JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1347–1356

Received January 25, 2001
Accepted July 3, 2001
ISSN 0022-3573

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NN, UK

Nicolette S. L. Perry, Peter J. Houghton, Julia Sampson, Anthony E. Theobald

G. K. T. Centre for Biomedical Sciences, Division of Pharmacology and Therapeutics, King's College London, Guy's Campus, London Bridge, London, SE1 1U2, UK

Stephen Hart, J. Robin S. Hoult, Patricia Evans, Peter Jenner

School of Applied Science, South Bank University, Southwark Campus, 103 Borough Road, London, SE1 0AA, UK

Maria Lis-Balchin

MRC Neurochemical Pathology Unit, Newcastle General Hospital, Westgate Road, Newcastle Upon Tyne, NE4 6BE, UK

Elaine K. Perry

G.K.T. Centre for Biomedical Sciences, Division of Endocrinology and Reproduction, King's College London, Guy's Campus, London Bridge, London, SE1 1U2, UK

Stuart Milligan

Correspondence: P. J. Houghton, Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London. SE1 9NN, UK. E-mail: peter.houghton@kcl.ac.uk

Funding and acknowledgements: Funding provided by Shire Pharmaceuticals for a PhD studentship (N. S. L. Perry) is gratefully acknowledged. The National Parkinson's Foundation is acknowledged for the use of materials.

In-vitro activity of *S. lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer's disease

Nicolette S. L. Perry, Peter J. Houghton, Julia Sampson, Anthony E. Theobald, Stephen Hart, Maria Lis-Balchin, J. Robin S. Hoult, Patricia Evans, Peter Jenner, Stuart Milligan and Elaine K. Perry

Abstract

Salvia lavandulaefolia Vahl. (Spanish sage) essential oil and individual monoterpenoid constituents have been shown to inhibit the enzyme acetylcholinesterase in-vitro and in-vivo. This activity is relevant to the treatment of Alzheimer's disease, since anticholinesterase drugs are currently the only drugs available to treat Alzheimer's disease. Other activities relevant to Alzheimer's disease include antioxidant, anti-inflammatory and estrogenic effects. Results of in-vitro tests for these activities are reported here for *S. lavandulaefolia* extracts, the essential oil and its major constituents. Antioxidant activity (inhibition of bovine brain liposome peroxidation) was found in the EtOH extract of the dried herb (5 mg mL^{-1}) and the monoterpenoids (0.1 M) α- and β-pinene and 1,8-cineole. Thujone and geraniol had lower antioxidant effects, while camphor had no antioxidant effects. Possible anti-inflammatory activity (eicosanoid inhibition in rat leucocytes) was found in the EtOH extract (50 μg mL^{-1}) and was shown by the monoterpenoids α-pinene and geraniol (0.2 mM), but not 1,8-cineole, thujone or camphor. Possible estrogenic activity (via induction of β-galactosidase activity in yeast cells) was found in the essential oil (0.01 mg mL^{-1}) and the monoterpenoid geraniol (0.1–2 mM). 1,8-Cineole, α- and β-pinene and thujone did not exhibit estrogenic activity in this analysis. These results demonstrate that *S. lavandulaefolia*, its essential oil and some chemical constituents have properties relevant to the treatment of Alzheimer's disease and provide further data supporting the value of carrying out clinical studies in patients with Alzheimer's disease using this plant species.

Introduction

At present, the only drugs licensed to treat Alzheimer's disease in Western medicine are tacrine, donepezil, rivastigmine and galantamine. These are anticholinesterases, which have a limited dose range (Small et al 1997; Tariot et al 2000). The continuing search for novel anticholinesterases from plants as therapeutic agents for dementia and other CNS disorders is based on the need for agents targeted to brain areas affected, with reduced toxicity and side-effects. Additional therapeutic strategies currently being explored include the protective role that oestrogen may play in neurodegeneration and limiting the role of free radical and inflammatory damage.

In recent years, oxidative stress and/or a failure of defence mechanisms have been described in the pathological changes that occur in Alzheimer's disease (Murray and Lynch 1998; Pratico & Delanty 2000). For example, alterations in the content

and metabolism of phospholipids is associated with the pattern of neuronal death in Alzheimer's disease (Frölich & Riederer 1995). There is also a suggested role of oxidants in the cross-linking process of β-amyloid (Aβ) aggregation and Aβ itself may promote free radical damage (Carr et al 1997; Varadarajan et al 2001). In-vitro studies have shown that the antioxidant α-tocopherol (vitamin E) reduced the toxicity of Aβ protein in nerve culture systems (Murray & Lynch 1998). This was supported by a clinical study where the progression of disease in patients with moderately severe impairment from Alzheimer's disease was significantly reduced when they were treated with vitamin E (Sano et al 1997).

Of particular relevance to this study, where a plant extract is involved, are the recent reports of well-designed controlled trials involving patients with Alzheimer's disease who were treated with a standardized Ginkgo extract (an extract of the leaves of *Ginkgo biloba*) and who showed a significant improvement in cognitive performance, memory and social functioning compared with those receiving a placebo (Kanowski et al 1997; Le Bars et al 1997; Brautigam et al 1998). The beneficial effects observed were ascribed to the antioxidant di- and sesquiterpenes present, such as bilobalide and the ginkgolides. However, it should be noted that flavonoids and organic acids are also present, which also have an antioxidant effect and, in addition, inhibit eicosanoid synthesis. Thus, an overall effect may be owing to several types of constituents acting in different ways.

