

Antimicrobial agents from plants: antibacterial activity of plant volatile oils

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7288/7/99: received 2 July 1999 and accepted 28 September 1999

H.J.D. DORMAN AND S.G. DEANS. 2000. The volatile oils of black pepper [*Piper nigrum* L. (Piperaceae)], clove [*Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae)], geranium [*Pelargonium graveolens* L'Herit (Geraniaceae)], nutmeg [*Myristica fragrans* Houtt. (Myristicaceae)], oregano [*Origanum vulgare* ssp. *hirtum* (Link) Letsw. (Lamiaceae)] and thyme [*Thymus vulgaris* L. (Lamiaceae)] were assessed for antibacterial activity against 25 different genera of bacteria. These included animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degrees of growth inhibition.

INTRODUCTION

The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s (Martindale 1910; Hoffman & Evans 1911). Plant volatile oils are generally isolated from nonwoody plant material by distillation methods, usually steam or hydrodistillation, and are variable mixtures of principally terpenoids, specifically monoterpenes [C₁₀] and sesquiterpenes [C₁₅] although diterpenes [C₂₀] may also be present, and a variety of low molecular weight aliphatic hydrocarbons (linear, ramified, saturated and unsaturated), acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpropanoids. Terpenes are amongst the chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants. Most terpenes are derived from the condensation of branched five-carbon isoprene units and are categorized according to the number of these units present in the carbon skeleton (Dorman 1999).

The antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been assessed (Lis-Balchin & Deans 1997) and reviewed (Janssen *et al.* 1987; Jain & Kar 1971; Inouye *et al.* 1983; Garg & Dengre 1986; Ríos *et al.* 1987; Sherif *et al.* 1987; Deans & Svoboda 1988,

1989; Cruz *et al.* 1989; Recio *et al.* 1989; Crespo *et al.* 1990; Carson *et al.* 1995; Larrondo *et al.* 1995; Pattnaik *et al.* 1995; Carson *et al.* 1996; Nenoff *et al.* 1996; Ríos *et al.* 1988). It is clear from these studies that these plant secondary metabolites have potential in medical procedures and applications in the cosmetic, food (Ueda *et al.* 1982; Shelef 1983; Jay & Rivers 1984; Gallardo *et al.* 1987; Baratta *et al.* 1998a,b; Youdim *et al.* 1999) and pharmaceutical industries (Janssen *et al.* 1988; Pélissier *et al.* 1994; Shapiro *et al.* 1994; Cai & Wu 1996).

Investigations into the antimicrobial activities, mode of action and potential uses of plant volatile oils have regained momentum. There appears to be a revival in the use of traditional approaches to protecting livestock and food from disease, pests and spoilage in industrial countries. This is especially true in regard to plant volatile oils and their antimicrobial evaluation, as can be seen from the comprehensive range of organisms against which volatile oils have been tested. These have included food spoiling organisms (Zaika *et al.* 1983, 1984b; Connor & Beuchat 1984a; Janssen *et al.* 1988; Ouattara *et al.* 1997) and food poisoning organisms (Beuchat 1976; Tharib *et al.* 1983; Deans & Ritchie 1987; Lis-Balchin & Deans 1997), spoilage and mycotoxigenic filamentous fungi (Knobloch *et al.* 1989), pathogenic and dimorphic yeasts (Boonchild & Flegel 1982; Ghannoum 1988) and animal and plant viruses (Ieven *et al.* 1982; Romerio *et al.* 1989).

The aims of the present investigation were to assess the antimicrobial activities of the test volatile oils and compare these to the effect of the antibiotics upon bacterial growth; to assess the components determined to be present in the volatile

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oils where available; to use these data to deduce which components are likely to contribute to the activities of the whole oils and to determine any structural relationships between the components and their antibacterial activity.

MATERIALS AND METHODS

The volatile oils of black pepper [*Piper nigrum* L. (Piperaceae)], clove [*Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae)], geranium [*Pelargonium graveolens* L'Herit (Geraniaceae)], nutmeg [*Myristica fragrans* Houtt. (Myristicaceae)], oregano [*Origanum vulgare* ssp. *hirtum* (Link) Letsw. (Lamiaceae)] and thyme [*Thymus vulgaris* L. (Lamiaceae)] were screened for antimicrobial activity using an agar diffusion technique (Deans & Ritchie 1987) against 25 microorganisms of significant importance (Table 1). In addition, 21 authentic terpenoids and the phenylpropanoid eugenol, commonly found in these volatile oils, were also screened for activity (Table 2).

Plant material

The volatile oils used in this study were isolated by hydro-distillation using essential oil distillation apparatus ('Quick Fit', British Pharmacopoeia, BDH, UK). The individual phytoconstituents were purchased either from Sigma (UK) or Fluka (UK) Chemicals.

Bacterial strains

Twenty-five bacterial strains were used to assess the antibacterial properties of the test samples, nine Gram-positive and 16 Gram-negative bacteria. Twenty-four out of 25 bacterial strains were maintained on Iso-Sensitest agar slopes [CM 471] (Oxoid, UK) at room temperature. *Clostridium sporogenes* was maintained in cooked meat broth under anaerobic conditions. All strains were subcultured every 2 weeks. The sources of the strains used are listed in Table 1.

