Antifungal and Antibacterial Activities of *Araucaria araucana* (Mol.) K. Koch Heartwood Lignans

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Five lignans (secoisolariciresinol, pinoresinol, eudesmin, lariciresinol, and lariciresinol-4-methyl ether) were isolated from an MeOH extract from *Araucaria araucana* (Mol.) K. Koch wood for the first time in this species and their structures determined with spectroscopic methods. The antimicrobial activities of these compounds were determined for the bacteria *Citrobacter* sp., *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and for the white rooting and staining fungi *Mucor miehei*, *Paecilomyces variotii*, *Ceratocystis pilifera*, *Trametes versicolor*, and *Penicillium notatum*, and in addition, the MeOH extract was evaluated against *Aspergillus niger*, *Candida albicans*, *Fusarium moniliforme*, *F. sporotrichum* and *Trichophyton mentagrophytes*. The most sensitive bacteria against pinoresinol were the Gram-positive. However, secoisolariciresinol exhibited a significant antifungal activity on fungi of white rooting and wood staining and this compound completely inhibited the mycelial growth of *T. versicolor* and *C. pilifera* at 300 and 400 μg per disc, respectively, whereas pinoresinol showed a moderate inhibitory activity. On the other hand, the MeOH extract had the highest activity against rooting and staining and pathogenic fungi as well as *T. versicolor*, *Fusarium* spp. and *Trichophyton mentagrophytes*, inhibiting completely the growth at 400 μg per disc.

Key words: Araucaria araucana, Lignans, Antibacterial Activity

Introduction

The Araucariaceae is divided into two genera, *Agatis* and *Araucaria*, including 38 species distributed throughout the southern hemisphere. The genus *Araucaria* Juss. based on 18 species distributed in New Guinea, eastern Australia, New Zealand, Norfolk Islands, New Caledony, southern Brazil, southwestern Argentina, and Chile (Armesto *et al.*, 1995). Species of *Araucaria* Juss. are distributed in four sections: Eutacta, Intermedia, Bunya, and Columbea. Only two species belong to the Columbea section: *Araucaria araucana* growing in southern and southwestern Chile and Argentina, respectively, and *A. angustifolia* (Bert.) O. Kuntze growing in Brazil, northeastern Argentina, and eastern Paraguay (Veblen, 1982).

In Chile the biggest population is found in the Andean mountain range between 37°24′S and 40°03′S. Another smaller population is found in the coastal mountain range, limited to the area between 37°30′S and 38°30′S. These two populations are separated by Chile's longitudinal valley (Villagrán *et al.*, 1998). The coastal *Araucaria araucana* populations grow at altitudes between 600 and 1400 m, where they receive annual precipitation of 1500–2500 mm. The Andean populations are found between 900–1700 m and receive annual precipitation of 1200 mm in Argentina and 4000 mm in Chile. *A. araucana* is the genus' unique template species. It is a long-life conifer, which can grow to 50 m in height and over 2 m in diameter (Muñoz-Pizarro, 1959). The durable

nature of wood of the *Araucaria* species is used for high-quality manufactured products. Natural durability, or decay resistance, is defined as the wood's ability to resist biological degradation (Eaton and Hale, 1993).

The degradation of wood, also known as "breakdown", is a natural process in forests and forestry products, in which the main components of wood, cellulose, hemicellulose, and lignins, decompose (Croteau *et al.*, 2000). This rather complex process involves bacteria, fungi, and insects that use the degraded components as carbon and energy sources. Plants generally counteract pathogenic attacks with structural strategies or physical barriers that impede the pathogen's penetration into and propagation within the wood. Furthermore, plant cells and tissues contain biochemical barriers that synthesize substances that are toxic to the pathogen or that create inhibitory conditions for the pathogen's development. These substances are found in the group known as extractives (Rhoades, 1979; Thomson, 1978).

The term "wood extractives" includes a sizeable number of compounds that can be obtained from plants by solvent extraction. In a narrow sense, extractives are those compounds soluble in either neutral or acidic organic solvents. However, water-soluble sugars and compounds can also be extractives (Fengel and Wegener, 1984). In woody plants, these extractive compounds are found in the resiniferous channels. In xylem rays and at a cellular level, extractives can be found in the middle lamella, intercellular spaces, tracheid walls, and bast fibres (Cambie *et al.*, 1984). Heartwood extractives are comprised of a heterogeneous group of chemical compounds, including terpenoids, tropolones, flavonoids, stilbenes, and other aromatic compounds, and in addition the significance of heartwood extractives for natural durability has been demonstrated (Croteau *et al.*, 2000). There are many studies about the chemical structure, toxicity, and specificity of various heartwood substances (Celimene *et al.*, 1999; DeBell *et al.*, 1997; Schultz and Nicholas, 2002).