The contribution of inflammatory damage to degeneration in Alzheimer's disease has been supported by results of epidemiological studies providing evidence that non-steroidal anti-inflammatory drugs (NSAIDs) may be beneficial and even protective to patients with Alzheimer's disease (Aisen 1996; Breitner 1996; Cooper et al 2000). There has been controversy over potential bias and confounding variables in these epidemiological findings. However, it is clear that patients suffering from rheumatoid arthritis and who have taken NSAIDs have a decreased risk or a decrease in the progression of Alzheimer's disease, and in one study showed a reduction in the number of Aβ plaques (Breitner 1996). In addition, a variety of cells involved in the inflammatory response have been reported to be elevated in Alzheimer's disease (Rogers 1995; de Vries et al 1997; Cooper et al 2000).

A further line of therapy that is currently being explored involves the possible protective role of estrogenic agents in preventing or delaying neurodegeneration in Alzheimer's disease (BirkHäuser et al 2000). Some studies have shown that post-menopausal women are more likely to develop Alzheimer's disease than men, and that women on hormone replacement therapy (HRT) are reported to have a decreased (40 %) risk of developing Alzheimer's disease (Green et al 1998). However, some, but not all, clinical studies have shown estrogen to be ineffective in halting the cognitive decline of, for example, mild to moderate Alzheimer's disease in older women (Mulnard et al 2000).

Among European medicinal plants, the traditional uses of sage (*Salvia* species) indicate that it may have all the above properties. *Salvia officinalis* L. is widely used commercially in foodstuffs for its antioxidant properties (Aruoma 1996; Culvelier et al 1996). Many *Salvia* species and their constituents are reputed to have anti-inflammatory action (Bingol & Sener 1995) and *Salvia* has been reported for centuries to have oestrogenic properties (Bartram 1995). The present study was undertaken to investigate the antioxidant, anti-inflammatory and estrogenic effects of *S. lavandulaefolia* in vitro. Estrogenic activity has not been scientifically investigated in this species until now.

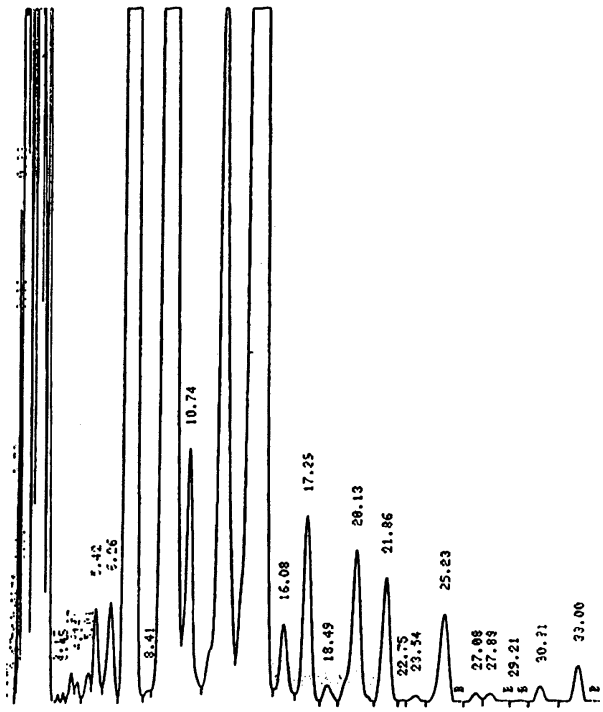
Materials and Methods

Materials

S. lavandulaefolia essential oil was obtained from Baldwins & Co. (London, UK). The gas chromatography profile showing the main constituents of the essential oil is shown in Figure 1 (Alltech fused silica capillary column, 20 M Carbowax; 60–279°C at 2°C min^{-1} + 80 min; injection and FID temperature, 290°C; injection volume, 0.1 μL). Cotswold Health Products Ltd (Tabernacle Road, Wotton-under-Edge, Gloucestershire, UK) kindly donated dried authenticated *S. lavandulaefolia* grown organically in the Murcia region of Spain. The herb (200 g) was extracted using vacuum-liquid chromatography in 96 % EtOH and this extract was evaporated and partitioned between $CHCl_3$ and H_2O. The major monoterpenoid constituents of the essential oil borneol (camphor, 1,8-cineole, geraniol, linalool, $\alpha + \beta$ pinene and thujone) were obtained from Sigma (Poole, Dorset, UK) and Aldrich Chemical Co. (Gillingham, Dorset, UK).

Antioxidant assay

Antioxidant activity of the essential oil and monoterpenoids was assessed using membrane lipid peroxidation according to the method of Aruoma et al (1996). Peroxidation in ox brain phospholipid liposomes was



Monoterpenoid constituent[a]	Relative percentage peak[b] (% in essential oil)
α-Pinene (**5**)	5
β-Pinene (**6**)	12
1,8-Cineole (**7**)	17
Thujone (**9**)	0.28
Camphor (**4**)	27
Geraniol (**8**)	0.4

[a]Identities of compounds determined from the retention times
[b] Relative percentages as internal normalisation of total peaks observed.

Figure 1 Gas chromatography profile and content of major constituents of *S. lavandulaefolia* essential oil (BN 25).

induced by the addition of the Fe^{3+} and ascorbate. The extent of peroxidation in the presence of the test substances was detected by addition of thiobarbituric acid (TBA) to produce the TBA/2-malondialdehyde adduct and the amount of this chromogen formed was measured by absorbance at 532 nm. Extracts and the monoterpenoids α- and β-pinene, 1,8-cineole, camphor, geraniol and thujone, of *S. lavandulaefolia* essential oil (in 96% EtOH) were tested at final concentrations of < 0.1 M (monoterpenoids) and 5 mg mL^{-1} (extracts). The results are presented as percent inhibition relative to the control in the absence of any antioxidant and compared with inhibition by the known antioxidant propyl gallate.

