | 0.09 | 0.12 | 0.09 | 0.25 | 1.0 | 0.25 | 0.25 | 0.06 |
| Cymbopogon martinii | Palmarosa | EO | L | 0.12 | 0.12 | 0.09 | 0.25 | 0.09 | 0.25 | $> 2 \cdot 0$ | 0.5 | 0.25 | 0.12 |
| Cymbopogon nardus | Citronella | EO | L | 0.25 | nd | 0.12 | 1.0 | 0.5 | 1.0 | $> 2 \cdot 0$ | > 2.0 | $> 2 \cdot 0$ | 0.25 |
| Daucus carota | Carrot seed | EO | S | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 2.0 | 2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 1.0 |
| Eucalyptus polybractea | | EO | LT | 1.0 | 0.5 | 1.0 | 2.0 | 1.0 | 2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 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 | | 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 | В | >2.0 | 1.0 | 2.0 | 2.0 | > 2.0 | > 2.0 | > 2.0 | 2.0 | > 2.0 | 2.0 |
| Lavandula angustifolia | | 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 | | FO | NT | > 2.0 | > 2.0 | > 2.0 | $> 2 \cdot 0$ | > 2.0 | > 2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | > 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 | Η | 0.5 | nd | 0.5 | 2.0 | 0.5 | 1.0 | $> 2 \cdot 0$ | 1.0 | 2.0 | 1.0 |
| Mentha spicata | Spearmint | EO | Η | 0.25 | 0.25 | 0.12 | 2.0 | 0.25 | 0.5 | $> 2 \cdot 0$ | 0.5 | 0.25 | 0.25 |
| Ocimum basilicum | Basil | EO | Н | 0.5 | 0.5 | 0.5 | $> 2 \cdot 0$ | 0.5 | 2.0 | $> 2 \cdot 0$ | 2.0 | $> 2 \cdot 0$ | 2.0 |
| Oenothera biennis | Evening primrose | FO | S | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Origanum majorana | Marjoram | EO | Н | 0.25 | 0.25 | 0.25 | 2.0 | 0.25 | 0.5 | $> 2 \cdot 0$ | 0.5 | 0.5 | 0.5 |
| Origanum vulgare | Oregano | EO | Н | 0.12 | 0.12 | 0.12 | 0.25 | 0.12 | 0.12 | 2.0 | 0.12 | 0.25 | 0.12 |
| Pelargonium graveolens | | EO | Н | 0.25 | 0.25 | 0.12 | 0.5 | | > 2.0 | $> 2 \cdot 0$ | > 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 or | est 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 | В | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 1.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Pogostemon patchouli | Patchouli | EO | L | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.5 | 0.12 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.25 |
| Prunus armeniaca | Apricot kernel | FO | S | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Prunus dulcis | Sweet almond | FO | \mathbf{S} | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Rosmarinus officinalis | Rosemary | EO | Н | 1.0 | 0.5 | 1.0 | $> 2 \cdot 0$ | 1.0 | 2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 1.0 |
| Salvia officinalis | Sage | EO | Н | 0.5 | 0.5 | 0.5 | 2.0 | 0.5 | 2.0 | $> 2 \cdot 0$ | 2.0 | 1.0 | 1.0 |
| Salvia sclarea | Clary sage | EO | Н | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ |
| Santalum album | Sandalwood | EO | W | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.06 | 0.25 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.12 |
| Syzygium aromaticum | Clove | EO | BD | 0.25 | nd | 0.12 | 0.5 | 0.25 | 0.25 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.25 | 0.25 |
| Thymus vulgaris | Thyme | EO | Н | 0.12 | 0.12 | 0.12 | 0.5 | 0.12 | 0.25 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.25 | 0.25 |
| Vetiveria zizanioides | Vetiver | EO | L | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.12 | 0.12 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 0.06 |
| Zingiber officinale | Ginger | EO | RH | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | >2.0 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | 2.0 |

^{*}EO, essential oil; FO, fixed oil; R, resin.

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 10⁸ cfu ml⁻¹ for bacteria and 10⁷ 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 $1-2 \mu l$ spots containing approximately 10⁴ 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.

[†] 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.

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 $\leq 2.0\%$ (v/v). Rosewood, coriander, palmarosa, tea tree, niaouli, peppermint, spearmint, sage and marjoram inhibited all organisms except *Ps. aeruginosa* at $\leq 2.0\%$ (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 $\leq 2.0\%$ (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 $\leq 2.0\%$ (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 scien-

tifically 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

| | Staphyloco | ccus aureus | Escherich | hia 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 | 0.5 | 1.0 | 0.25 | 0.25 | 0.5 | 1.0 | |
| (Tasmanian) | | | | | | | |
| 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 | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | $> 2 \cdot 0$ | |
| Prunus dulcis | > 8.0 | > 8.0 | > 8.0 | $> 8 \cdot 0$ | $> 8 \cdot 0$ | > 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 preexisting, 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.