Our studies of the chemical structure of secondary metabolites from wood and bark extractives of some gymnosperm species with biological activities show the presence of a series of compounds including terpenes and lignans (Silva and Bittner, 1986; Flores *et al.*, 2001; Calderón *et al.*, 2001; Kubo *et al.*, 2003a, b; Torres *et al.*, 2003; Céspedes *et al.*, 2000; 2004). Lignans are plant secondary metabolites which are constituted of both two cinnamic or ferulic acid residues widely distributed in terrestrial plants; the formation of these compounds involves a direct stereochemically controlled coupling of coniferyl alcohol-derived moieties (Lewis *et al.*, 1999). This may require the presence of two stereoselective enzymes with different activities and subsequent processes are dependent on the regiochemistry and stereochemistry of the initial linkage (Davin *et al.*, 1992; Umezawa *et al.*, 1990; Weinges and Spänig, 1967; Seigler, 1997). Analytical methods for the detection and study of lignans have been reviewed (Lewis *et al.*, 1999).

In contrast, neolignans are distributed in the limited plant families Pinaceae and Cupressaceae (Castro *et al.*, 1996). The most well-known and representative lignan is podophyllotoxin because it is an important parent compound of the potent antitumor agent etoposide. However, most known lignans do not possess potent biological properties. Their studies are limited to structure determination, biodegradation and biosynthetic pathways (Miyazawa, 2001; Carpinella *et al.*, 2003; Schmitt and Petersen, 2002; Kim *et al.*, 2002). In some cases, lignans are responsible for the wood's natural resistance to the action of insects, fungi, and bacteria. Thus, these compounds could have applications as pesticides in wood preservation (Keith *et al.*, 1974; Kubo *et al.*, 1992), and as allelochemicals (Whittaker and Feeny, 1971), insecticidal (Gao *et al.*, 2004; Nascimento *et al.*, 2004), larvicidal (Park *et al.*, 2005), antimalarial (Zhang *et al.*, 2001), antifeedant (Garcia *et al.*, 2000), anti-inflammatory (Cho *et al.*, 2001), antioxidant (Kato *et al.*, 1998), nematicidal (Suga *et al.*, 1993), estrogenic agents (Mazur and Adlercreutz, 1998), and exhibit a variety of other biological activities, *e.g.* COX-2 inhibitory, anti-tumor, antimitotic and antiviral activities (MacRae and Towers, 1984; Seigler, 1997).

Some species of the Araucariaceae family have been studied in Australia, Brazil, Japan, New Zealand, and Chile (Ohashi *et al.*, 1992; Fonseca *et al.*, 2000). As a contribution to the knowledge and continuing the work with native gymnosperm woods, the aim of this work was to evaluate the antibacterial and antifungal activity of the MeOH extract and lignans present in *A. araucana* wood extractives.

Experimental

Plant material

Wood samples of *A. araucana* were collected in the native forests in ecological areas of southern Chile, specifically at the "Cordillera de Nahuelbuta", VIII Region, in November 1999. Voucher specimens are deposited in the herbarium collection of Botanic Department (CONC), Universidad de Concepción, Concepción City, Chile.

Isolation and general procedures

The heartwood (10 kg) was separated mechanically and finely chopped. It was extracted three times, using methanol at 40 °C for 48 h. The crude extract was then evaporated to dryness under vacuum conditions. The total heartwood extract was partitioned between water/MeOH (1:1) and ether according to previously reported procedures (Mazur and Adlercreutz, 1998), the ether fractions were separated and the aqueous MeOH (extract M) phase was concentrated. This last phase was fractionated by fast CC (silica gel 60, 0.063–0.200 mm particle size, 1 g extract:20 g silica gel). The column was eluted with increasing polarity solvent mixtures to obtain a total of seven fractions: F-1 to F-7.