Table 1 Percentage inhibition of bovine liposome lipid peroxidation by crude extracts and commercially obtained constituents of *S. lavandulaefolia* essential oil and the synthetic antioxidant propyl gallate.

Extract/compound	Percentage inhibition
Ethanol extract of *S. lavandulaefolia* (5 mg mL^{-1})	77 ± 8*
Aqueous fraction from ethanol extract of *S. lavandulaefolia* (5 mg mL^{-1})	78 ± 6*
Chloroform fraction from ethanol extract of *S. lavandulaefolia* (5 mg mL^{-1})	72 ± 8*
Camphor (**4**) (100 mM)	-37 ± 20[a]
Camphor (**4**) (10 mM)	-10 ± 5[a]
α-Pinene (**5**) (100 mM)	67 ± 12*
β-Pinene (**6**) (100 mM)	38 ± 4*
1,8-Cineole (**7**) (100 mM)	42 ± 3*
Geraniol (**8**) (100 mM)	29 ± 20
Thujone (**9**) (100 mM)	22 ± 4
Thujone (**9**) (10 mM)	3 ± 1
Propyl gallate (positive control) 10 μM	100

Data are mean ± s.d, n = 6. [a]Pro-oxidant activity. *$P < 0.05$ significant difference compared with control.

Assay to examine anti-inflammatory activity

Inhibition of the formation of pro-inflammatory eicosanoids, thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4) (measured by radioimmunoassay) derived from the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism, respectively, was carried out using calcium-ionophore-stimulated rat leucocytes. The method used was a modification of that of Moroney et al (1988), which has been used for several other types of plant constituents. The EtOH, $CHCl_3$ and H_2O extracts and the monoterpenoids camphor, 1,8-cineole, geraniol, α-pinene and thujone of *S. lavandulaefolia* essential oil were tested in EtOH at a final concentration of 200 μM (pure compounds) and 50 μg mL^{-1} (extracts). The data are presented as the mean (mean ± s.d., n = 3) percentage inhibition of the control (A23187), where $< 50\%$ was considered active inhibition and 50–70% was considered weak inhibition.

Assay to examine binding to estrogen receptor

The method of analysis, a recombinant estrogen-inducible screen developed in yeast (*Saccharomyces cerevisiae*), was a modification of that described by Routledge & Sumpter (1996). The yeast genome

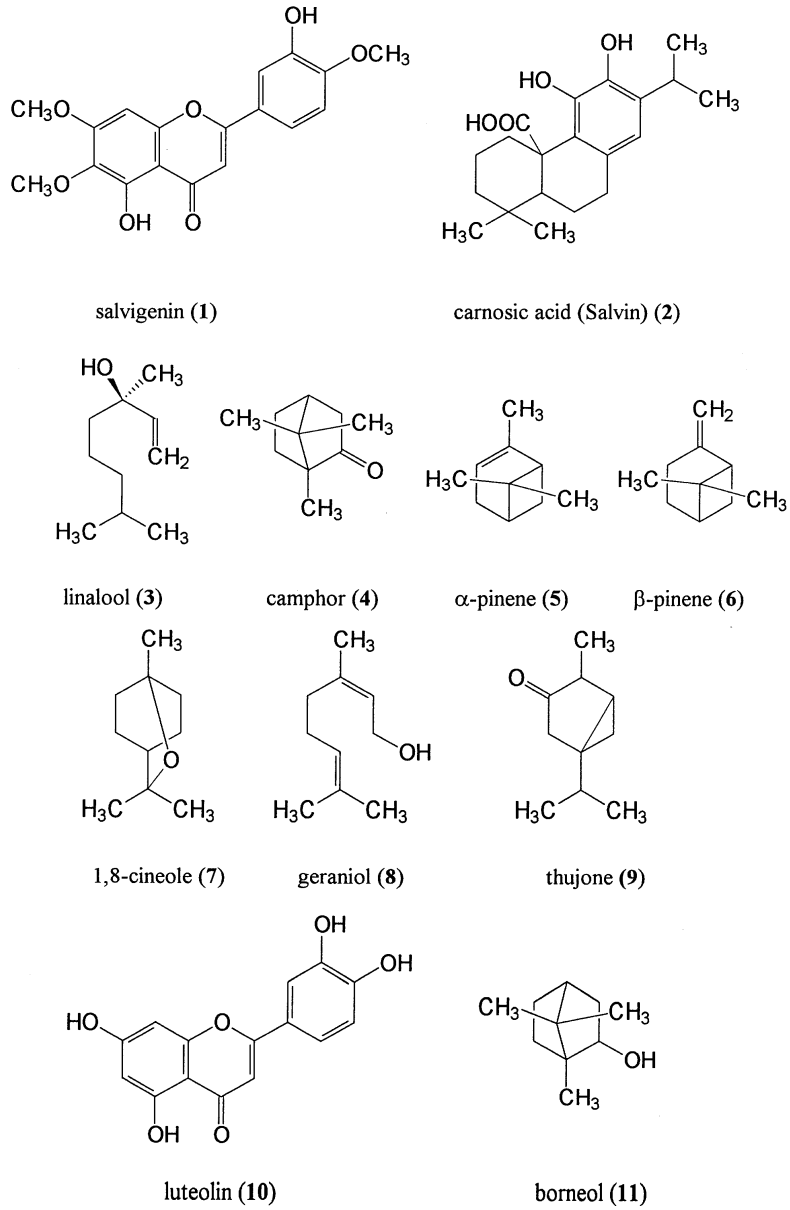


Figure 2 Anti-inflammatory (**5**, **9**, **10**), antioxidant (**1**, **2**, **3**, **5**, **6**, **7**, **8**) and oestrogenic (**8**) constituents of *Salvia* species.