Assessment of inhibition of bacterial growth

The measurement of growth inhibition was carried out in agreement with the method of Deans & Ritchie (1987) using Iso-Sensitest agar. Cells from cultures grown on Iso-Sensitest slopes were inoculated using a sterile loop into fresh Iso-Sensitest broth and incubated overnight at 25 °C (10 ml volume, 10⁵ ml⁻¹ final concentration). In the case of the *Clostridium* culture, a universal containing 20 ml of meat extract broth was boiled for 20 min and allowed to cool in order to

create anaerobic conditions, and subsequently was incubated with a loopful of broth from the original inoculated culture. Next, 1 ml amounts of each culture were pipetted into separate sterile Petri dishes to which 20 ml amounts of molten Iso-Sensitest agar (45 °C) were added. Once set, wells of 4 mm diameter were made in the centre of each agar plate using a Pharmacia gel punch (Uppsala, Sweden), into which 15 µl test substance was added. The plates were then left undisturbed to allow diffusion of the sample into the agar, and incubated inverted in the dark at 25 °C for 48 h. Following this, zones of growth inhibition were measured using Vernier calipers.

RESULTS

Antibacterial activity of plant volatile oils

The antibacterial activities of the plant volatile oils presented in Table 1 are in general agreement with previously reported studies on the volatile oils of *P. nigrum* (Deans & Ritchie 1987; Ouattara *et al.* 1997), *S. aromaticum* (Deans *et al.* 1995; Cai & Wu 1996; Hao *et al.* 1998; Smith-Palmer *et al.* 1998), *P. graveolens* (Pattnaik *et al.* 1996), *M. fragrans*, *O. vulgare* (Kivanc & Akgül 1986) and *T. vulgaris* (Kivanc & Akgül 1986; Smith-Palmer *et al.* 1998). All the bacterial strains demonstrated some degree of sensitivity to the plant volatile oils tested, although the growth of a number of bacteria were uninhibited by specific volatile oils. Zaika (1988) proposed that Gram-positive bacteria are more resistant than Gram-negative bacteria to the antibacterial properties of plant volatile oils which is in contrast to the hypothesis proposed by Deans that the susceptibility of bacteria to plant volatile oils and the Gram reaction appears to have little influence on growth inhibition (Deans & Ritchie 1987; Deans *et al.* 1995). The volatile oils of *O. vulgare* ssp. *hirtum*, *P. nigrum*, *S. aromaticum* and *M. fragrans* did appear to be equally effective against both Gram-positive and Gram-negative microorganisms, in contrast to Zaika (1988), Hussein (1990) and Smith-Palmer *et al.* (1998). However, *P. graveolens* and *T. vulgaris* volatile oils appeared preferentially more active with respect to Gram reaction, exerting greater inhibitory activity against Gram-positive organisms.

Table 1 summarizes the antibacterial activity of the volatile oils. From this, the oil with the widest spectrum of activity was found to be *T. vulgaris*, followed by *O. vulgare* ssp. *hirtum*, *S. aromaticum*, *M. fragrans*, *P. nigrum*, *P. graveolens*, in that order. Table 2 summarizes the antibacterial activity of the individual oil components. From this, the component with the widest spectrum of activity was found to be thymol followed by carvacrol, α -terpineol, terpinen-4-ol, eugenol, (\pm)-linalool, (-)-thujone, δ -3-carene, *cis*-hex-3-an-1-ol, geranyl acetate, (*cis* + *trans*) citral, nerol, geraniol, menthone,

Table 1 Zones of growth inhibition (mm) showing antibacterial activity for a number of selected plant volatile oils; well diameter 4.0 mm