Compound purification was carried out from the seven fractions, using chromatographic techniques of routine (*i.e.* CC, TLC, HPLC, GLC, and GC-MS). The purity of compounds was controlled by HPLC with a diode arrangement LC 10 A-VP detector.

The isolated and purified compounds were characterized and identified through their spectroscopic data and compared with authentic samples. The melting points were determined on a Kofler block. Infrared spectra were determined using an IR-408 SHIMADZU spectrophotometer. Ultraviolet spectra were determined with an UV-160 SHIMADZU spectrometer. NMR spectra (^{1}H and ^{13}C) were recorded with an AM-400 BRUKER spectrometer (at 250 and 62.5 MHz, respectively) and mass spectra were carried out in a 5972 series Hewlett Packard mass spectrometer. The GC/MS technique (MS detection at 70 eV) was performed under the following conditions: Column: HP-5, 30 m × 0.25 mm × 0.25 μm; temperature: 100 °C isothermal for 5 min, with 10 degree increments per min up to 275 °C, which was held constant for 20 min; split injection: 100:1; injector temperature: 275 °C; detector temperature: 300 °C; and helium carrier.

Physical data of lignans

Secoisolariciresinol (**1**): M.p. 112–114 °C. – MS: *m/z* (rel. int.) = 362 [M$^+$] (21), 344 (8), 326 (2), 311 (2), 259 (2), 220 (2), 206 (4), 189 (9), 163 (4), 151 (35), 137 (100), 122 (13), 94 (10), 77 (5), 65 (4), 51 (4). – ^{1}H NMR ($CDCl_3$): δ = 1.60 (2H, m, H-8, H-8′), 2.75 (4H, br, H-7, H-7′), 3.49 (4H, m, H-9, H-9′), 3.87 (3H, s, OMe), 3.90 (3H, s, OMe), 4.75 (H, Br, OH), 6.89–6.90 (6H, m, aromatic protons).

Lariciresinol (**2**): M.p. 162–164 °C. – MS: *m/z* (rel. int.) = 360 [M$^+$] (52), 345 (5), 329 (4), 311 (5), 241 (3), 221 (15), 219 (15), 194 (35), 175 (18), 151 (50), 137 (100), 122 (17), 93 (12), 77 (12), 65 (10), 51 (5). – ^{1}H NMR ($CDCl_3$): δ = 2.07–2.63 (4H, m, H-7, H-8, H-8′), 3.49 (4H, m, H-9, H-9′), 3.80 (6H, s, 2OMe), 5.5 (1H, br, H-7′), 6.55–6.85 (6H, m, aromatic protons).

Pinoresinol (**3**): M.p. 118–120 °C. – MS: *m/z* (rel. int.) ($C_{20}O_6H_{22}$) = 358 [M$^+$] (24), 344 (62), 330 (5), 302 (4), 266 (2), 253 (2), 237 (2), 208 (5), 189 (6), 163 (4), 151 (57), 137 (100), 122 (15), 106 (10), 94 (9), 77 (7), 69 (5), 51 (5). – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.11 (2H, m, H-1, H-5), 3.86 (2H, q, J = 9.2, 3.8 Hz, H-4, H-8α), 3.88 (6H, s, ArOMe), 4.21 (2H, m, J = 9.2, 6.9 Hz, H-4, H-8β), 4.9 (2H, s, 2OH), 4.80 (2H, d, J = 4.2 Hz, H-2, H-6), 6.80–6.91 (6H, m, H-2′, 5′, 6′).

4′-Methoxy-pinoresinol (**4**): Oil. – MS: *m/z* (rel. int.) = 370 [M$^+$] (21), 339 (11), 324 (2), 309 (2), 284 (2), 271 (3), 248 (1), 235 (4), 219 (11), 203 (20), 189 (13), 177 (37), 265 (64), 151(100), 137 (48), 122 (17), 103 (10), 77 (12), 65 (9), 55 (5). – ^{1}H NMR ($CDCl_3$): δ = 3.25 (2H, m, H-1, H-5), 3.88 (3H, s, OMe), 3.89 (3H, s, OMe), 3.91 (3H, s, OMe), 4.05 (2H, d, J = 4 Hz, H-4, H-8), 4.13–4.33, (2H, m, C4, C8), 4.50, (1H, s, OH), 4.75 (1H, d, J = 4 Hz, H-6), 4.83 (1H, d, J = 3.5 Hz, H-2), 6.80–6.91 (6H, m, aromatic protons).

