

FUNGISTATIC ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM *Peumus boldus* Mol., *Laureliopsis philippiana* (Looser) Schodde AND *Laurelia sempervirens* (Ruiz & Pav.) Tul. (CHILEAN MONIMIACEAE)

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ABSTRACT

Components of essential oils from the Chilean Monimiaceae, boldo (*Peumus boldus* Mol.), tepa (*Laureliopsis philippiana* (Looser) Schodde), and laurel (*Laurelia sempervirens* (Ruiz & Pav.) Tul.) were determined using Gas Chromatography- Mass Spectrometry (GCMS) and fungistatic activity of the essential oils was tested against *Rhizoctonia solani* Kühn (Donk), *Pythium irregulare* Buisman, *Ceratocystis pilifera* (Fr.) C. Moreau, *Phragmidium violaceum* (Schultz) G. Winter, and *Fusarium oxysporum* Schldt. The essential oils of the Monimiaceae species shared some common components; all three had the 3-carene, α -phellandrene, and α -pinene terpenes. *L. philippiana* and *L. sempervirens* also had safrole. The main components were ascaridol in *P. boldus* oil, 3-carene in *L. philippiana*, and safrole in *L. sempervirens*. The essential oil from *L. sempervirens* showed the highest fungistatic activity with significant differences in dose as well as exposure. *P. violaceum* was the most sensitive strain and *P. irregulare* the most resistant of all the essential oils (*P. boldus* extract affected growth by only 19%). Therefore, essential oils from these three plants could be used to control the fungal strains studied.

Key words: Chilean native trees, chemical components, natural fungistatic activity, *in vitro* analysis.

INTRODUCTION

Chemical and biological studies are useful to understand and appreciate biodiversity. In general, isolating, identifying, and determining structures of new metabolites are fundamental to reveal their chemical potential, a first step to use, conserve, and protect them (Castillo, 1992). According to Niemeyer (1995), about 5% of all 5971 known species of Chilean flora (Marticorena, 1990) have been chemically studied.

Moreover, there is great interest to replace synthetic xenobiotics with similar acting natural compounds. It is important to determine secondary metabolites with fungicidal or fungistatic activity, since they allow the use of natural origin compounds that are generally species-specific, have low environmental persistence, and are biodegradable.

In Chile, chemical and biological studies of members of the Monimiaceae family: boldo (*Peumus boldus* Mol.),

tepa (*Laureliopsis philippiana* (Looser) Schodde), and laurel (*Laurelia sempervirens* (Ruiz & Pav.) Tul.) (Rodríguez and Quezada, 2001), have attempted to determine aporphinic and bisbenzylisoquinolinic types of alkaloid compounds (Hoffmann *et al.*, 1992; Vogel *et al.*, 1999; Montes *et al.*, 2001). Studies of *L. philippiana* and *L. sempervirens* revealed isoquinolinic alkaloids derived from aporphine, noraporphine, and bisbenzylisoquinolinic-type alkaloid compounds (Speisky and Cassels, 1994). Chemical studies of these plants have been mostly aimed at their use in popular medicine where α -pinene, β -pinene, p-cimole, linalole, limonene, ascaridole, benzyl benzoate, benzaldehyde, camphene, 1,8-cineole, α -hexylcinnamaldehyde, p-cymene, eugenol methyl ether, and safrole have been identified in their essential oils. The chemistry of *P. boldus* has been studied more and is used in popular medicine; its alkaloids and essential oils have been isolated with ascaridole as the main component (Zin and Weiss, 1998; Vogel *et al.*, 1999; Schrickel and Bittner, 2001). The most important component of the essential oil from *L. sempervirens* leaves is safrole (Montes *et al.*, 2001). Although information is lacking on the chemical and biological activity of *L. philippiana* (Arbert, 2002), it was found to contain asimilobine, anonaine, and norcoridine (Urzúa and Cassels, 1982).

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Bittner *et al.* (2008) previously tested the effect of essential oils from *Gomortega keule* (Molina) I. M. Johnst., *Laurelia sempervirens*, *Origanum vulgare* L., *Eucalyptus globulus* Labill., and *Thymus vulgaris* L. on the *Sitophilus zeamais* and *Acanthoscelides obtectus* (Coleoptera) granary weevils obtaining promissory results that suggest their use in grain storage pest control.