ACKNOWLEDGEMENTS

The authors thank Sunspirit Oils Pty Ltd, Byron Bay, NSW, Australia for providing essential oils and plant extracts. This work was supported by Australian Bodycare Corporation Pty Ltd, Currumbin, QLD, Australia and, in part, by a grant from the Rural Industries Research and Development Corporation (UWA-24 A).

REFERENCES

Carson, C.F., Hammer, K.A. and Riley, T.V. (1995) Broth micro-

- dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Microbios* 82, 181–185.
- Deans, S.G. and Ritchie, G. (1987) Antibacterial properties of plant essential oils. *International Journal of Food Microbiology* 5, 165–180
- Hammer, K.A., Carson, C.F. and Riley, T.V. (1996) Susceptibility of transient and commensal skin flora to the essential oil of *Mela-leuca alternifolia* (tea tree oil). *American Journal of Infection Control* 24, 186–189.
- Hili, P., Evans, C.S. and Veness, R.G. (1997) Antimicrobial action of essential oils: the effect of dimethylsulfoxide on the activity of cinnamon oil. *Letters in Applied Microbiology* 24, 269–275.
- Hoffman, D.L. (1987) *The Herb User's Guide*. Wellingborough, UK: Thorsons Publishing Group.
- Janssen, A.M., Scheffer, J.J.C. and Baerheim Svendsen, A. (1987) Antimicrobial activity of essential oils: a 1976–86 literature review. Aspects of the test methods. *Planta Medica* 53, 395–398.
- Jones, F.A. (1996) Herbs useful plants. Their role in history and today. European Journal of Gastroenterology and Hepatology 8, 1227–1231.
- Lawless, J. (1995) The Illustrated Encyclopedia of Essential Oils. Shaftesbury, UK: Element Books Ltd.
- Lis-Balchin, M. and Deans, S.G. (1997) Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *Journal of Applied Bacteriology* 82, 759–762.
- Mann, C.M. and Markham, J.L. (1998) A new method for determining the minimum inhibitory concentration of essential oils. Journal of Applied Microbiology 84, 538–544.
- Mishra, A.K. and Dubey, N.K. (1994) Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology* 60, 1101–1105.
- Mitscher, L.A., Drake, S., Gollapudi, S.R. and Okwute, S.K. (1987)

- A modern look at folkloric use of anti-infective agents. *Journal of Natural Products* **50**, 1025–1040.
- Morris, J.A., Khettry, A. and Seitz, E.W. (1979) Antimicrobial activity of aroma chemicals and essential oils. *Journal of the Ameri*can Oil Chemists' Society 56, 595–603.
- Newall, C.A., Anderson, L.A. and Phillipson, J.D. (1996) Herbal Medicines, a Guide for Health-Care Professionals. London: The Pharmaceutical Press.
- Remmal, A., Bouchikhi, T., Rhayour, K., Ettayebi, M. and Tantaoui-Elaraki, A. (1993) Improved method for the determination of antimicrobial activity of essential oils in the agar medium. *Journal of Essential Oil Research* 5, 179–184.
- Reynolds, J.E.F. (1996) Martindale the Extra Pharmacopoeia 31st edn. London: Royal Pharmaceutical Society of Great Britain.
- Rios, J.L., Recio, M.C. and Villar, A. (1988) Screening methods for natural products with antimicrobial activity: a review of the literature. *Journal of Ethnopharmacology* 23, 127–149.
- Ross, S.A., El-Keltawi, N.E. and Megalla, S.E. (1980) Antimicrobial activity of some Egyptian aromatic plants. *Fitoterapia* 51, 201– 205.
- Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1995) Antimicrobial activity of mint essential oils. *Journal of Agricultural and Food Chemistry* 43, 2384–2388.
- Smith, M.D. and Navilliat, P.L. (1997) A new protocol for antimicrobial testing of oils. *Journal of Microbiological Methods* 28, 21–24.
- Smith-Palmer, A., Stewart, J. and Fyfe, L. (1998) Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology* 26, 118–122.
- Windholz, M., Budavari, S., Blumetti, R.F. and Otterbein, E.S. (1983) *The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals* 10th edn. Rahway, NJ: Merck and Co. Inc.
- Yousef, R.T. and Tawil, G.G. (1980) Antimicrobial activity of volatile oils. *Die Pharmazie* 35, 698–701.