Eudesmin (**5**): M.p. 107–109 °C. – MS: *m/z* (rel. int.) = 386 [M$^+$] (82), 371 (3), 355 (9), 337 (2), 325 (2), 230 (2), 287 (3), 265 (2), 248 (2), 235 (5), 219 (15), 189 (12), 177 (68), 165 (100), 151 (54), 135 (15), 107 (6), 95 (8), 77 (10), 55 (5). – ^{1}H NMR ($CDCl_3$): δ = 3.09–3.14 (2H, m, H-1, H-5), 3.86 (3H, s, Ome), 3.87 (3H, s, OMe), 3.90 (3H, s, OMe), 3.92 (3H, s, OMe), 4.23–4.29 (4H, m, H-4, H-8), 4.76 (2H, d, J = 4 Hz, H-2, H-6), 6.82–6.91 (6H, m, aromatic protons).

Evaluation of biological activity (microorganisms and growth medium)

The antibacterial and antifungal activities of the M extract and lignans **1**, **2**, **3**, and **5** obtained from the wood of the *Araucaria* species were determined; due to a very little amount of **4** this compound was not used. Testing paper discs (6 mm, Whatman) were impregnated with 10 μl solution containing 100 μg of each compound to perform the test against *Citrobacter* sp., *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC25922), *Micrococcus luteus*, *Pseudomonas aeruginosa* (ATCC27853), and *Staphylococcus aureus* (ATCC6538p) (Table I), and the fungi strains *Mucor miehei*, *Paecilomyces variotii* (ATCC74097), *Ceratocystis pilifera* (ATCC60758), *Trametes versicolor* (ATCC64311), and *Penicillium notatum* (Table III). *Aspergillus niger* (ATCC64958), *Candida albicans* (ATCC96110), *Fusarium moniliforme* (ATCC96574), *F. sporotrichum* (wild-type), and *Trichophyton mentagrophytes* (ATCC9972) (Table IV) were assayed against the M extract only.

Bacteria were grown in Brain Heart Infusion Broth (Bioxon, Mexico City, Mexico: 112–1) for stock cultures and Mueller-Hinton Broth (Bioxon: 260–1) was used as a test medium due to its low interaction with the assayed compounds. The antibacterial activity of the extract and pure compounds was assessed with the disc diffusion method using Mueller-Hinton agar (Baron and Finegold, 1995) by determining inhibition zones at different dilutions of compounds and extract. For pure compounds filter paper discs (6 mm i.d., Whatman) with were impregnated with 10 μl of a 10 μg/μl solution of each sample, and for the crude total extract the filter paper discs were impregnated with 10 μl of a 40 μg/μl solution of sample. The discs were placed in petri dishes containing the test organisms. Cultures were incubated at 37 °C, and after 24 h, the diameter of inhibition zone was determined (mm). The mean value from at least three different experiments was used for statistical analysis and each experiment was done in triplicate. The treatments were evaluated with a completely randomized design. The treatments were subjected to a one-way analysis of variance (ANOVA) and means were compared with the Student-Newman-Keuls (SNK) test ($P = 0.05$) using the Microcal Origin 6.2 Microsoft statistical program. Gentamicin was used as positive control.

The antifungal property of the extract and compounds was tested by the agar-well diffusion method using Sabouraud Dextrose Agar. Standard reference antibiotics were used in order to control the sensitivity of the tested microorganisms, that were inoculated in Czapek-Dox broth medium. Plates containing only the culture medium, with the addition or not of the solvents (methanol or water 10 μl/sensidisk), were used as viability controls for each fungus studied. The fungi inocula (10 μl of 3×10^6 spores/ml) were placed in a hole (0.4 mm^2) made in the center of each petri dish after solidification of the medium. The doses of the extract were 2 mg/sensidisk; positive control was 10 μg/sensidisk ketoconazole; negative control was each one of used solvents (water and methanol 10 μl/sensidisk). The cultures were incubated at 28 °C for 14 d and controlled every 24 h. Inhibition of radial mycelial growth diameters were measured daily and recorded as mean percentages (%) of growth (Wang and Ng, 2002).

