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Activity of Australian grown *Lavandula* spp. essential oils against *Aspergillus nidulans*, *Trichophyton mentagrophytes*, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*

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ABSTRACT

In this study the antifungal activity of eight essential oils and three hydrosols (aqueous distillates) from Australian grown lavenders was determined. The oils and hydrosols were assayed against four fungi, *Aspergillus nidulans*, *Trichophyton mentagrophytes*, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*, using a disc diffusion method. No evidence of antifungal activity was observed with any of the hydrosols. In contrast, all oils displayed some antifungal activity. *Lavandula angustifolia* and the three examples of *Lavandula* x *intermedia* oil demonstrated the greatest effect against *A. nidulans* and *T. mentagrophytes* while *L. stoechas* was particularly effective against the two agricultural fungi, *L. maculans* and *S. sclerotiorum*. No significant difference was observed between the antifungal activity of *L. angustifolia* oils derived from European and Australian grown plants. These results suggest that the oils from various *Lavandula* species may be useful in the treatment of fungal infections.

KEYWORD INDEX: *Lavandula angustifolia*; *Lavandula allardii*, *Lavandula intermedia*, *Lavandula heterophylla*, *Lavandula stoechas*, laminaceae, lavender, essential oil composition, linalool, linalyl acetate, camphor, 1,8-cineole, fenchone, hydrosols, antifungal activity

INTRODUCTION

Lavender essential oils are widely used in the aromatherapy industry and research has shown that they possess a range of biological properties that may make them useful in a medical environment (1). Increasingly lavender hydrosols are also being advocated for a variety of therapeutic uses (2). Hydrosols or distillate waters are a by-product of steam distillation of essential oils and contain variable amounts of essential oil as well as other dissolved compounds (3). These products are generally discarded although there has been some attempt to recover oils from the hydrosols to enrich the primary essential oil (4).

As with other essential oils, much of the lavender research has been targeted to the antimicrobial properties of the oil, in part as a response to increasing resistance of microorganisms to conventional pharmaceuticals (5, 6). The antibacterial activity of lavender oils has been demonstrated by many researchers including Lis-Balchin et al. (7), Svoboda and Deans (8) and Goren et al. (9). Other researchers have investigated the antifungal activity of lavender essential oils. For example, Inouye et al. (10, 11) have demonstrated that *Lavandula angustifolia* oil is effective against a range of fungi including *Trichophyton rubrum* and *Aspergillus fumigatus*. Lavender essential oils have also been shown to inhibit the growth of *Aspergillus niger*, *Aspergillus ochraceus* and *Fusarium culmorum*, with inhibition ranging from 29-93% (7).

The majority of research into lavender oils have used *L. angustifolia*, also known as *L. officinalis*, *L. vera*, or 'true' lavender, as this is the oil preferred by the perfume industry due to its low camphor content (1). However, other lavender oils such as *L. x intermedia* (lavandin), *L. stoechas* and *L. x allardii* are also grown and distilled, particularly in geographical areas such as mainland Australia not well suited to large-scale production of *L. angustifolia*. Although these oils are generally viewed as having less commercial value than *L. angustifolia* oils they may have therapeutically useful properties (1, 12). In this study we have investigated the activity of Australian grown lavender oils against four fungi to determine which may have potential as antifungal agents.

EXPERIMENTAL

Other than the *L. angustifolia* oils which were purchased commercially (Bronson & Jacobs Pty Ltd., Sydney and Norfolk Lavender, UK) essential oils and hydrosols were obtained directly from local growers/distillers. The oils used in this study were: *L. angustifolia* (2 samples; 1 European (A) and 1 Australian grown (B)), *L. x allardii* (2 samples – A & B), *L. x intermedia* 'Grosso', *L. x intermedia* 'Seal', *L. x intermedia* 'Miss Donnington', *L. x heterophylla*, *L. stoechas* 'Avonview. Hydrosols (aqueous distillate) were also obtained for *L. x allardii*, *L. x intermedia* 'Seal' and *L. x intermedia* 'Miss Donnington'. Each oil or hydrosol was assayed against *Aspergillus nidulans*, *Trichophyton mentagrophytes*, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*. Organisms were originally obtained from the culture collection of the University of New South Wales, Australia. Essential oil and hydrosol compositions were determined by GC/MS analysis (outsourced to Australian Botanical Products, Australia).