Bacterial strain	Source	<i>Myristica fragrans</i>	<i>Origanum vulgare</i>	<i>Palaemonium graveolens</i>	<i>Piper nigrum</i>	<i>Syzygium aromaticum</i>	<i>Thymus vulgaris</i>
<i>Acinetobacter calcoacetic</i>	NCIB 8250	12.7 ± 1.3	52.2 ± 1.5	13.0 ± 0.3	12.3 ± 2.0	10.3 ± 0.2	30.7 ± 0.5
<i>Aeromonas hydrophila</i>	NCTC 8049	> 90.0	> 90.0	No inhibition	> 90.0	11.7 ± 1.1	> 90.0
<i>Alcaligenes faecalis</i>	NCIB 8156	9.0 ± 0.3	33.6 ± 0.1	No inhibition	7.1 ± 0.8	23.1 ± 0.6	53.8 ± 1.2
<i>Bacillus subtilis</i>	NCIB 3610	7.0 ± 0.4	20.5 ± 0.4	11.4 ± 0.6	9.5 ± 0.6	21.1 ± 0.1	23.4 ± 1.2
<i>Beneckea natriegens</i>	ATCC 14048	10.0 ± 1.2	37.1 ± 3.2	11.0 ± 0.7	10.8 ± 0.7	15.8 ± 0.7	> 90.0
<i>Brevibacterium linens</i>	NCIB 8456	22.2 ± 0.3	> 90.0	7.6 ± 0.1	15.9 ± 1.0	29.8 ± 0.1	> 90.0
<i>Brochothrix thermosphacta</i>	Sausage meat	9.7 ± 0.7	31.2 ± 0.8	8.6 ± 0.5	7.2 ± 0.1	11.1 ± 0.1	> 90.0
<i>Citrobacter freundii</i>	NCIB 11490	12.8 ± 0.1	29.6 ± 0.8	16.0 ± 2.0	12.0 ± 1.6	14.1 ± 2.6	> 90.0
<i>Clostridium sporogenes</i>	NCIB 10696	No inhibition	> 90.0	7.8 ± 0.6	8.7 ± 0.3	13.4 ± 0.5	> 90.0
<i>Enterococcus faecalis</i>	NCTC 775	18.5 ± 1.2	17.9 ± 0.8	19.8 ± 2.1	8.8 ± 0.9	15.5 ± 0.6	41.8 ± 0.8
<i>Enterobacter aerogenes</i>	NCTC 10006	No inhibition	14.6 ± 0.1	No inhibition	No inhibition	7.8 ± 1.1	15.2 ± 0.7
<i>Erwinia carotovora</i>	NCPPB 312	14.1 ± 2.6	31.2 ± 1.4	No inhibition	12.9 ± 1.0	11.7 ± 0.4	35.8 ± 4.4
<i>Escherichia coli</i>	NCIB 8879	10.4 ± 0.1	29.5 ± 3.4	No inhibition	7.3 ± 0.4	13.6 ± 0.3	32.4 ± 0.1
<i>Flavobacterium suaveolens</i>	NCIB 8992	16.9 ± 0.9	9.4 ± 0.7	30.9 ± 5.4	10.1 ± 0.1	14.4 ± 0.2	> 90.0
<i>Klebsiella pneumoniae</i>	NCIB 418	16.9 ± 0.9	19.0 ± 1.5	13.8 ± 0.2	No inhibition	9.1 ± 0.1	31.8 ± 0.5
<i>Lactobacillus plantarum</i>	NCDO 343	No inhibition	23.8 ± 0.3	No inhibition	No inhibition	28.5 ± 1.0	26.3 ± 0.4
<i>Leuconostoc cremoris</i>	NCDO 543	No inhibition	> 90.0	16.7 ± 2.3	16.3 ± 0.8	18.7 ± 0.6	> 90.0
<i>Micrococcus luteus</i>	NCIB 8165	11.7 ± 0.3	21.5 ± 0.1	13.3 ± 0.4	12.4 ± 0.1	14.8 ± 0.8	> 90.0
<i>Moraxella</i> sp.	NCIB 10762	6.4 ± 0.2	31.4 ± 1.9	No inhibition	5.4 ± 0.2	15.8 ± 0.8	29.0 ± 5.6
<i>Proteus vulgaris</i>	NCIB 4175	10.0 ± 1.1	44.6 ± 4.9	No inhibition	7.1 ± 0.3	9.1 ± 0.6	> 90.0
<i>Pseudomonas aeruginosa</i>	NCIB 950	No inhibition	> 90.0	19.4 ± 0.1	7.7 ± 0.9	14.0 ± 1.9	33.5 ± 2.0
<i>Salmonella pullorum</i>	NCTC 10704	8.4 ± 0.5	46.0 ± 6.7	6.9 ± 0.6	7.1 ± 0.2	14.0 ± 0.8	> 90.0
<i>Serratia marcescens</i>	NCIB 1377	8.2 ± 0.3	18.9 ± 0.4	8.5 ± 0.4	7.5 ± 0.4	21.6 ± 0.9	39.1 ± 0.8
<i>Staphylococcus aureus</i>	NCIB 6571	24.6 ± 0.4	17.6 ± 0.5	13.6 ± 0.3	14.5 ± 0.3	14.9 ± 0.1	> 90.0
<i>Yersinia enterocolitica</i>	NCTC 10460	7.3 ± 0.4	33.9 ± 0.4	No inhibition	11.7 ± 2.2	13.7 ± 0.1	25.5 ± 2.9

Source of bacterial strains: NCIB, National Collection of Industrial Bacteria; NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection; NCPPB, National Collection of Plant Pathogenic Bacteria; NCDO, National Collection of Dairy Organisms. Values for zone of growth inhibition are presented as mean ± SEM.