Antifungal assays (IC_{50}) and minimum fungicide concentration (MFC)

These tests were carried out to analyze the fungicidal activity exhibited by each compound and extract, while further studying their fungistatic activity. For quantitative assays of the extract, three doses were added to potato dextrose agar (4 ml) at 45 °C, mixed rapidly and poured into 3 separate 6 cm petri dishes. After the agar had cooled down to room temperature, a small amount of mycelia (1×1 mm^2) was inoculated, the same amount to each plate. Buffer only was employed for a negative control. After incubation at 23 °C for 72 h, the area of the mycelial colony was measured and the inhibition of fungal growth and hence the IC_{50} values were determined. For the extract and compounds, 14 d after the beginning of the assay, a circle of agar around the central hole was obtained, as well as the mycelium of fungi from these plates that exhibited negative growth. At the end of this period, the MFC values were recorded (Wang and Ng, 2002).

Statistical analysis

Data shown in the tables are average results obtained by means of three replicates and independent experiments and are presented as average ± standard errors of the mean (SEM). Data were subjected to analysis of variance (ANOVA) with significant differences between means identified by GLM procedures. Results are given in the text

as probability values, with $p < 0.05$ adopted as the criterion of significance; differences between treatment means were established with a Student-Newman-Keuls (SNK) test. The I_{50} values for each activity were calculated by PROBIT analyses based on percentage of inhibition obtained at each concentration of the samples. I_{50} is the concentration producing 50% inhibition. The complete statistical analysis was performed by means of the MicroCal Origin 6.0 statistical and graphs PC program, Microsoft Mexico City, Mexico.

Results and Discussion

In our screening program looking for biological activities of plants from template regions it was found that *A. araucana* showed antifungal and antibacterial activity in a preliminary trial. Based on this information and in the high resistance to insect and pathogen attack of this wood we have carried out biodirected phytochemical studies on the heartwood of *A. araucana* tree.

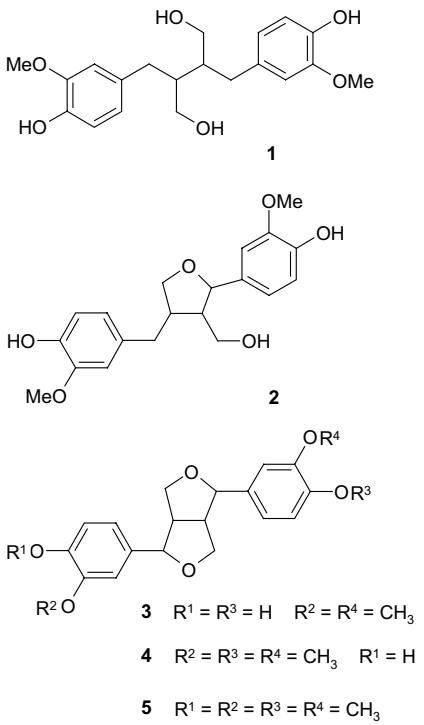

Fig. 1. Lignan chemical structures: secoisolariciresinol (**1**), lariciresinol (**2**), pinoresinol (**3**), 4′-methoxy-pinoresinol (**4**) and eudesmin (**5**).

From the M extract five lignans were isolated and identified from the heartwood of the Chilean Araucariaceae species *A. araucana*, secoisolariciresinol (**1**), lariciresinol (**2**), pinoresinol (**3**), 4′-methoxy-pinoresinol (**4**), and eudesmin (**5**) (Fig. 1). The structures of these compounds were established by co-chromatography with standards and spectroscopic methods. It is the first report about the occurrence of these lignans in *A. araucana*.

The evaluation of antibacterial activity was carried out against Gram-positive (*B. subtilis*, *Citrobacter* sp., *M. luteus* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeroginosa*) bacteria, all compounds assayed were active against all Gram-positive bacteria and the most active compound was pinoresinol. Table I shows the zone (mm) and percentage of inhibition of the assayed compounds and M extract for bacteria resulted with antimicrobial activity. After the evaluation of the results, we found that the M extract showed the highest inhibitory activity against the tested Gram-positive bacteria and did not show the same activity against the tested Gram-negative bacteria, since the zone (mm) diameters were greater than shown by the assayed compounds.

The M extract showed a variable composition of lignans, being secoisolariciresinol (32.99%) the most abundant/common lignan in the *A. araucana* extractives, followed by eudesmin (18.24%), lariciresinol (10.09%), pinoresinol (7.32%), and 4′-methoxy-pinoresinol (2.96%), together with other secondary metabolites such as mono, di- and triterpenes, the lignans being the most abundant in these extractives (Table II). These lignans have also been reported for other species of the *Araucaria* genus (Ohashi *et al.*, 1992; Fonseca *et al.*, 2000).