Ceratocystis pilifera is found in Chile on numerous pine species, especially *Pinus radiata* D. Don, as well as on the surface of sawed lumber causing blue stain (bluing) (Parra *et al.*, 2001). *Fusarium oxysporum* frequently causes problems in forest nurseries by affecting seeds and/or seedlings causing “damping off” (Alvarez and Nishijima, 1987) where necrotic rings correspond to collapsed parenchymatic cells. This results from the secretion of characteristic toxins of the *Fusarium* genus species and its capacity to decompose cellulose. A *formae speciale* (f.sp.) that explains a special disease in *Pinus* species does not yet exist in spite of the economic importance of these diseases in Chilean nurseries (Webster and Weber, 2007). *Rhizoctonia solani* and representatives of the *Pythium* genus (generally controlled by fungicides) also cause “damping off” in seedlings (Butin and Peredo, 1986). *Phragmidium violaceum* is a compulsory parasite on several *Rubus* species and causes rust (Oehrens and González, 1974).

Alternative low cost, effective, and species-specific control methods should be found that do not leave permanent toxic residues in the environment. Previous studies of essential oils from aromatic plants such as *Ocimum canum* Sims, *Citrus medica* (L.) (Dubey *et al.*, 1983), *Pimpinella anisum* (L.) (Shukla y Tripathi, 1987), *Cinnamomum camphora* (L.) J. Presl (Mishra *et al.*, 1991), *Cymbopogon citratus* (DC.) Stapf (Mishra y Dubey, 1994), and *Chenopodium ambrosioides* L. (Mishra *et al.*, 2002) have demonstrated their strong fungicidal activity.

Becerra *et al.* (2002) and Solis *et al.* (2004) have shown antifungal activity of natural terpenes isolated from extractives of Chilean gymnosperm species from the Podocarpaceae and Cupressaceae families, respectively.

The objective of this study was to determine chemical components and *in vitro* fungistatic potential of essential oils from three native Chilean plant species (*Peumus boldus*, *Laureliopsis philippiana*, and *Laurelia sempervirens*) on important pathogens such as *Rhizoctonia solani*, *Pythium irregulare*, *Ceratocystis pilifera*, *Phragmidium violaceum*, and *Fusarium oxysporum*.

MATERIALS AND METHODS

Biological material

Fresh material was collected from *L. philippiana*, *L. sempervirens*, and *P. boldus* (Monimiaceae) on 2 June,

2003 in the Huachi sector of Santa Bárbara (37°31' S, 71°51' W), Bío-Bío Region, Chile. Leaves were placed in plastic bags, labelled to indicate species, site, date, and collector after which they were taken to the Natural Products Chemical Laboratory of the Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. Identification was confirmed by comparing reference material for each species with material from the herbarium of the Universidad de Concepción.

The strains of *C. pilifera*, *F. oxysporum*, and *P. violaceum* were isolated from pine species in nurseries of the Bío-Bío Region damaged by these organisms and were classified by Dr. R. Weber from the Fruit Research Station (OVB) in Jork, Germany. The *R. solani* and *P. irregulare* strains were kindly donated by Dr. R. Weber. All the strains are maintained in permanent cultures in the Natural Products Chemical Laboratory.

Extraction of essential oils

Leaves from each species under study were snipped and essences were extracted by hydrodistillation as established by Montes *et al.* (2001) and Céspedes *et al.* (2002).

Identification of essential oils

Water was eliminated from the essential oils by treating them with sodium sulfate anhydrous. The composition of the essential oils was determined with a Gas Chromatograph Mass Spectrometer (GCMS) (Hewlett Packard, series II 5890, Avondale, Arizona, USA), using an HP-5MS Capillary Column (5%-diphenyl-95%-dimethylsiloxane), ID (internal diameter) 0.25 mm, length 30 m, df (film thickness) 0.25 µm. Relative percentages of the essential oil components were obtained for each species by using helium as a mobile phase with a mass detector (Hewlett Packard, Mod 5972 series, Avondale, Arizona, USA).

Evaluation of fungistatic activity

Essential oils were directly assayed to each fungus with 30% and 50% dilution and undiluted (100%). A control was used for each case by not exposing the fungus to any extract.