Fungi were grown on either V8 juice agar (*L. maculans*) or Sabaurouds dextrose (SAB) agar (*A. nidulans*, *T. mentagrophytes* and *S. sclerotiorum*) with 6 mm plugs of actively growing fungus removed from each fungal colony for use in the assay. For each assay 2 fungal plugs were placed onto a petri dish containing either V8 juice agar (Gibco, Australia) or SAB agar (Gibco, Australia) such that the plugs were separated by 46 mm. A single sterile 6 mm paper disc (Oxoid, UK), onto which was pipetted 10 μL of oil or hydrosol, was placed in the centre of the petri dish (20 mm from each fungal plug) and the plates immediately sealed with Parafilm. 10 μL of nanopure water was added to the disc in the control plates. Plates were incubated at 25°C and the growth of the fungi measured, in mm, at regular intervals until the fungi in the control plates had reached the centre disc. All assays were repeated three times.

Data was analyzed using GraphPad Prism (Version 4) with differences between samples evaluated using an ANOVA with Tukey's multiple comparison post-test. Differences were deemed to be significant when $p<0.05$.

RESULTS AND DISCUSSION

The main constituents of the essential oils and hydrosols used in this study are shown in Table I. Linalyl acetate, linalool, camphor, 1,8-cineole were the major constituents of the oils with fenchone being present only in *L. stoechas* oil. The two samples of *L. angustifolia* had similar compositions other than the European grown sample of *L. angustifolia* which had a slightly lower level of linalool. Likewise, the two samples of Australian grown *L. x allardii* differed only minimally in their composition.

The effects of the oils and hydrosols on the four fungi used in this study are shown in Figures 1 and 2. None of the hydrosols tested in this study had a statistically significant effect on the growth of the fungi.

All oils assayed inhibited the growth of *A. nidulans* at all time points ($p<0.01$) (Figure 2). By day 6 some statistically significant differences between the various oils were noted; *L. x allardii* (A) v *L. intermedia* 'Miss Donnington', *L. x intermedia* 'Seal', *L. x intermedia* 'Grosso', *L. angustifolia* (all $p<0.01$); *L. x allardii* (B) v *L. angustifolia* (A) ($p<0.05$), *L. angustifolia* (B) ($p<0.01$) *L. x intermedia* 'Miss Donnington' ($p<0.01$), *L. x intermedia* 'Seal', *L. x intermedia* 'Grosso' (both $p<0.001$); *L. x heterophylla* v *L. intermedia* 'Seal' and *L. x intermedia* 'Grosso' (both $p<0.05$). In addition there was significantly greater inhibition of growth with *L. x allardii* (A) compared to *L. x allardii* (B) ($p<0.05$). Overall the *L. angustifolia* and *L. x intermedia* oils provided the greatest growth inhibition of *A. nidulans* resulting in an approximate 50% reduction in growth by day 6.

Similarly the growth of *T. mentagrophytes* was inhibited by all oils tested ($p<0.01$) at 2, 4, 7 and 9 days growth (Figure 2) and resulted in growth inhibition of between 57% (*L. x allardii* (A)) and 86% (*L. angustifolia* (A)) relative to controls on day 9. Statistically significant differences in growth inhibition noted at day 9 were: *L. x allardii* (A) v *L. x intermedia* 'Seal', *L. x intermedia* 'Grosso', *L. x intermedia* 'Miss Donnington', *L. angustifolia* (A) (all $p<0.001$); *L. x allardii* (B) v *L. x intermedia* 'Seal', *L. x intermedia* 'Grosso', *L. x intermedia* 'Miss Donnington', *L. angustifolia* (A) (all $p<0.001$); *L. x heterophylla* v *L. x intermedia* 'Seal', *L. x intermedia* 'Grosso', *L. x intermedia* 'Miss

Donnington' (all $p<0.01$), *L. angustifolia* (A)($p<0.05$); *L. angustifolia* (B) v *L. x allardii* (A) & (B) (both $p<0.01$).