Table 2a Zones of growth inhibition (mm) showing antibacterial activity for a number of selected plant volatile oil components; well diameter 4.0 mm

Bacterial strain	1	2	3	4	5	6	7
<i>Acinetobacter calcoaceticus</i>	7.0 ± 1.1	10.0 ± 0.2	45.3 ± 1.3	No inhibition	7.9 ± 0.9	15.4 ± 0.3	6.1 ± 0.2
<i>Aeromonas hydrophila</i>	8.2 ± 0.5	11.1 ± 0.8	37.7 ± 2.4	No inhibition	7.9 ± 0.3	17.0 ± 0.4	6.4 ± 0.5
<i>Alcaligenes faecalis</i>	No inhibition	14.4 ± 0.7	21.8 ± 1.6	5.9 ± 0.7	8.4 ± 0.4	12.3 ± 0.5	7.0 ± 0.2
<i>Bacillus subtilis</i>	10.4 ± 0.5	9.5 ± 0.3	39.5 ± 1.0	No inhibition	5.9 ± 0.3	21.8 ± 0.4	6.4 ± 0.6
<i>Beneckea natrigens</i>	9.1 ± 0.5	10.3 ± 0.2	14.1 ± 0.3	6.8 ± 0.3	7.3 ± 0.7	20.8 ± 1.8	6.2 ± 0.4
<i>Brevibacterium linens</i>	6.7 ± 0.4	9.1 ± 0.5	21.7 ± 0.5	No inhibition	7.5 ± 0.3	12.7 ± 0.1	7.3 ± 0.6
<i>Brocothrix thermosphacta</i>	7.4 ± 0.4	No inhibition	25.5 ± 1.0	No inhibition	6.1 ± 0.1	14.1 ± 0.2	7.4 ± 0.9
<i>Citrobacter freundii</i>	No inhibition	No inhibition	17.7 ± 0.1	No inhibition	6.9 ± 0.1	9.1 ± 0.3	9.2 ± 0.2
<i>Clostridium sporogenes</i>	No inhibition	16.6 ± 0.5	20.3 ± 0.7	8.9 ± 0.5	12.6 ± 0.2	9.7 ± 0.1	12.9 ± 0.5
<i>Enterococcus faecalis</i>	No inhibition	11.3 ± 0.7	21.2 ± 0.4	No inhibition	21.6 ± 0.1	10.0 ± 0.1	12.9 ± 0.9
<i>Enterobacter aerogenes</i>	No inhibition	12.0 ± 0.8	18.5 ± 0.8	No inhibition	6.2 ± 0.7	9.9 ± 0.1	6.4 ± 0.5
<i>Erwinia carotovora</i>	No inhibition	11.0 ± 0.4	15.5 ± 0.7	No inhibition	10.2 ± 0.1	10.0 ± 0.5	8.1 ± 0.2
<i>Escherichia coli</i>	6.7 ± 0.3	13.5 ± 1.2	29.2 ± 0.2	5.9 ± 0.6	11.0 ± 0.2	13.3 ± 0.2	9.7 ± 0.7
<i>Flavobacterium suaveolens</i>	7.0 ± 0.1	10.9 ± 0.4	26.0 ± 1.8	5.1 ± 0.5	6.6 ± 0.1	11.6 ± 0.6	7.0 ± 0.6
<i>Klebsiella pneumoniae</i>	No inhibition	11.7 ± 0.6	23.6 ± 0.1	7.1 ± 0.3	8.8 ± 0.2	10.9 ± 0.3	No inhibition
<i>Lactobacillus plantarum</i>	No inhibition	No inhibition	18.7 ± 0.7	6.3 ± 0.7	7.9 ± 0.4	21.5 ± 0.6	6.2 ± 0.4
<i>Leuconostoc cremoris</i>	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
<i>Micrococcus luteus</i>	No inhibition	11.1 ± 0.4	26.6 ± 0.5	No inhibition	6.7 ± 0.4	11.7 ± 0.7	6.1 ± 0.1
<i>Moraxella</i> sp.	No inhibition	12.3 ± 0.7	21.6 ± 0.1	No inhibition	6.5 ± 0.4	10.1 ± 0.6	6.1 ± 0.1
<i>Proteus vulgaris</i>	No inhibition	11.1 ± 0.4	26.5 ± 1.6	6.2 ± 0.1	6.9 ± 0.1	8.3 ± 0.3	5.6 ± 0.3
<i>Pseudomonas aeruginosa</i>	No inhibition	10.6 ± 2.0	26.0 ± 0.4	No inhibition	6.6 ± 0.1	15.5 ± 0.6	5.7 ± 0.3
<i>Salmonella pullorum</i>	No inhibition	13.8 ± 0.8	27.1 ± 0.7	5.0 ± 0.5	12.0 ± 0.1	12.9 ± 0.1	6.3 ± 0.2
<i>Serratia marcescens</i>	5.4 ± 0.6	8.0 ± 1.2	22.5 ± 0.9	No inhibition	6.0 ± 0.6	22.9 ± 0.8	5.7 ± 0.1
<i>Staphylococcus aureus</i>	6.9 ± 0.2	11.3 ± 0.6	20.2 ± 0.5	No inhibition	4.9 ± 0.1	11.5 ± 0.5	5.2 ± 0.1
<i>Yersinia enterocolitica</i>	No inhibition	15.4 ± 0.2	22.4 ± 1.2	No inhibition	9.0 ± 0.6	11.6 ± 0.4	8.0 ± 0.2

Values for zone of growth inhibition are presented as mean ± SEM. 1, Borneol; 2, δ -3-carene; 3, carvacrol; 4, carvacrol methyl ester; 5, *cis/trans* citral; 6, eugenol; 7, geraniol.