Qualitative and quantitative assays of fungicidal activity were done using Woodward and De Groot 1999 by adding a 1 cm diameter of each fungal growth (three replicates) in the center of a Petri dish containing culture medium with agar, saccharose, malt and yeast extracts (Merck™), or other nutrients according to the requirements of each fungus (NCCLS, 2000). Paper filter discs (Whatman Nr 3, 6 mm diameter) impregnated with increasing dilutions (30, 50, and 100%) of different extracts obtained from the three species were placed around the fungi. These were

then incubated at 24 °C (per requirements of each species) (Woodward and De Groot, 1999) for 21 d and the diameter of their inhibition halos was measured alternately every 3rd and 4th d (Woodward and De Groot, 1999). These activities were considered to be positive when inhibitory action of the fungal growth was observed and negative when it was not. The percentage of growth inhibition was calculated by measuring the control halo (untreated fungi), which was set at 100%, and comparing it to the growth of the treated fungi. The percentage was calculated with the slope drawn for the measurements taken every 3rd and 4th d (3, 7, 10, 14, 17 and 21 d) and comparing them with the control halo.

Data analysis

In order to determine significant differences in toxicity among treatments, a two-way ANOVA was carried out using the Statistica 6.0 software package (Statsoft, 2008). The results showed significant differences at $p < 0.01$ according to the Tukey test for time and the Dunnett test for dose.

RESULTS

The gas-mass analysis of the oils showed 18 main derived components, 13 in *P. boldus*, 8 in *L. philippiana*, and 6 in *L. sempervirens*. The three Monimiaceae species had the 3-carene, α -phellandrene, and α -pinene terpenes. The main components were ascaridole in *P. boldus* oil

(34.80%), 3-carene in *L. philippiana* oil (53.81%), and safrol in *L. sempervirens* oil (69.30%) (Table 1).

The results of the fungistatic activity of the three concentrations of essential oils from the three species studied are presented in Table 2.

The growth rate of *P. violaceum* was slowed 62% by the essential oil from *L. philippiana* (undiluted), 61% (50% dilution), and 60% (30% dilution). In *F. oxysporum*, these rates were 59.6% (undiluted), 59.2% (50% dilution), and 58.2% (30% dilution). In *R. solani* and *C. pilifera* growth rates were slowed 51% (undiluted), 46.2% (50% dilution), 42% (30% dilution) and 48.5% (undiluted), 48.4% (50% dilution), 47% (30% dilution) respectively (Table 2).

According to ANOVA, the effects of different doses and times of exposure were significant at $p < 0.01$ (Tables 2 and 3). Of the three plants studied, *L. philippiana* showed significant differences in relation to time and dose treatments, in contrast to other plant differences that were mainly due to exposure times. *Laureliopsis philippiana* showed the highest growth inhibition activity of tested fungi, being more effective in *P. violaceum* with 62% inhibition using undiluted essential oils, 61% inhibition (50% diluted) and 60% inhibition (30% diluted). For *F. oxysporum*, the levels of inhibition were 59.6% (undiluted), 59.2% (50% diluted), and 58.2% (30% diluted). Differences were significant in both fungi for time and dose. There was no significant difference in exposure time for *L. philippiana*, which showed the same

Table 1. Main components of the essential oils from *Peumus boldus*, *Laureliopsis philippiana*, and *Laurelia sempervirens*.

Compound	<i>Peumus boldus</i>	<i>Laureliopsis philippiana</i>		<i>Laurelia sempervirens</i>
			%	
Ascaridole	34.80	-	-	-
3-Carene	1.81	53.81		1.97
α -Phellandrene	1.01	2.95		0.92
β -Phellandrene	5.43	-		1.98
1,2-Dimethoxy-4-(2-propenyl)-phenol	-	10.58		-
Safrole	-	2.33		69.30
α -1-Methyl-3-cyclohexane	-	5.86		-
α -Pinene	3.19	2.30		0.39
β -Pinene	1.02	-		-
Limonene	16.10	-		-
Eucalyptol (cineole)	11.95	14.76		-
α -Terpineol	8.90	-		-
β -Myrcene	0.37	-		-
p-Cymol	7.85	-		-
Linalool	1.03	-		-
Nerolidol	4.95	-		-
α -1-Methyl-3-cyclohexadiene	-	5.14		-
1-Methyl-4-1-methyl ethyl cyclohexane	-	-		18.55
Other components	1.59	2.27		6.89

fungus growth inhibition throughout the treatment whereas there were significant differences in exposure time in *L. sempervirens* and *P. boldus*, on average between 7 and 10 d when the greatest inhibition occurred in the tested fungi (Table 2). It should be noted that *P. irregulare* was the fungus that showed the greatest resistance to treatment with essential oils of the three plants.