In view of the potent activity of the M extract against bacteria, this extract and compounds **1**, **2**, **3**, and **5** were assayed against different fungal strains. Due to the little amount obtained of compounds **1**, **2**, **3**, and **5** these were evaluated only against bacteria and rooting-staining fungi. However, the M extract was used in all measurements.

Compounds **1**, **2**, **3**, and **5** and the M extract were assayed against *M. miehei*, *P. variotii*, *C. piliferа*, *T. versicolor* and *P. notatum*, that are rooting and staining fungi of wood (Table III). The activity level showed by compounds **1**, **2**, **3**, and **5** against these fungi was relatively high compared with the positive control ketoconazole (data not shown), being the most active secoisolariciresinol (**1**) with an

Table I. Antibacterial growth inhibition activity on bacteria inoculum by lignans of *Araucaria araucana*[a].

Compound	*Citrobacter* sp.	%	*B. subtilis*	%	*M. luteus*	%	*P. aeruginosa*	%	*E. coli*	%	*S. aureus*	%
Pinoresinol (**3**)	8.3 ± 1.15a	18.4	10.3 ± 0.57a	22.9	9.6 ± 0.57a	21.3	n.a.[b]	–	n.a.	–	8.6 ± 0.57a	19.1
Eudesmin (**5**)	7.3 ± 0.57b	16.2	8.3 ± 0.57b	18.4	7.3 ± 0.57b	16.2	n.a.	–	n.a.	–	8.3 ± 0.57a	18.4
Laricíresinol (**2**)	7.6 ± 0.57b	16.9	8.6 ± 0.57b	19.1	7.6 ± 0.57b	16.9	n.a.	–	n.a.	–	7.0 ± 1.11b	15.6
Secoisolariciresinol (**1**)	6.9 ± 0.61b	15.3	8.1 ± 0.51b	18.1	6.9 ± 0.49b	15.4	n.a.	–	n.a.	–	6.8 ± 0.98b	15.0
MeOH extract	8.57 ± 1.12a	19.0	13.4 ± 0.64a	29.9	10.4 ± 0.61a	23.0	n.a.	–	n.a.	–	9.9 ± 1.3d	22.0
Gentamicin	22.6 ± 0.57c	50.2	22.0 ± 1.0c	48.9	22.0 ± 1.0c	48.9	20.3 ± 0.57c	45.1	21.0 ± 1.0c	46.7	21.0 ± 1.0c	46.7

Mean of three replicates. Means followed by the same letter within a column are not significantly different in a Student-Newman-Keuls (SNK) test (treatments are compared by concentration to control), 95% confidence limits.

[a] Inhibitory effects at 100 μg per disc are represented as mm of growth [mean value of diameter of inhibition zone (mm) ± standard error of $N = 21$ and its significant difference from the control $p < 0.01$].

[b] Activity not present.

inhibition of 45% at 100 μg/ml per disc against *T. versicolor* and 41.5% against *C. pilifera* (Table III). In addition, this compound showed a total inhibition (100%) against these two fungi at 400 μg/ml per disc (data not show). Similar effects were showed by the M extract inhibiting completely the mycelial growth of these two fungi at 300 μg/ml per disc; the mycelial growth of *M. miehei* and *P. variotii* was also inhibited completely at 400 μg/ml per disc by this extract and the growth of *P. notatum* was only partially inhibited (39%) by the M extract at this concentration (data not shown).

On the other hand, the M extract was assayed against the human pathogen fungi *C. albicans* (unicellular, fungi of vaginal mucus) and *T. mentagrophytes* (dermatophytic fungi) and the phytopathogens *A. niger* (stored grains), *F. moniliforme* (stored grains), *F. sporotrichum* and *R. solani* (beans pest). The growth of *T. mentagrophytes*, *A. niger*, *F. moniliforme* and *F. sporotrichum* was completely inhibited in the range of 2500 to 4000 μg/ml (MFC) and the IC_{50} values resulted were of half concentration (Table IV).