β -pinene, *R*(+)-limonene, α -pinene, α -terpinene, borneol, (+)-sabinene, γ -terpinene, citronellal ~ terpinolene, 1,8-cineole, bornyl acetate, carvacrol methyl ether, myrcene, β -caryophyllene, α -bisabolol, α -phellandrene, α -humulene, β -ocimene, aromadendrene, *p*-cymene, in that order.

DISCUSSION

The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components. A correlation of the antimicrobial activity of the compounds tested and their relative percentage composition in the plant volatile oils used in this study, with their chemical structure, functional groups and configuration, suggests a number of observations.

The components with phenolic structures, such as carva-

crol, eugenol and thymol, were highly active against the test microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used (Pelczar *et al.* 1988). These compounds were strongly active despite their relatively low capacity to dissolve in water, which is in agreement with published data (Nadal *et al.* 1973; Suresh *et al.* 1992; Lattaoui & Tantaoui-Elaraki 1994; Mahmoud 1994; Meena & Sethi 1994; Shapiro *et al.* 1994; Belaiche *et al.* 1995; Jeongmok *et al.* 1995; Charai *et al.* 1996; Sivropoulou *et al.* 1996; Hili *et al.* 1997; Lis-Balchin & Deans 1997).

The importance of the hydroxyl group in the phenolic structure was confirmed in terms of activity when carvacrol was compared to its methyl ether. Furthermore, the relative position of the hydroxyl group exerted an influence upon the components effectiveness as seen in the difference in activity between carvacrol and thymol against Gram-negative and Gram-positive bacteria. Furthermore, the significance of the

Table 2b Zones of growth inhibition (mm) showing antibacterial activity for a number of selected plant volatile oil components; well diameter 4.0 mm

Bacterial strain	8	9	10	11	12	13	14
<i>Acinetobacter calcoacetica</i>	10.3 ± 0.3	8.1 ± 0.1	No inhibition	9.3 ± 0.5	9.7 ± 2.3	11.4 ± 0.5	No inhibition
<i>Aeromonas hydrophila</i>	9.0 ± 0.4	8.5 ± 0.3	No inhibition	11.5 ± 0.9	7.0 ± 0.4	7.7 ± 0.1	No inhibition
<i>Alcaligenes faecalis</i>	10.5 ± 0.1	9.3 ± 0.6	No inhibition	12.1 ± 0.4	6.2 ± 0.5	7.1 ± 0.4	No inhibition
<i>Bacillus subtilis</i>	10.8 ± 0.2	6.4 ± 0.7	No inhibition	14.0 ± 0.8	7.1 ± 0.3	12.4 ± 0.2	No inhibition
<i>Beneckea natriegens</i>	10.8 ± 0.1	7.6 ± 0.5	No inhibition	11.4 ± 0.3	5.9 ± 0.4	11.3 ± 0.5	No inhibition
<i>Brevibacterium linens</i>	12.5 ± 0.8	8.1 ± 0.2	No inhibition	12.5 ± 0.7	No inhibition	11.7 ± 0.6	No inhibition
<i>Brocothrix thermosphacta</i>	9.2 ± 0.2	24.0 ± 0.6	No inhibition	8.1 ± 0.4	6.8 ± 0.4	9.0 ± 0.9	No inhibition
<i>Citrobacter freundii</i>	6.8 ± 0.8	9.7 ± 0.1	7.8 ± 0.1	27.5 ± 1.9	7.8 ± 0.6	7.8 ± 0.4	6.0 ± 0.3
<i>Clostridium sporogenes</i>	20.4 ± 0.4	7.8 ± 0.2	10.3 ± 0.1	20.3 ± 0.4	10.7 ± 0.3	No inhibition	5.7 ± 0.1
<i>Enterococcus faecalis</i>	7.5 ± 0.6	8.9 ± 1.1	No inhibition	16.7 ± 1.1	No inhibition	No inhibition	9.2 ± 0.1
<i>Enterobacter aerogenes</i>	7.6 ± 0.2	6.5 ± 0.2	7.1 ± 0.2	9.7 ± 0.5	6.3 ± 0.1	7.2 ± 0.5	No inhibition
<i>Erwinia carotovora</i>	8.7 ± 1.2	9.3 ± 0.9	7.4 ± 0.1	12.3 ± 0.8	6.5 ± 0.5	7.7 ± 1.2	8.7 ± 0.7
<i>Escherichia coli</i>	11.0 ± 0.2	12.0 ± 0.8	11.2 ± 0.3	13.8 ± 0.3	6.6 ± 0.2	7.6 ± 0.6	8.9 ± 0.5
<i>Flavobacterium suaveolens</i>	11.0 ± 0.6	10.5 ± 0.2	10.6 ± 0.1	15.7 ± 2.4	5.8 ± 0.3	7.0 ± 0.4	6.5 ± 0.8
<i>Klebsiella pneumoniae</i>	7.8 ± 0.4	10.7 ± 0.5	7.0 ± 0.1	12.6 ± 0.3	5.9 ± 0.4	No inhibition	8.1 ± 0.1
<i>Lactobacillus plantarum</i>	12.9 ± 1.7	16.7 ± 2.9	No inhibition	25.3 ± 0.9	8.8 ± 0.7	19.1 ± 0.1	No inhibition
<i>Leuconostoc cremoris</i>	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
<i>Micrococcus luteus</i>	8.0 ± 0.9	12.8 ± 0.8	No inhibition	13.4 ± 0.8	7.1 ± 0.3	7.4 ± 0.3	7.6 ± 0.8
<i>Moraxella</i> sp.	9.0 ± 0.6	6.7 ± 0.2	7.9 ± 0.4	10.3 ± 0.9	6.9 ± 0.5	No inhibition	6.2 ± 0.4
<i>Proteus vulgaris</i>	9.8 ± 0.1	8.2 ± 0.1	7.4 ± 0.5	12.2 ± 0.9	6.2 ± 0.1	No inhibition	7.5 ± 0.1
<i>Pseudomonas aeruginosa</i>	6.5 ± 0.3	8.4 ± 0.3	No inhibition	No inhibition	No inhibition	13.6 ± 1.0	No inhibition
<i>Salmonella pullorum</i>	8.7 ± 0.4	12.0 ± 0.7	11.2 ± 0.6	7.5 ± 0.5	6.2 ± 0.6	No inhibition	7.9 ± 0.5
<i>Serratia marcescens</i>	6.8 ± 0.1	12.4 ± 0.9	6.5 ± 0.1	8.8 ± 0.1	7.1 ± 0.4	8.5 ± 1.0	No inhibition
<i>Staphylococcus aureus</i>	6.6 ± 0.6	8.2 ± 0.3	No inhibition	9.0 ± 0.4	10.2 ± 1.0	9.4 ± 0.4	8.3 ± 0.1
<i>Yersinia enterocolitica</i>	8.2 ± 1.0	11.5 ± 1.1	7.1 ± 0.2	9.5 ± 0.9	8.0 ± 0.2	7.1 ± 0.2	6.6 ± 0.6