DISCUSSION

Of the main terpenes found in the essential oils of *P. boldus*, *L. philippiana* and *L. sempervirens*, the components ascaridole, 3-carene, and α -phellandrene

were previously described for *P. boldus* (Gupta, 1995; Schrickel and Bittner, 2001; Montes *et al.*, 2001) and safrole for *L. sempervirens* (Montes *et al.*, 2001).

The results of this study concur with the research cited above, but also revealed safrole in *L. philippiana* and 1,2-dimethoxy-4-(2-propenyl)-phenol was found exclusively in this species. Eucalyptol was ascertained in *P. boldus* and *L. philippiana* essential oils. Finally, *L. philippiana* revealed the presence of α -1-methyl-3-cyclohexadiene, previously described for *Persea lingue* (Ruiz & Pav.) and Nees, a member of the Lauraceae family, which is close to the Monimiaceae family (Marticorena and Rodríguez, 2001).

Table 2. Inhibition of growth rates (%) of fungi exposed to three concentrations of the essential oils extracted from *Peumus boldus*, *Laureliopsis philippiana*, and *Laurelia sempervirens*.

Species	Control	<i>P. boldus</i>			<i>L. philippiana</i>			<i>L. sempervirens</i>		
		Concentration %								
		100	50	30	100	50	30	100	50	30
<i>Ceratocystis pilifera</i>	0.0	0.0	0.0	0.0	48.5	48.4	47.0	28.4	25.2	25.0
<i>Phragmidium violaceum</i>	0.0	45.0	45.0	27.4	62.0	61.0	60.0	36.0	13.0	11.0
<i>Fusarium oxysporum</i>	0.0	25.2	24.4	24.0	59.6	59.2	58.2	3.5	3.0	2.6
<i>Pythium irregulare</i>	0.0	19.0	0.0	0.0	9.0	8.0	7.0	6.0	0.0	0.0
<i>Rhizoctonia solani</i>	0.0	26.0	23.3	21.0	51.0	46.2	42.0	6.10	6.0	5.2

Table 3. Results of ANOVA for essential oil dose and exposure time on *Fusarium oxysporum*, *Phragmidium violaceum*, *Ceratocystis pilifera*, *Rhizoctonia solani* and *Pythium irregulare*.

	DF	<i>Laureliopsis philippiana</i>		<i>Laurelia sempervirens</i>		<i>Peumus boldus</i>	
		Mean Square	F	Mean Square	F	Mean Square	F
Time	5	4032.57	22.76*	6837.57	2032.74*	5560.85	53141*
Dose	3	2494.68	14.08*	3.36	1.00	25.41	2.43
Dose x Time	15	177.18		3.36		10.46	
Time	5	3910.39	49.65*	6225.58	116.20*	6225.58	116.20*
Dose	3	1362.93	17.31*	420.66	7.85	420.66	7.85
Dose x Time	15	78.75		53.58		53.58	
Time	5	5272.15	32.58*	5863.19	108.11*	4537.50	843.10*
Dose	3	420.90	2.60	120.90	2.23	5.38	1.00
Dose x Time	15	161.84		54.23		5.38	
Time	5	3925.73	87.04*	6854.65	109.56*	5451.00	117.78*
Dose	3	1073.18	23.79*	137.57	2.20	212.74	4.60
Dose x Time	15	45.10		62.57		46.28	
Time	5	4251.72	584.48*	4316.63	1398.81*	4451.63	622.74*
Dose	3	17.01	2.34	6.84	2.22	25.07	3.51
Dose x Time	15	7.27		3.09		7.15	

* Values differ significantly at $p < 0.01$. DF: degrees of freedom. F: Statistic F.