contained an integrated human estrogen receptor (hER) and expression plasmids containing estrogen-responsive sequences (ERE). ERE control the expression of the gene *lac*-Z, which encodes the enzyme β-galactosidase. Thus, in the presence of an active ligand, the hER interacts with transcription factors to modulate gene expression and β-galactosidase is secreted into the medium, where it metabolizes a chromogenic substrate (chlorophenol red-β-D-galacto-pyranoside) into a red product measured by absorbance at 540 nm. 17β-Estradiol was used as a positive control (62–1000 nM). The volatile monoterpenoids (α- and β-pinene, 1,8-cineole, geraniol and thujone; final concn 0.001–2.25 mM) and essential oil (final concn 0.01–1.25 μL mL^{-1}) of *S. lavandulaefolia* were added to the well already containing 200 μL yeast culture; extracts (in 96% EtOH) were tested at a final concentration of 1–5 mg mL^{-1}. The data are presented as the mean $\pm$ s.d. relative to the control for yeast cells with EtOH and yeast alone.

Statistics

All results are presented as the mean ± s.d. of three or more experiments. Student's *t*-test was used to assess the significance of differences between control and test activities and $P < 0.05$ was regarded as being statistically significant.

Results

Antioxidant assay

The EtOH extract, the H_2O and $CHCl_3$ layers of the EtOH extract (5 mg mL^{-1}) and the monoterpenoids (0.1 M) α and β-pinene (10–15% of essential oil), 1,8-cineole (17% of essential oil), geraniol and thujone (< 1% of essential oil) showed significant antioxidant effects in protecting bovine liposome peroxidation ($P < 0.05$) (Table 1). Camphor (27% of essential oil) exhibited pro-oxidant activity in this analysis (Figure 2). Propyl gallate gave 100% inhibition at 10 μM.

Assay for eicosanoid synthesis inhibition

The total oil was not soluble enough to be used in the test system. The EtOH extract and the $CHCl_3$ layer of the dried *S. lavandulaefolia* herb were selective for LTB_4 over TXB_2 (with 50 μg mL^{-1} giving 60 ± 5.6 and 78 ± 4.6% inhibition of the control, respectively) (Table 2). The effect of the total oil would therefore be expected to be weak, but in favour of inhibition of 5-lipoxygenase inhibition, since α-pinene (200 μM; 5% of essential oil) showed weak selectivity for LTB_4, giving 48 ± 15.5% inhibition of the control, whereas geraniol (200 μM; < 1% of essential oil) showed weak selectivity for TXB_2 giving 46 ± 7.8% inhibition of the control. The monoterpenoids 1,8-cineole, thujone, and camphor (at 200 μM) and the H_2O layer of the EtOH extract did not show significant inhibition of either eicosanoid in this analysis.

Estrogenic assay

The essential oil demonstrated weak estrogenic activity over a range of concentrations tested in the same assay, although higher concentrations killed the yeast cells as shown by a decrease in absorbance at higher concentrations (Tables 3, 4). The estrogenic activity of the essential oil was not significant compared with the control since the variation in activity between cells was high and dose-dependent activity could not be obtained.

There was weak, but significant ($P < 0.02$), estrogenic activity by 1.25 mg mL^{-1} of the EtOH extract of *S. lavandulaefolia*, although the highest concentration affected cell growth. There was also weak significant estrogenic activity at the highest concentration of the H_2O layer of the EtOH extract ($P < 0.003$). No significant estrogenic activity was shown by the $CHCl_3$ layer of the EtOH extract and all concentrations tested affected cell growth.

Of the five monoterpenoid constituents of *S.*

Table 2 Eicosanoid production in rat leucocytes by extracts and commercially obtained constituents of *S. lavandulaefolia* essential oil.

Extract/compound	Percentage LTB_4 released by A23187 alone	Percentage TXB_2 released by A23187 alone
Ethanol extract of *S. lavandulaefolia* (50 μg mL^{-1})	73 ± 4	39 ± 3*
Aqueous fraction from ethanol extract of *S. lavandulaefolia* (50 μg mL^{-1})	71 ± 6	22 ± 2*
Chloroform fraction from ethanol extract of *S. lavandulaefolia* (50 μg mL^{-1})	83 ± 8	61 ± 8*
Camphor (**4**) (200 μM)	96 ± 2	97 ± 17
α-Pinene (**5**) (200 μM)	93 ± 7	51 ± 10*
1,8-Cineole (**7**) (200 μM)	101 ± 3	105 ± 13
Geraniol (**8**) (200 μM)	52 ± 6*	112 ± 4
Thujone (**9**) (200 μM)	95 ± 4	89 ± 6
Indometacin (positive control) (5 μM)	99 ± 2	6 ± 1*
ZM211965 (positive control) (5 μM)	5 ± 1*	100 ± 2

Note: a low value indicates strong inhibition. Data are mean ± s.d., n = 6. *$P < 0.05$, significant difference compared with control.

Table 3 Binding to estradiol receptors in yeast by extracts and essential oil of *S. lavandulaefolia* as shown by induction of β-galactosidase activity.