For both *A. nidulans* and *T. mentagrophytes*, the oils showing the greatest inhibition of fungal growth were the *L. x intermedia* and *L. angustifolia* oils (Figure 2). In contrast to these two fungi, not all oils inhibited the growth of *L. maculans*. From day 2 to day 4 all oils inhibited growth of *L. maculans* ($p<0.01$)(Figure 3) however from day 5 to 7 this inhibitory effect was lost with some oils such that by day 7 the only oils to show an inhibitory effect were *L. x allardii* (A) and (B), *L. x heterophylla*, *L. x intermedia* 'Miss Donnington', *L. x intermedia* 'Grossa' and *L. stoechas* (all $p<0.01$). The greatest inhibition at day 7 was observed with *L. stoechas*, which produced a 64% reduction in growth compared to the control. Statistically significant differences were observed between oils on day 7 were: *L. stoechas* v all other samples ($p<0.001$), *L. angustifolia* (A) v *L. x allardii* (A), *L. x intermedia* 'Grosso' (both $p<0.01$), L. x allardii (B), *L. x intermedia* 'Miss Donnington, *L. x heterophylla* ($p<0.001$); *L. angustifolia* (B) v *L. x allardii* (B) ($p<0.001$), *L. x heterophylla*, *L. x intermedia* 'Miss Donnington' (both $p<0.01$); *L. x allardii* (B) v *L. x intermedia* 'Seal', *L. x intermedia* 'Miss Donnington', *L. x intermedia* 'Grosso', *L. x heterophylla*, *L. x allardii* (A) (all $p<0.001$); *L. x intermedia* 'Seal' v *L. x intermedia* 'Grosso', *L. x intermedia* 'Miss Donnington', *L. x heterophylla*, *L. x allardii* (A) (all $p<0.001$).

All oils initially inhibited the growth of *S. sclerotiorum* ($p<0.001$ at both 18 and 26 h); however, by 42 h only the fungi exposed to four oils continued to show a significant growth inhibition relative to the control; *L. x intermedia* 'Seal' ($p<0.01$), *L. x intermedia* 'Miss Donnington' ($p<0.01$), *L. stoechas* ($p<0.01$) and *L. angustifolia* (B) ($p<0.05$) (Figure 3). The greatest inhibition was observed with *L. stoechas* ($p<0.001$ relative to other oils) with growth approximately 50% of the controls.

This study demonstrates that lavender oils have an antifungal action against fungi of both medical and agricultural importance. Despite the increasing use of hydrosols for therapeutic purposes (2) we found no evidence for an antifungal activity in the hydrosols tested. The *L. angustifolia* and *L. x intermedia* oils were, in general, the best antifungal oils particularly for the medically significant fungi, *A. nidulans* and *T. mentagrophytes*. *Lavandula stoechas* oil was particularly effective against the two agricultural fungi, *L. maculans* and *S. sclerotiorum*. However no oil completely inhibited fungal growth with the greatest inhibition seen with the *L. angustifolia* oils against *T. mentagrophytes*. We observed little difference in antifungal activity between the Australian and European grown *L. angustifolia* oils.

There are no published reports of the activity of lavender essential oils against *L. maculans, S. sclerotiorum* and *A. nidulans;* however the results from this study are consistent with data for other aspergillus species and for *T. mentagrophytes*. *Lavandula angustifolia* oil has been previously noted to inhibit the growth of *A. niger* (82% inhibition), *A. ochraceus* (90% inhibition) *(8), A. fumigatus, T. mentagrophytes* and *T. rubrum* (10, 11, 13). Lavandin (type unknown) was also shown to have strong activity against *A. niger* and *A. ochraceus* (7) which is consistent with our finding that *L. x intermedia* oils were among those oils with the best activity against *A. nidulans*. The lavandin used in the study by Lis-Balchin et al. (7) does not have the same composition as any oil used in this study; with the closest match being *L. x intermedia* 'Grosso'.

There is no obvious correlation between antifungal activity and essential oil composition. For example, both *L. x allardii* oils used in this study had similar composition with respect to the oil's major chemical components; however, *L. x allardii* (B) produced a 46% greater inhibition of *L. maculans* than sample (A) of this oil. Further, although composition of the three *L. x intermedia* oils used in this study was dissimilar, all three oils displayed similar antifungal activity against both *A. nidulans* and *T. mentagrophytes*. Lis-Balchin et al. (7) also failed to show a strong correlation between linalool and linalyl acetate content and antifungal activity. The lack of correlation between the major oil components and antifungal activity suggests that the different susceptibilities of the fungi

may be related to either the minor components of the oil or differences in the cell wall/cell membrane of the fungi themselves.