Table 4. Results of multiple comparisons with means and standard error (SE) for exposure time and dilution of essential oil o *Fusarium oxysporum*, *Phragmidium violaceum*, *Ceratocystis pilifera*, *Rhizoctonia solani*, and *Pythium irregulare*.

		n	<i>Laureliopsis philippiana</i>		<i>Laurelia sempervirens</i>		<i>Peumus boldus</i>	
			Mean	SE	Mean	SE	Mean	SE
<i>Fusarium oxysporum</i>								
Time, d	3	4	1.25	12.50	0.00	0.00	5.31*	0.31
	7	4	20.00	116.70	48.44*	22.46	54.06*	0.94
	10	4	44.06	165.63	93.75*	0.00	82.81*	38.65
	14	4	49.38	168.75	100.00*	0.00	94.38	18.75
	17	4	65.00	117.70	100.00	0.00	100.00	0.00
	21	4	90.63	31.25	100.00	0.00	100.00	0.00
Dilution, %	100	6	34.17	129.37	73.13	170.59	70.83	151.85
	50	6	34.79	129.60	73.33	169.92	71.88	150.68
	30	6	35.63	132.12	73.54	169.26	72.71	149.53
	Control	6	75.63	158.37	74.79	165.84	75.63	158.37
<i>Phragmidium violaceum</i>								
Time, d	3	4	3.73	0.75	1.28	12.82	5.97*	0.00
	7	4	36.57	108.06	31.09*	95.71	52.24*	55.51
	10	4	61.19	134.74	67.31*	66.31	77.99*	74.72
	14	4	76.49	79.07	91.67*	43.63	82.46	60.13
	17	4	79.10	70.01	94.23	34.92	90.30	33.65
	21	4	84.33	53.64	96.80	19.23	94.78	21.54
Dilution, %	100	6	47.02	119.45	55.34	158.01	61.44	128.19
	50	6	50.00	123.91	60.90	165.87	62.69	131.87
	30	6	51.24	123.54	63.46	173.46	65.92	136.60
	Control	6	79.35	155.66	75.21	154.51	79.10	154.97
<i>Ceratocystis pilifera</i>								
Time, d	3	4	10.31*	24.67	4.38	43.75	17.50*	28.41
	7	4	52.50*	159.75	74.06*	88.59	100.00*	0.00
	10	4	80.63	67.99	99.38*	0.63	100.00	0.00
	14	4	100.00	0.00	100.00	0.00	100.00	0.00
	17	4	100.00	0.00	100.00	0.00	100.00	0.00
	21	4	100.00	0.00	100.00	0.00	100.00	0.00
Dilution, %	100	6	68.13	164.82	76.25	165.55	85.42	145.83
	50	6	69.38	160.01	77.92	164.62	85.63	143.75
	30	6	71.88	155.85	78.13	164.37	86.46	135.42
	Control	6	86.25	137.50	86.25	137.50	87.50	125.00
<i>Rhizoctonia solani</i>								
Time, d	3	4	1.25	12.50	3.75*	0.72	5.00*	0.00
	7	4	38.44*	72.42	30.94*	97.28	43.13*	56.25
	10	4	52.50	91.00	67.50*	41.77	63.44*	60.25
	14	4	71.88	94.30	100.00*	0.00	81.88	65.65
	17	4	75.94	82.82	100.00	0.00	100.00	0.00
	21	4	86.88	53.40	100.00	0.00	100.00	0.00
Dose, %	100	6	44.58	111.56	64.17	178.96	60.63	151.72
	50	6	48.33	121.01	64.38	178.94	62.92	151.72
	30	6	50.83	131.13	65.42	172.98	64.58	154.03
	Control	6	74.17	152.98	74.17	152.98	74.17	152.98
<i>Pythium irregulare</i>								
Time, d	3	4	15.63*	11.97	17.50*	0.88	15.63*	29.09
	7	4	75.31*	24.14	82.50*	15.31	85.63*	18.75
	10	4	88.75*	12.50	98.44*	15.63	95.31*	17.95
	14	4	96.56*	21.27	100.00	0.00	100.00	0.00
	17	4	100.00	0.00	100.00	0.00	100.00	0.00
	21	4	100.00	0.00	100.00	0.00	100.00	0.00
Dilution, %	100	6	77.08	132.71	81.88	132.59	80.21	144.06
	50	6	79.17	129.21	83.75	132.68	82.71	139.36
	30	6	80.42	131.25	84.17	132.75	82.92	137.30
	Control	6	80.83	140.34	82.50	138.89	85.21	124.60

*Values differ significantly according to Tukey for time and Dunnett for dose (p < 0.01).

The 3-carene, α -phellandrene, and α -pinene terpenes were present in the three essential oils. Although this can be explained by the fact that the species all belong to the same family (Monimiaceae), they nonetheless showed some specificity in some main components as well as others that were found in lesser concentrations.

