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Antimycotic activity of 5′-prenylisoflavanones of the plant *Geoffroea decorticans*, against *Aspergillus* species

Emma N. Quiroga [1], Diego A. Sampietro [1,2], Melina A. Sgariglia [2], José R. Soberón [1,2], Marta A. Vattuone *[,1,3]

Cátedra de Fitoquímica. Instituto de Estudios Vegetales "Dr. Antonio R. Sampietro", Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471 (4000) San Miguel de Tucumán, Argentina

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ABSTRACT

The antifungal activity of the ethanolic extract (EE), (3R)-5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone (**1**) and (3R)-7-2′-3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone (**2**) isolated from *Geoffroea decorticans* was evaluated against four different species of *Aspergillus*. Their effect was compared with that displayed by synthetic products. The antifungal activity was assayed by bioautography, hyphal radial growth, hyphal extent and microdilution in liquid medium. The percentage of hyphal radial growth inhibition produced by EE varied between 18.4 ± 0.1 and 39.6 ± 0.2 for *Aspergillus nomius* VSC23 and *Aspergillus nomius* 13137, respectively; and the same value for **1** and **2** were between 31.2 ± 0.1–60.8 ± 1.5 and 28.9 ± 0.7–57.2 ± 0.6 for *Aspergillus flavus* (IEV 018) and *Aspergillus nomius* 13137, respectively. The values of MIC/MFC determined for EE, **1** and **2** were compared with the actions of ascorbic and sorbic acids, and clotrimazole. The sequence of antifungal potency was clotrimazole > **1** > **2** > ascorbic acid > sorbic acid > EE. Consequently, EE as well as the purified substances from *Geoffroea decorticans* would be used as biopesticides against *Aspergillus* species. The cytotoxicity was evaluated.

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1. Introduction

Fungi are a major cause of spoilage of stored grains and probably rank second only to insects as a cause of plant diseases, producing heavy loss of plant products. Pathogenic fungi alone cause nearly 20% reduction in the yield of major food and cash crops (Agrios, 2000). One-third of global agricultural production is reportedly destroyed each year by different pests and diseases (Maqbool et al., 1988). To avoid the incidence of yield losses due to plant diseases, a variety of control measures are presently in use. Physical methods, use of sunlight and UV radiations, among others, but the most commonly known means of controlling fungal diseases in fields and green houses and sometimes in storage is through the use of chemical compounds that are toxic to fungi. The use of chemicals has been found very effective in controlling plant fungal diseases but some major problems threaten the continued use of fungicides. Firstly, some fungi have developed resistance to chemicals. This needs higher doses of the product or the development of new chemicals to replace those to which fungi are resistant. Secondly, some fungicides are not readily biodegradable and tend to persist for years in the environment. This leads to a third problem, the detrimental effects of chemicals on organisms other than target fungi (Brady, 1984). Many research workers have tried to find safe and economical control of fungal growth by using extracts of different plant parts (Singh et al., 1980; Sumbali and Mehrotra, 1981; Bhowmick and Chaudhary, 1982; Vir and Sharma, 1985). Fungal growth can be inhibited by fungistatics of which propionic, acetic, ascorbic and sorbic acids are the most commonly used (Paster et al., 1999). These antifungals work by acidifying the cytoplasmic content of fungal cells (Hazan et al., 2004; Lambert and Stratford, 1999). Many plant secondary metabolites do have biological activity and may be toxic to microorganisms (antibiotics), plants (phytotoxins) or animals (mycotoxins) (Haschek et al., 2002). Many fungi produce mycotoxins as aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisins, fusaric acid, and penitrem that are mutagenic, teratogenic, and hepatotoxic secondary metabolites (Ali et al., 2005). Fungi belonging to the genus *Aspergillus* (*A. flavus*, *A. parasiticus* and *A. nomius*) produce aflatoxins that have been considered as probable human carcinogens by the International Agency for Research on Cancer (Xiulan et al., 2006). Besides *Aspergillus* are important agents in the biodeterioration of foods, feeds and the raw materials used in their manufacture. *Aspergillus* growth and mycotoxin incidence in foods and feeds are relatively high in tropical and subtropical regions, where climatic conditions favour the growth of moulds (Rustom, 1997). Consequently, there are strong needs for novel antifungal compounds applicable to foods and feeds as preservatives. These compounds must

* Corresponding author.
E-mail addresses: sampietro@tucbbs.com.ar (D.A. Sampietro), mvattuone@fbqf.unt.edu.ar (M.A. Vattuone).

[1] Investigators of the Universidad Nacional de Tucumán, Argentina.
[2] Fellowship holders of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.
[3] Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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have high activity against fungi and low biotoxicity to human beings and animals. From this view point, plants used in traditional medicine are promising sources.