Further research is required to determine which component(s) of the oils are responsible for the antifungal effect and the contribution that oil vapor makes to this activity. Linalool, a major component of many lavender oils, is known to be effective at inhibiting the growth of a range of fungi (14). Edris and Farrag (15) have shown that linalool in vapor form can suppress the growth of *S. sclerotiorum* but was not fungicidal. These authors suggest that the action of vapors is in part due to agar absorption of volatile oil components; the converse probably also applies. That is, in disc diffusion assays of volatile substances inhibitory activity is probably due to diffusion of hydrophilic oil components through the agar as well as volatile components acting directly on the fungal mycelia.

This study has demonstrated that some Australian grown lavender oils have potential as antifungal agents for both medical and agricultural application and revealed that the floral source of the oil can have a major impact on the effectiveness against different fungi.

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Table I. Percentage composition of essential oils and hydrosols used in the assay of antifungal activity. This list is a subset of the most abundant 18 compounds identified (a total of 103 compounds were identified).

		Essential oil					
Non-polar RI		*L. angustifolia* (A)	*L angustifolia* (B)	*L. x allardii* (A)	*L. x allardii* (B)	*L. x intermedia* 'Grosso'	*L. x intermedia* 'Miss Donnington'
939	alpha-pinene	0.1	0.2	5.0	4.7	1.0	
980	beta-pinene	0.1	0.1	5.8	4.7	1.1	
1031	beta-phellandrene			9.0	7.8		
1033	1,8-cineole	1.1	1.5	38.7	34.2	10.9	11.6
1040	(Z)-beta-ocimene	1.0	1.8	1.2	1.3	1.9	
1074	cis-linalool oxide (furan)	0.3	0.1	0.1	0.1	0.1	4.6
1087	fenchone				tr		
1088	trans-linalool oxide	0.2					4.2

	(furan)						
1098	linalool	29.1	36.2	4.9	4.2	34.3	12.1
1143	camphor	0.3	1.0	13.4	17.1	7.3	20.3
1165	borneol	1.1	0.8	0.4	0.7	1.6	6.0
1177	terpinen-4-ol	2.3	1.5	0.5	0.6	2.3	
1183	cryptone and p-cymen-8-ol	0.4		0.6	0.9		0.6
1186	(Z)-3-hexenyl butyrate	0.2					1.9
1189	alpha-terpineol	0.5	0.5	1.2	1.5	0.9	
1257	linalyl acetate	41.3	40.4			23.6	9.3
1289	lavandulyl acetate	6.1	1.2			2.2	0.2
1581	caryophyllene oxide	0.9	0.2	0.7	0.9		1.1

Table I continued

		Essential oil			Hydrosol		
		L. x intermedia 'Seal'	*L. x heterophylla*	*L. stoechas* 'Avonview'	*L. x allardii*	*L. x intermedia* 'Miss Donnington'	*L. x intermedia* 'Seal'
939	alpha-pinene	0.7		1.1			
980	beta-pinene	1.3	0.3				
1031	beta-phellandrene	7.9					
1033	1,8-cineole	15.3	49.1	9.2	23.3	1.7	tr
1040	(Z)-beta-ocimene	6.5					
1074	cis-linalool oxide (furan)	0.1	0.8		3.1	tr	2.8
1088	trans-linalool oxide (furan)		1.0		2.5	tr	3.2

1098	linalool	36.1	1.5	1.9	11.9	19.9	19.0
1143	camphor	0.7	20.8	48.6	39.2	17.5	2.4
1165	borneol	0.7	1.1	0.2	1.5	31.8	5.2
1177	terpinen-4-ol	3.5		0.3	3.8	2.3	14.0
1183	cryptone and p-cymen-8-ol		0.7		2.8	7.5	7.1
1186	(Z)-3-hexenyl butyrate		0.2				
1187	fenchone			21.9	tr	tr	
1189	alpha-terpineol	1.0	0.6	0.5	6.3	10.1	24.0
1257	linalyl acetate	5.8		0.4			
1289	lavandulyl acetate	0.8					
1581	caryophyllene oxide		1.0				

Figure 1. Growth of *A. nidulans* and *T. metagrophytes* in the presence of lavender essential oils. Data is graphed as mean ± standard deviation.

Figure 2. Growth of *L. maculans* and *S. sclerotiorum* in the presence of lavender essential oils. Data is graphed as mean ± standard deviation.