Values for zone of growth inhibition are presented as mean ± SEM. 8, Geranyl acetate; 9, *cis*-hex-3-en-1-ol; 10, *R*(+)-limonene; 11, (±)-linalool; 12, menthone; 13, nerol; 14, α -pinene.

phenolic ring was demonstrated by the lack of activity of the monoterpene cyclic hydrocarbon *p*-cymene. The high activity of the phenolic components may be further explained in terms of the alkyl substitution into the phenol nucleus, which is known to enhance the antimicrobial activity of phenols (Pelczar *et al.* 1988). The introduction of alkylation has been proposed to alter the distribution ratio between the aqueous and the nonaqueous phases (including bacterial phases) by reducing the surface tension or altering the species selectivity. Alkyl substituted phenolic compounds form phenoxyl radicals which interact with isomeric alkyl substituents (Pauli & Knobloch 1987). This does not occur with etherified/esterified isomeric molecules, possibly explaining their relative lack of activity.

The presence of an acetate moiety in the structure appeared to increase the activity of the parent compound. In the case of geraniol, the geranyl acetate demonstrated an increase in activity against the test microorganisms (Table 2). Only *Cl. sporogenes* was found to be more resistant to the acetate. A similar tendency was identified in the case of borneol and

borneol acetate (Table 2). Borneol was less active than the acetate except against *Aeromonas hydrophila*, *Bacillus subtilis*, *Beneckea natriegens*, *Escherichia coli*, *Flavobacterium suaveolens* and *Serratia marcescens* but only the acetate was capable of affecting the growth of the bacterium *Micrococcus luteus*.

Alcohols are known to possess bactericidal rather than bacteriostatic activity against vegetative cells. The alcohol terpenoids in this study did exhibit activity against the test microorganisms, potentially acting as either protein denaturing agents (Pelczar *et al.* 1988), solvents or dehydrating agents.

Aldehydes, notably formaldehyde and glutaraldehyde, are known to possess powerful antimicrobial activity. It has been proposed that an aldehyde group conjugated to a carbon to carbon double bond is a highly electronegative arrangement, which may explain their activity (Moleyar & Narasimham 1986), suggesting an increase in electronegativity increases the antibacterial activity (Kurita *et al.* 1979, 1981). Such electronegative compounds may interfere in biological processes involving electron transfer and react with vital nitrogen

Table 2c Zones of growth inhibition (mm) showing antibacterial activity for a number of selected plant volatile oil components; well diameter 4.0 mm