Extract/compound	Concentration	Absorbance at 540 nm
Ethanol extract of *S. lavandulaefolia*	5 mg mL^{-1}	0.096 ± 0.011
	2.5 mg mL^{-1}	0.146 ± 0.004
	1.25 mg mL^{-1}	0.149 ± 0.030*
	1 mg mL^{-1}	0.125 ± 0.010
Aqueous fraction from ethanol extract of *S. lavandulaefolia*	5 mg mL^{-1}	0.156 ± 0.005*
	2.5 mg mL^{-1}	0.121 ± 0.007
	1.25 mg mL^{-1}	0.107 ± 0.006
	1 mg mL^{-1}	0.111 ± 0.002
Chloroform fraction from ethanol extract of *S. lavandulaefolia*	5 mg mL^{-1}	0.002 ± 0.003
	2.5 mg mL^{-1}	0.045 ± 0.001
	1.25 mg mL^{-1}	0.101 ± 0.001
	1 mg mL^{-1}	0.096 ± 0.002
Essential oil	1.25 mg mL^{-1}	0.096 ± 0.070
	0.625 mg mL^{-1}	0.142 ± 0.035
	0.313 mg mL^{-1}	0.217 ± 0.130
	0.25 mg mL^{-1}	0.162 ± 0.061
	0.125 mg mL^{-1}	0.092 ± 0.013
	0.0125 mg mL^{-1}	0.224 ± 0.104
	1.25 μg mL^{-1}	0.181 ± 0.081
	0.125 μg mL^{-1}	0.112 ± 0.071
Estradiol (positive control)	62 nM	0.275 ± 0.002
	1000 nM	0.342 ± 0.001

Data are mean $\pm$ s.d., n = 3. *$P < 0.05$ significant difference compared with control.

lavandulaefolia essential oil screened, only geraniol (0.1–2 mM; < 1% of essential oil) exhibited estrogenic activity ($P < 0.001$) in this analysis, which was weak in comparison with 17β-estradiol (1000 nM). The highest concentrations of all the monoterpenoids interfered with cell growth.

Discussion

Antioxidant activity

The results confirm the reputed antioxidant effects of *Salvia* in the species *S. lavandulaefolia* (Huang et al 1996). Antioxidant effects were present in the EtOH extracts and five out of the six monoterpenoids tested in this analysis (Table 1). Since the concentration of extracts and monoterpenoids were high (5 mg mL^{-1} and 0.1 M, respectively), the inhibition can be considered weak, but significant ($P < 0.05$), in comparison with the synthetic antioxidant propyl gallate (100% inhibition at 10 μM). However, more potent compounds may be present in small quantities. Given that the monoterpenoids with antioxidant activity in this study are present in the essential oil at a slightly higher relative percentage (collectively over 30%) than camphor (27% of essential oil), the pro-oxidant activity of camphor may not have an effect if the whole essential oil were tested, but any synergistic effects are unknown.

Although the constituents responsible for the antioxidant activity of the extracts tested here remain to be identified, carvacrol has been reported to be an antioxidant agent present in *S. lavandulaefolia* (ssp. *oxyodon*) (Dorman et al 1995; Adam et al 1998). *Salvia* constituents such as flavonoids (e.g. salvigenin; **1**), diterpenoids (e.g. carnosic acid; **2**) (Graven et al 1992; Dorman et al 1995) and monoterpenoids (e.g. linalool; **3**) (< 1% of essential oil) have previously been shown to be antioxidants and these could be present in the ethanolic extract (Aruoma et al 1996; Culvelier et al 1996; Kang et al 1997). The monoterpenoid camphor (**4**) (27% of the essential oil) exhibited pro-oxidant activity on bovine liposomes, whereas α- and β-pinene (**5** and **6**, respectively), 1,8-cineole (**7**), geraniol (**8**) and thujone (**9**) (collectively 35% of the essential oil) exhibited antioxidant activity. Further structure–activity relationships of the constituent monoterpenoids of the essential oil of *S. lavandulaefolia* are required. It would also be important to establish their

Table 4 Binding to estradiol receptors in yeast by major constituents of the essential oil of *S. lavandulaefolia* as shown by induction of β-galactosidase activity (n = 3).

Compound	Concentration (mM)	Absorbance at 540 nm
α-Pinene (**5**)	0.001	0.161 ± 0.010
	0.01	0.115 ± 0.009
	0.1	0.160 ± 0.001
	0.2	0.107 ± 0.004
	2.25	0.002 ± 0.000
β-Pinene (**6**)	0.001	0.108 ± 0.009
	0.01	0.095 ± 0.005
	0.1	0.090 ± 0.000
	0.2	0.108 ± 0.002
	2.25	0.003 ± 0.003
1,8-Cineole (**7**)	0.001	0.135 ± 0.002
	0.01	0.116 ± 0.002
	0.1	0.148 ± 0.001
	0.2	0.120 ± 0.003
	2.25	0.118 ± 0.001
Geraniol (**8**)	0.001	0.146 ± 0.004
	0.01	0.132 ± 0.008
	0.1	0.282 ± 0.104*
	0.2	0.465 ± 0.171*
	2.25	0.008 ± 0.007
Thujone (**9**)	0.001	0.008 ± 0.004
	0.01	0.155 ± 0.001
	0.1	0.107 ± 0.003
	0.2	0.100 ± 0.120
	2.25	0.006 ± 0.006
Estradiol (positive control)	62 nM	0.279 ± 0.003
	1000 nM	0.343 ± 0.002

Data are mean ± s.d., n = 3. *$P < 0.001$ compared with control.

activity in different oxidative systems to determine other possible mechanisms of action, such as protection against oxidative DNA damage (Halliwell 1995), since compounds may exhibit antioxidant activity in one system, but show pro-oxidant activity in others (Masaki et al 1995; Aruoma et al 1996).