Each of the species studied had a main component, ascaridole (34.80%) in *P. boldus*, safrole (69.30%) in *L. sempervirens*, and 3-carene (53.81%) in *L. philippiana*. The greatest concentrations were found in these components, and they were thought to be responsible for the responses obtained in the biological activity assays.

Overall, the greatest growth inhibition was produced by the essential oil from *L. philippiana* at all doses and for all species of fungi tested. The exception was *P. irregulare*, which exhibited an average inhibition of about 50% in the strains of fungi treated. The 1,2-dimethoxy-4-(2-propenyl)-phenol compound, known to be one of recognized toxic activity, was found only in *L. philippiana* and could be attributed to fungistatic activity (Pérez and Ubera, 2006). However, we believe that given the chemical composition of the essential oils of the species tested, this activity would be a result of a synergetic effect between the presence of phenolic compounds and those characterized as terpenes.

On the other hand, essential oil from *P. boldus* presented an activity inhibition that did not exceed 30% on the average, even though it had a greater diversity in its terpene composition, but no 1,2-dimethoxy-4-(2-propenyl)-phenol, which could be responsible for the increased fungistatic activity measured.

It would also be interesting to study the effect of the essential oil and crude extract of these plants on medically important fungi in order to develop new anti-fungal or fungistatic agents for preventive treatment of serious fungal disease infections in animals and human beings.

CONCLUSIONS

The analysis of all the essential oils of the three studied species revealed the following main components: ascaridole, 3-carene, α -phellandrene, β -phellandrene, 1,2-dimethoxy-4-(2-propenyl)-phenol, safrole, $\alpha\alpha$ -1 methyl-3-cyclohexane, α -pinene, β -pinene, limonene, eucalyptol, α -terpineol, β -myrcene, p-cymol, linalool, nerolidol, 1-methyl-4-(1-methylethyl)-cyclohexene, as well as others that were found in lower concentrations.

All the dilutions of the *Laureliopsis philippiana* essential oil reduced growth rates around 50% in all the fungi species studied, except for *Pythium irregulare* which had an inhibition rate lower than 10% with the different dilutions used.

The essential oil from *Laurelia sempervirens* showed the lowest biological activity in all the tests.

Finally, considering the results obtained in this study, it can be concluded that the essential oils from *Laureliopsis philippiana* and *Peumus boldus* showed the best fungistatic activity. This will benefit humans in the production of specific and environmentally friendly pesticide compounds.

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RESUMEN

Actividad fungistática de extractos de aceites esenciales de *Peumus boldus* Mol., *Laureliopsis philippiana* (Looser) Schodde y *Laurelia sempervirens* (Ruiz & Pav.) Tul. (Monimiaceae chilenas). Se determinaron los compuestos de aceites esenciales de Monimiaceae chilenas, boldo (*Peumus boldus* Mol.), tepa (*Laureliopsis philippiana* (Looser) Schodde), y laurel (*Laurelia sempervirens* (Ruiz & Pav.) Tul.) a través de cromatografía de gas con espectrometría de masas (CG-EM) y se midió la actividad fungistática de los aceites sobre los hongos *Rhizoctonia solani* Kühn (Donk), *Pythium irregulare* Buisman, *Ceratocystis pilifera* (Fr.) C. Moreau, *Phragmidium violaceum* (Schultz) Winter y *Fusarium oxysporum* Schltdl. Los aceites esenciales de las especies de Monimiaceae tienen algunos compuestos en común; en las especies estudiadas se encontró que todos tenían los terpenos 3-careno, α -felandreno, y α -pineno. *L. philippiana* y *L. sempervirens* además tienen safrol. En cambio, ascaridol fue el principal compuesto en el aceite de *P. boldus*, 3-careno en *L. philippiana* y safrol en *L. sempervirens*. El aceite esencial de *L. sempervirens* presentó la mejor actividad fungistática contra las cepas tratadas, con diferencias significativas tanto en dosis como en tiempo de exposición. *P. violaceum* fue la cepa más sensible a los aceites esenciales y *P. irregulare* la más resistente (el extracto de *P. boldus* detuvo el crecimiento sólo un 19%). Por lo tanto, los aceites esenciales de todos estos árboles podrían ser usados como controladores de las cepas de hongos estudiadas.

Palabras clave: árboles nativos chilenos, compuestos químicos, actividad fungistática natural, análisis *in vitro*.

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