Bacterial strain	15	16	17	18	19	20	21
<i>Acinetobacter calcoaceticus</i>	11.2 ± 0.5	7.8 ± 0.8	No inhibition	14.5 ± 0.4	18.7 ± 0.3	8.7 ± 0.5	29.8 ± 1.5
<i>Aeromonas hydrophila</i>	7.1 ± 0.3	No inhibition	No inhibition	24.4 ± 2.1	16.7 ± 0.9	12.2 ± 0.1	26.8 ± 1.3
<i>Alcaligenes faecalis</i>	7.8 ± 0.5	7.7 ± 0.4	No inhibition	21.3 ± 1.3	19.3 ± 0.4	13.5 ± 0.9	32.5 ± 1.2
<i>Bacillus subtilis</i>	No inhibition	7.4 ± 0.1	No inhibition	13.1 ± 0.3	28.8 ± 0.4	12.6 ± 0.3	39.2 ± 2.2
<i>Beneckea natrigens</i>	6.5 ± 0.7	7.7 ± 1.0	6.4 ± 0.1	29.9 ± 0.8	17.6 ± 0.8	9.5 ± 0.4	50.1 ± 1.6
<i>Brevibacterium linens</i>	No inhibition	6.5 ± 0.2	6.1 ± 0.1	10.3 ± 0.1	18.4 ± 0.6	9.8 ± 0.8	42.0 ± 2.0
<i>Brocothrix thermosphacta</i>	5.9 ± 0.7	7.6 ± 0.4	No inhibition	7.7 ± 0.1	11.3 ± 0.2	12.0 ± 0.3	29.3 ± 1.3
<i>Citrobacter freundii</i>	5.9 ± 0.1	9.5 ± 0.4	No inhibition	18.3 ± 0.4	15.4 ± 1.1	12.9 ± 0.4	46.5 ± 0.7
<i>Clostridium sporogenes</i>	7.5 ± 0.2	No inhibition	8.8 ± 0.3	9.7 ± 1.7	No inhibition	18.1 ± 2.2	No inhibition
<i>Enterococcus faecalis</i>	7.8 ± 0.2	No inhibition	No inhibition	10.7 ± 0.7	13.4 ± 1.1	13.3 ± 1.0	26.3 ± 3.6
<i>Enterobacter aerogenes</i>	No inhibition	7.3 ± 0.8	No inhibition	17.3 ± 0.4	21.6 ± 1.0	10.7 ± 0.2	30.5 ± 0.4
<i>Erwinia carotovora</i>	No inhibition	No inhibition	6.3 ± 0.7	14.8 ± 0.7	20.1 ± 0.3	11.0 ± 1.2	32.0 ± 1.0
<i>Escherichia coli</i>	7.8 ± 0.8	No inhibition	6.1 ± 0.7	14.4 ± 0.3	16.3 ± 0.3	12.2 ± 0.5	34.3 ± 5.4
<i>Flavobacterium suaveolens</i>	8.4 ± 0.5	No inhibition	No inhibition	13.4 ± 0.3	21.2 ± 0.2	11.8 ± 0.4	25.8 ± 0.8
<i>Klebsiella pneumoniae</i>	7.9 ± 0.4	7.4 ± 0.5	7.9 ± 0.3	10.7 ± 0.9	19.1 ± 0.1	11.1 ± 0.1	40.0 ± 3.4
<i>Lactobacillus plantarum</i>	No inhibition	No inhibition	9.4 ± 0.1	14.7 ± 0.5	29.1 ± 0.8	13.4 ± 1.2	>90.0
<i>Leuconostoc cremoris</i>	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
<i>Micrococcus luteus</i>	6.3 ± 0.3	No inhibition	No inhibition	12.6 ± 0.7	11.2 ± 0.6	11.3 ± 0.7	53.1 ± 1.3
<i>Moraxella</i> sp.	4.8 ± 0.8	No inhibition	5.5 ± 0.1	11.4 ± 1.4	18.2 ± 0.2	10.0 ± 0.8	39.1 ± 0.9
<i>Proteus vulgaris</i>	6.6 ± 0.1	No inhibition	5.6 ± 0.1	9.7 ± 1.8	20.2 ± 0.2	12.6 ± 0.2	33.5 ± 6.2
<i>Pseudomonas aeruginosa</i>	6.5 ± 0.1	No inhibition	6.3 ± 0.4	17.0 ± 1.7	8.9 ± 0.7	9.3 ± 0.7	13.4 ± 1.4
<i>Salmonella pullorum</i>	6.0 ± 0.2	No inhibition	16.5 ± 1.1	14.9 ± 0.1	19.2 ± 0.2	11.5 ± 0.2	31.5 ± 0.3
<i>Serratia marcescens</i>	5.4 ± 0.1	No inhibition	No inhibition	15.2 ± 0.4	11.9 ± 0.3	7.5 ± 0.4	42.6 ± 0.5
<i>Staphylococcus aureus</i>	7.4 ± 0.2	No inhibition	No inhibition	13.7 ± 0.5	18.2 ± 0.1	9.4 ± 0.1	31.6 ± 0.4
<i>Yersinia enterocolitica</i>	5.8 ± 0.3	No inhibition	No inhibition	8.6 ± 0.2	20.4 ± 0.4	9.0 ± 0.3	27.7 ± 0.1

Values for zone of growth inhibition are presented as mean ± SEM. 15, β-pinene; 16, (+)-sabinene; 17, α-terpinene; 18, terpinen-4-ol; 19, α-terpineole; 20, (-)-thujone; 21, thymol.

components, e.g. proteins and nucleic acids and therefore inhibit the growth of the microorganisms. The aldehydes *cis* + *trans* citral displayed moderate activity against the test microorganisms while citronellal was only active against *B. subtilis*, *Cl. sporogenes*, *Fl. suaveolens*, *M. luteus* and *Pseudomonas aeruginosa* (Table 2).