Anti-inflammatory activity

Many *Salvia* species and their constituents are reputed to have anti-inflammatory activity and these results support the use of *Salvia* species in traditional medicine to treat inflammation (Bartram 1995; Hernandez-Perez 1995). The *S. lavandulaefolia* extracts showed only weak inhibition of eicosanoid synthesis, although there may be more potent constituents present in minute quantities. Whereas geraniol (< 1% of the essential oil) showed weak selectivity for TXB_2, the EtOH and $CHCl_3$ extracts and α-pinene (5% of the essential oil and previously reported to show anti-inflammatory action; Bingöl & Sener 1995), showed weak selectivity for LTB_4. LTB_4 is produced via the enzyme 5-lipoxygenase, the gene of which is up-regulated during neurodegeneration, and although the role of this inflammatory mediator in Alzheimer's disease is not entirely apparent, selective inhibition over cyclooxygenase may be therapeutically relevant (Sugaya et al 2000). The three other monoterpenoids tested (collectively 44% of essential oil) did not show anti-inflammatory activity in this analysis.

The compounds responsible for the inhibition of eicosanoid synthesis shown by the EtOH and $CHCl_3$ extracts tested in this study remain to be identified. Most of the anti-inflammatory activity of plants, so far reported, has been attributed to the presence of phenolics, which are widely distributed in nature. Anti-inflammatory constituents of *Salvia* species, so far identified, include flavonoids (e.g. luteolin; **10**) and terpenoids (e.g. carvacrol, rosmarinic acid and α-pinene, a number of which are present in *S. lavandulaefolia* (Wager et al 1986; Bingöl & Sener 1995; Cuvelier et al 1996). Many essential oils have been used for the treatment of rheumatism and inflammation and some monoterpenoids have been found to inhibit cyclooxygenase (Wagner et al 1986). Wagner et al (1986) and Bingöl & Sener (1995) concluded that for anti-inflammatory action there has to be at least 1 hydroxyl group. In this study geraniol, but not α-pinene, contains a hydroxyl group. It is of interest that this structural requirement is also important for many naturally occurring antioxidant and oestrogenic actions (Bouchet et al 1998). Further to this, anti-inflammatory flavonoids containing hydroxyl functions have been shown both to scavenge reactive oxygen species and to promote their formation, depending on the experimental conditions (Abelson & Simon 1994). Screening in other anti-inflammatory assays, which may use other inflammatory mediators, may determine other mechanisms of action that target mechanisms specific to inflammation in Alzheimer's disease, such as complement Aβ interactions (Santos & Rao 1997).

Estrogenic activity

Estrogenic activity was present in the EtOH extract and the essential oil of *S. lavandulaefolia* (Table 3). No dose–response activity was obtained from the essential oil. An explanation may lie in the volatility of components of the oil that may have evaporated and spread their activity to surrounding cells across the plate. Thus, it was interesting to note significant estrogenic activity in those wells surrounding the higher concentrations of

geraniol (data not shown), a monoterpenoid present only in small amounts in the essential oil (Figure 1). The highest concentration of geraniol affected cell growth, causing lysis of cells (Table 4); however the growth of cells surrounding this concentration produced estrogenic activity (data not shown). It can be suggested that a quantity of geraniol was transferred (owing to its volatility) into surrounding cells that originally contained no geraniol. This could also be seen at the lower concentrations of geraniol where the estrogenic activity in surrounding cells was also affected by the nearby concentration of geraniol. Repeat and carefully controlled experiments would be necessary to confirm the activity of the essential oil, extracts and geraniol and to determine, for example, dose–response activity. It is interesting that geraniol is a recruiting pheromone for honeybees that can be detected over 7 miles away and that pheromones act synergistically to produce their effect (Harborne & Tomas-Barberan 1991).

The mechanism of action of estrogenic agents has been related to a phenolic ring in the molecule and many plant extracts exhibit estrogenic action because they contain isoflavonoids. However, it is unlikely that flavonoids are present in the essential oil. Studies have shown a structure–activity association between the most effective (neuroprotective) effects of estrogen (specifically β-estradiol) with the hydroxyl groups (2, 3 or 4) on the steroid A-ring (Simpkins et al 1997; Xiao & Becker 1998). Although not phenolic in nature, of the monoterpenes tested here, only geraniol contains a hydroxyl group and, in addition, it lacks the cyclic ring structure present in the four other monoterpenoids tested (α- and β-pinene, 1,8-cineole and thujone). It would be of interest to test other monoterpenoid structures to establish any structure–activity relationships. The results obtained here would also need further assessment in other estrogenic assays, such as one using human cells (Littlefield et al 1990), to determine their mechanism of action in more detail. Compounds that produce estrogenic activity via the hER developed in yeast may not produce activity in an in-vivo environment (Green et al 1998) where compounds can be metabolized and the metabolites can have more, less, no, or different effects (such as through regulation of post-transcriptional events and other non-genomic events).

It is known that stimulation of estrogen receptors can produce different pharmacological effects (Jordan 1998) and thus potential estrogenic agents may also have other activities. In addition, the antioxidant effect (against Aβ, hydrogen peroxide and glutamate damage) of β-estradiol (which is also dependent on the presence of the hydroxyl group) is independent of an activation of estrogen receptors (Behl et al 1997), and estrogens have been found to stimulate prostaglandin biosynthesis (via cyclooxygenase) (Gambassi et al 1996). Thus, it is becoming increasingly evident that there may be a possibility of a combined or even synergistic biological effect from many of the plant-derived chemicals that exhibit antioxidant, anti-inflammatory and estrogenic actions, which may be associated with the presence of a ring structure (especially if it is phenolic in nature) and the presence of hydroxyl groups (Jiménez et al 1995; Green et al 1998).