Interestingly, in the M extract, the high percentage of secoisolariciresinol and eudesmin, together with a very low percentage of pinoresinol and laricíresinol is noteworthy. On the other hand, it is possible to observe the absence of compounds with methylene dioxy terminal as in sesamin for instance, that show a possible absence of an enzymatic pool for an oxidative cyclization. A similar situation can be observed in other trees from gymnosperms; facts are reported by Gang *et al.* (1997).

The activity showed by these lignans could be due to the antioxidant capacity of the components of the M extract, according to the reported by Schultz and Nicholas (2002). The four compounds assayed showed inhibitory effects against some of rooting-staining, human and phytopathogenic fungi. The presence of compounds **1**–**5** has already been described in many angiosperms and gymnosperms and it is well-established that they have different biological properties (Gang *et al.*, 1997), but their fungitoxic and bactericidal activities have not been described in the literature, to the best of our knowledge. However, among the above mentioned compounds, only compounds **1** and **3** showed a significant fungal inhibitory effect in our guided bioassay procedure; in addition, extract M emerged as the substance responsible for the antifungal activity of *A. araucana*.

Table II. Composition of *Araucaria araucana* wood extractives (% from the total MeOH extract, based on residual dry weight).

Species	Fatty acids	Monoterpenes and sesquiterpenes	Diterpenes	Triterpenes	Lignans and phenols	Others
Araucaria araucana	4.62	9.19	3.51	2.40	71.05	9.23

Table III. Antifungal growth inhibition activity on fungi inoculum by lignans of *Araucaria araucana*[a].

Compound	*M. miehei*	%	*P. variotii*	%	*C. pilifera*	%	*T. versicolor*	%	*P. notatum*
Pinoresinol (**3**)	7.33 ± 0.57a	16.3	n.a.[b]	–	16.0 ± 1.0c	35.6	15.0 ± 1.0c	33.3	n.a.
Eudesmin (**5**)	11.6 ± 0.57c	25.8	8.33 ± 0.57b	18.4	12.33 ± 1.15b	27.4	15.1 ± 1.1c	33.6	n.a.
Lariciresinol (**2**)	8.0 ± 1.10b	17.8	7.61 ± 0.57a	16.9	11.6 ± 0.57b	25.8	15.3 ± 0.57c	34.0	n.a.
Secoisolariciresinol (**1**)	13.4 ± 0.74d	29.7	9.91 ± 1.11b	21.9	18.65 ± 0.65c	41.5	20.3 ± 1.3d	45.1	n.a.
MeOH extract	15.2 ± 0.88d	33.9	13.0 ± 1.32c	28.7	22.30 ± 1.14d	49.5	25.7 ± 1.4d	57.1	n.a.

Mean of three replicates. Means followed by the same letter within a column are not significantly different in a Student-Newman-Keuls (SNK) test (treatments are compared by concentration to control), 95% confidence limits.

[a] Inhibitory effects at 100 μg per disc is represented as mm of growth [mean value of diameter of inhibition zone (mm) ± standard error of $N = 21$ and its significant difference from the control $p < 0.01$].

[b] Activity not present.

Table IV. Antifungal bioassay of the MeOH extract from *Araucaria araucana*. Evaluation of IC_{50} and minimum fungicidal concentration (MFC). (Values from the total MeOH extract, based on residual dry weight, the determination is explained in the text.)

Fungus	IC_{50} [μg/ml]	MFC[a] [μg/ml]
Candida albicans	1250	2500
Trichophyton mentagrophytes	1500	3000
Aspergillus niger	2000	4000
Fusarium moniliforme	2000	4000
Fusarium sporotrichum	2000	4000
Rhizoctonia solani	1500	3000

Results, the average of three replicates, were measured at 14 d after incubation.

[a] MFC is defined as the lowest concentration providing complete inhibition of fungal mycelial growth.

In summary, when the antifungal activity was assayed with the M extract the IC_{50} value was as low as that observed with the positive control ketoconazole (data not shown). This result showed a clear synergistic effect of the lignan composition of the M extract, which was not observed till now. The synergistic effect is one of the most important characteristics exhibited by natural extracts, increasing their efficacy in contrast to that which could be obtained with the equivalent amount of the active constituents alone.

These results reveal that lignans found in the *Araucaria araucana* extractives act on white rotting and wood staining fungi and show antibacterial and antifungal activities. This could indicate that such metabolites can play an important role in the wood's natural preservation.

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