A number of the components tested are ketones. The presence of an oxygen function in the framework increases the antimicrobial properties of terpenoids (Naigre *et al.* 1996). From this study, and by using the contact method, the bacteriostatic and fungistatic action of terpenoids was increased when carbonylated. Menthone was shown to have modest activity, *Cl. sporogenes* and *Staphylococcus aureus* being the most significantly affected (Table 2).

An increase in activity dependent upon the type of alkyl substituent incorporated into a nonphenolic ring structure appeared to occur in this study. An alkenyl substituent (1-methylethenyl) resulted in increased antibacterial activity, as seen in limonene [1-methyl-4-(1-methylethenyl)-cyclohexene], compared to an alkyl (1-methylethyl) substituent as in

p-cymene [1-methyl-4-(1-methylethyl)-benzene]. As shown in Table 2, the inclusion of a double bond increased the activity of limonene relative to *p*-cymene, which demonstrated no activity against the test bacteria. In addition, the susceptible organisms were principally Gram-negative, which suggests alkylation influences Gram reaction sensitivity of the bacteria. The importance of the antimicrobial activity of alkylated phenols in relation to phenol has been previously reported (Pelczar *et al.* 1988). Their data suggest that an allylic side chain seems to enhance the inhibitory effects of a component and chiefly against Gram-negative organisms.

Furthermore, the stereochemistry had an influence on bioactivity. It was observed that α-isomers are inactive relative to β-isomers, e.g. α-pinene; *cis*-isomers are inactive contrary to *trans*-isomers, e.g. geraniol and nerol; compounds with methyl-isopropyl cyclohexane rings are the most active; or unsaturation of the cyclohexane ring further increases the antibacterial activity, e.g. terpinolene, terpineol and terpineolene (Hinou *et al.* 1989).

Investigations into the effects of terpenoids upon isolated

bacterial membranes suggest that their activity is a function of the lipophilic properties of the constituent terpenes (Knobloch *et al.* 1986), the potency of their functional groups and their aqueous solubility (Knobloch *et al.* 1988). Their site of action appeared to be at the phospholipid bilayer, caused by biochemical mechanisms catalysed by the phospholipid bilayers of the cell. These processes include the inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme-dependent reactions (Knobloch *et al.* 1986). Their activity in whole cells appears more complex (Knobloch *et al.* 1988). Although a similar water solubility tendency is observed, specific statements on the action of single terpenoids *in vivo* have to be assessed singularly, taking into account not only the structure of the terpenoid, but also the chemical composition of the cell wall (Knobloch *et al.* 1988). The plant extracts clearly demonstrate antibacterial properties, although the mechanistic processes are poorly understood. These activities suggest potential use as chemotherapeutic agents, food preserving agents and disinfectants.

Chemotherapeutic agents, used orally or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious disease. The tested plant products appear to be effective against a wide spectrum of microorganisms, both pathogenic and nonpathogenic. Administered orally, these compounds may be able to control a wide range of microbes but there is also the possibility that they may cause an imbalance in the gut microflora, allowing opportunistic pathogenic coliforms to become established in the gastrointestinal tract with resultant deleterious effects. Further studies on therapeutic applications of volatile oils should be undertaken to investigate these issues, especially when considering the substantial number of analytical studies carried out on these natural products.

The volatile oils and their component volatility and lack of solubility make these plant extracts less appealing for general disinfectant applications. However, a role as disinfectants of rooms has been reportedly investigated in a classical study (Kellner & Kober 1954). Their volatility would be a distinct advantage in lowering microbial contamination in air and on difficult to reach surfaces. Although the minimum inhibitory concentrations for a selection of oils tested in a closed chamber were lower in the vapour phase (Inouye *et al.* 1983), evidence suggests that such applications may have merit (Taldykin 1979; Makarchuk *et al.* 1981).

As food preservatives, volatile oils may have their greatest potential use. Spices, which are used as integral ingredients in cuisine or added as flavouring agents to foods, are present in insufficient quantities for their antimicrobial properties to be significant. However, spices are often contaminated with bacterial and fungal spores due to their volatile oil content,

often with antimicrobial activity, being enclosed within oil glands and not being released onto the surface of the spice matter. Volatile oils, which often contain the principal aromatic and flavouring components of herbs and spices, if added to foodstuffs, would cause no loss of organoleptic properties, would retard microbial contamination and therefore reduce the onset of spoilage. In addition, small quantities would be required for this effect. Furthermore, evidence suggests that these oils possess strong antioxidant activities (Dorman 1999; Youdim *et al.* 1999), which are favourable properties to combat free radical-mediated organoleptic deterioration.

ACKNOWLEDGEMENTS

H.J.D.D. gratefully acknowledges financial support through a MAFF Postgraduate Studentship. S.A.C. received financial support from the Scottish Office Agriculture Environment and Fisheries Department.

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