Conclusions

It is evident that further investigations are required on the pharmacology of individual, combined monoterpenoids and essential oils to clarify their bioactivity. *S. lavandulaefolia* extracts and/or individual monoterpenoid constituents have anticholinesterase, antioxidant, anti-inflammatory and estrogenic effects and these activities are relevant to current Alzheimer's disease therapy. There are many other pharmacological targets that might be relevant to therapy in Alzheimer's disease, for example, enhancing other cholinergic targets such as choline acetyltransferase and cholinergic receptors, targeting the process involved in Aβ aggregation and other transmitter systems that are affected in Alzheimer's disease. With the growing wealth of pharmacological evidence for the reputed traditional uses of *Salvia* (*S. lavandulaefolia*), the absence of any reported adverse effects from its long use in food flavourings and that it has biological effects relevant to the cholinergic and other systems pertinent to Alzheimer's disease, a controlled clinical study of the essential oil from this species should now be undertaken to determine whether it has effects relevant to the treatment of Alzheimer's disease.

References

Abelson, J. N., Simon, M. I. (eds) (1994) *Methods in Enzymology*. Vol 234. *Oxygen Radicals in Biological Systems* (Part D). pp 443–445

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M. (1998) Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula augustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem.* **46**: 1739–1745

Aisen, P. S. (1996) Inflammation and Alzheimer's disease. *Mol. Chem. Neuropathol.* **28**: 83–88

Aruoma, O. I. (1996) Assessment of potential prooxidant and antioxidant actions. *JAOCS* **73**: 1617–1625

Aruoma, O. I., Spencer, J. P. E., Rossi, R., Aeschbach, R., Khan, H., Mahmood, N., Munoz, A., Murcia, A., Butler, J., Halliwell, B.

(1996) An evaluation of the antioxidant and antiviral action of the extracts of rosemary and provencal herbs. *Food Chem. Toxicol.* **34**: 449–456

Bartram, T. (1995) *Encyclopaedia of Herbal Medicine*. 1st edn. Grace Publishers, London, p. 379

Behl, C., Skutella, T., Lezoualch, F., Post, A., Widmann, M., Newton, C. J., Holsboer, F. (1997) Neuroprotection against oxidative stress by estrogens: structure–activity relationship. *Am. Soc. Pharmacol. Exp. Ther.* **51**: 535–541

Bingol, F., Sener, B. (1995) A review of terrestrial plants and marine organisms having antiinflammatory activity. *Int. J. Pharmacog.* **33**: 81–97

BirkHäuser, M. H., Strnad, J., Kämpp, C., Bahro, M. (2000) Oestrogens and Alzheimer's disease. *Int. J. Geriatr. Psychiatry* **15**: 600–609

Bouchet, N., Barrier, L., Fauconneau, B. (1998) Radical scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae). *Phytother. Res.* **12**: 159–162

Brautigam, M. R. H., Blommaert, F. A., Verleye, G., Castermans, J., Jansen Steur, E. N. H., Kleijnen, J. (1998) Treatment of age-related memory complaints with *Ginkgo biloba* extract: a randomised double blind placebo-controlled study. *Phytomedicine* **5**: 425–434

Breitner, J. C. S. (1996) Inflammatory processes and antiinflammatory drugs in Alzheimer's disease: a current appraisal. *Neurobiol. Aging* **17**: 789–794

Carr, D. B., Goate, A., Phil, D., Morris, J. C. (1997) Current concepts in the pathogenesis of Alzheimer's disease. *Am. J. Med.* **103**: 3S–10S

Cooper, N. R., Kalaria, R. N., McGeer, P. L., Rogers, J. (2000) Key issues in Alzheimer's disease inflammation. *Neurobiol. Aging* **21**: 451–453

Culvelier, M.-E., Richard, H., Berset, C. (1996) Antioxidant activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. *JAOCS* **73**: 645–652

de Vries, H. E., Kuiper, J., De Boer, A. G., Van Berkel, J. C., Breimer, D. (1997) The blood–brain barrier in neuroinflammatory diseases. *Pharmacol. Rev.* **49**: 143–155

Dorman, H. J. P., Deans, S. G., Noble, R. C. (1995) Evaluation *in vitro* of plant essential oils as natural antioxidants. *J. Essent. Oil. Res.* **7**: 645–651

Frölich, L., Riederer, P. (1995) Free radical mechanisms in dementia of Alzheimer type and the potential for antioxidant treatment. *Drug Res.* **45**: 443–446

Gambassi, G., Landi, R., Bernabei, R. (1996) Oestrogen and Alzheimer's disease. *Lancet* **348**: 1027–1030

Graven, E. H., Deans, S. G., Svoboda, K. P., Mavi, S., Gundidza, M. G (1992) Antimicrobial and antioxidative properties of the volatile (essential oil) of *Artemisia afra* Jacq. *Flav. Frag. J.* **7**: 121–123

Green, P. S., Gridley, K. E., Simpkins, J. W. (1998) Nuclear estrogen receptor-independent neuroprotection by estratrienes: a novel interaction with glutathione. *Neuroscience* **84**: 7–10

Halliwell, B. (1995) Antioxidant characterization: methodology and mechanism. *Biochem. Pharmacol.* **49**: 1341–1348

Harborne, J. B., Tomas-Barberan, F. A. (eds) (1991) *Ecological Chemistry and Biochemistry of Plants Terpenoids*. Clarendon Press, Oxford

Hernandez-Perez, M., Rabanal, R. M., Delatorre, M. C., Rodriguez, B. (1995) Analgesic, antiinflammatory, antipyretic and hematological effects of aethiopinene, an omicron-naphthoquinone diterpenoid from *Salvia aethiopis* roots and two hemisynthetic derivatives. *Planta Med.* **61**: 505–509

Huang, S. W., Frankel, E. N., Schwarz, K., Aeschbach, R., German, J. B. (1996) Antioxidant activity of carnosic acid and methyl carnosate in bulk oils and oil-in-water emulsions. *J. Agric. Food Chem.* **44**: 2951–2956

Jiménez, I., Jiménez, J., Gamez, M. J., Gonzalez, M., Sanchez de Medina, F., Zarzuelo, A. (1995) Effects of *Salvia lavandulifolia* Vahl. ssp. Oxyodon extract on pancreatic endocrine tissue in steptozotocin-diabetic rates. *Phytother. Res.* **9**: 536–537

Jordan, V. C. (1998) Designer estrogens. *Sci. Am.* **279**: 60–67

Kang, H. S., Chung, H. Y., Jung, J. H., Kang, S. S., Choi, J. S. (1997) Antioxidant effects of *Salvia miltiorrhiza*. *Arch. Pharmacol. Res.* **20**: 496–500

Kanowski, S., Herrmann, W. M., Stephan, K. T., Wierich, W., Horr, R. (1997) Proof of efficacy of the *Ginkgo biloba* special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. *Phytomedicine* **4**: 3–13

Le Bars, L. P., Katz , M. M., Berman, N., Itil, T. M., Freedman, A. M., Schatzberg, A. F. (1997) A placebo-controlled, double blind, randomized trial of an extract of *Ginkgo biloba* for dementia. *JAMA* **278**: 1327–1332

Littlefield, B. A., Gurpide, E., Markiewicz, L., McKinley, B., Hochberg R. B. (1990) A simple and sensitive microtiter plate estrogen bioassay based on stimulation of alkaline phosphatase in ishikawa cells: estrogenic action of delta 5 adrenal steroids. *Endocrinology* **127**: 2757–2762

Masaki, H., Sakaki, S., Atsumi, T., Sakurai, H. (1995) Active-oxygen scavenging activity of plant extracts. *Biol. Pharm. Bull.* **18**: 162–166

Moroney, M. A., Alcarez, M. J., Forder, R. A., Carey, R., Hoult, R. S. (1988) Selectivity of neutrophil 5-lipoxygenase and cyclooxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. *J. Pharm. Pharmacol.* **40**: 787–792

Mulnard, R. I., Cotman, C. W., Kawas, C., Van Dyck, C. A., Sano, H., Doody, R., Koss, E., Pfeiffer, E., Jin, S., Gamst, A., Grundman, M., Thomas, R., Mal, L. J. (2000) Estrogen replacement therapy for treatment of mild to moderate Alzheimers disease – A randomised controlled trial. *J. Am. Med. Ass.* **283**: 1007–1015

Murray, C. A., Lynch, M. A. (1998) Dietary supplementation with vitamin E reverses the age-related deficit in long term potentiation in dentate gyrus. *J. Biol. Chem.* **273**: 12 161–12 168

Pratico, D., Delanty, N. (2000) Oxidative injury in diseases of the central nervous system: focus on Alzheimer's disease. *Am. J. Med.* **109**: 577–585

Rogers, J. (1995) Inflammation as a pathogenic mechanism in Alzheimer's disease. *Drug Res.* **45**: 439–442

Routledge, E. J., Sumpter, J. P. (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.* **15**: 241–248

Sano, M., Ernesto, C., Thomas, R. G., Klauber, M. R., Schafer, K., Grundman, M., Woodbury, P., Growdon, J., Cotman, C. W., Pfeiffer, E., Schneider, L. S., Thal, L. J. (1997) A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N. Engl. J. Med.* **336**: 1216–1222

Santos, F. A., Rao, V. S. N. (1997) Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constituent of eucalyptus and rosemary oils. *Eur. J. Pharmacol.* **331**: 253–258

Simpkins, J. W., Green, P. S., Kelly, B. S., Gridley, K. E., Singh, M., Fiebre, N. C., Rajakumar, G. (1997) Role of estrogen replacement therapy in memory enhancement and the prevention of neuronal loss associated with Alzheimer's disease. *Am. J. Med.* **103**: 19S–25S

Small, G. W., Rabins, R. V., Barry, P. P., Buckholtz, N. S., DeKosky, S. T., Ferris, S. H., Finkel, S. I., Gwyther, L. P., Khachaturian, Z. S., Lebowitz, B. D., McRae, T. D., Morris, J. C., Oakley, F.,

Schneider, L. S., Streim, J. E., Sunderland, T., Teri, L. A., Tune, L. E. (1997) Diagnosis and treatment of Alzheimer disease and related disorders. *JAMA* **278**: 1363–1371

Sugaya, K., Uz, T., Kumar, V., Manev, H. (2000) New anti-inflammatory treatment strategy in Alzheimer's disease. *Jpn. J. Pharmacol.* **82**: 85–94

Tariot, P. N., Solomon, P. R., Morris, J. C., Kershaw, P., Lilienfeld, S., Ding, C. (2000) A 5-month, randomised placebo-controlled trial of galantamine in AD. *Am. Acad. Neurol.* **54**: 2269–2276

Varadarajan, S., Kanski, J., Aksenova, M., Lauderback, C., Butterfield, D. A. (2001) Different mechanisms of oxidative stress and neurotoxicity for Alzheimers A beta (1–42) and A beta (25–35). *J. Am. Chem. Soc.* **123**: 5625–5631

Wagner, H., Wierer, M., Bauer, R. (1986) In vitro inhibition of prostaglandin biosynthesis by essential oils and phenolic compounds. *Planta Med.* 184–187

Xiao, L., Becker, J. B. (1998) Effects of estrogen agonists on amphetamine-stimulated dopamine release. *Synapse* **2**: 379–391