Geoffroea decorticans is a middling-size tree (3–5 m height) when it grows as a pure grove, to a big tree till 10 m height when it is isolated. It belongs to the family Fabaceae, whose bark detaches itself in strips revealing lighter layers of the same hue. This species is widely distributed in the dry lands of the centre and north of Argentina (Cámpora 1913; Domínguez, 1928; Digilio and Legname 1966; Cabrera and Zardini 1978; Hieronymus, 1882; Amorín 1988; Boelcke, 1989). Its bark and flowers are used in folk medicine as antiasthmatic and emollient in the Northwestern of Argentina. The fruits are sweet and fragrant, though harsh to the palate; they are used as forage. Moreover, a syrup, frequently used to alleviate the cough, is prepared with them. In a previous work it was demonstrated the presence of the two known triterpenoids, lupeol and lupenone, with antifungal properties in the organic extracts of the stem bark of *G. decorticans* (Vila et al., 1998). We identified the same compounds in the ethereous extract of the aerial parts of this plant by comparison of their chromatographic and spectroscopic data with those of the literature (Razdan et al., 1988). In previous studies we demonstrated that ethanolic extracts of *G. decorticans* inhibit the growth of wood-destroying, and phytopathogenic fungi and of some yeast strains (Quiroga et al., 2001, 2004). These encouraged the study of its potential use in other applications (e.g. food and forage conservation) for substituting chemical preservations for food systems.

The purposes of this work were: a) to determine the *in vitro* antifungal activity of the alcoholic extract of *Geoffroea decorticans* against four *Aspergillus* strains isolated from animal feeds, b) to isolate and identify antifungal constituents from *Geoffroea decorticans*, c) to compare their inhibitory effect with the fungistatic activity of synthetic products used in food and feed protection.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Ascorbic and sorbic acids were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Plant material

Plant material was collected in an arid and semiarid regions of the Calchaquí Valley (Amaicha del Valle, Province of Tucumán) in northwestern Argentina at 1800–2000 m above sea level. The plant was identified as *Geoffroea decorticans* (Gill., ex Hook & Arn) Burkart, family Fabaceae, by specialists of the Instituto “Miguel Lillo”, Tucumán, Argentina. Voucher specimens are held for reference in the collection of the Fitoquímica Chair, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina. Leaves and twigs were dried in the shade in a well-ventilated room, ground to a coarse powder, and stored in closed flasks at −20 °C in the dark until they were used.

2.3. Extraction and isolation of active components

Ethereous and ethanolic extracts were prepared from dry aerial parts (leaves and stems) of *G. decorticans*. Briefly: the powder (10 g) was extracted with 100 ml ethyl ether by maceration at room temperature in a well-stoppered flask for two days, and filtered. The plant residue was extracted with 100 ml of 96% (v/v) ethanol by maceration at 37 °C for 72 h in the dark with slight agitation (in a shaker, 40 cycles/min). By paper filtration (Whatman No. 3 paper) we obtained the ethanolic extract (EE) that was evaporated to dryness under reduced pressure and weighted (1.58 g). This residue was partitioned with CH_2Cl_2. The extracted material (316 mg) was concentrated under reduced pressure at 35 °C, and chromatographed on a Silicagel column (4 cm diameter, 300 g, 60–200 mesh, Merck, Damstadt, Germany) eluted with hexane and with a gradient of hexane-ethyl acetate mixtures up to 100% ethyl acetate, to enhance the polarity. Fractions were analyzed by analytical and preparative thin layer chromatography (TLC) using Silicagel 60 F_{254} precoated alumina sheets (0.2 mm, and 1 mm, Merck, Damstadt, Germany) as stationary phase and hexane- ethyl acetate (3:2) as mobile phase. The separated components were visualized under ultraviolet light (254 and 366 nm, UV lamp model UV 5L-58 Mineralight Lamp), and spraying with vanillin-sulphuric acid and anisaldehyde/sulfuric acid for steroids and terpenes, methanolic potassium hydroxide for coumarins, natural products reagent (1% methanolic 2-aminoethyl diphenylborate) or aluminum chloride for phenolic compounds, Dragendorff's reagent for alkaloids, and 10% H_2SO_4 followed by heating (Wagner et al., 1984; Krebs et al., 1969). The bioactive bands of the preparative TLC were separately scraped from the air-dried plate, and eluted with HPLC grade methanol. The suspension was centrifuged (10 min at 5000 g), and the supernatant evaporated to dryness for further analysis. This process was repeated as many times as necessary.

2.4. Instrumental analysis

UV-visible spectra (200–600 nm) were carried out in a Beckman DU 650 spectrophotometer (Mabry et al., 1970). ^1H and ^{13}C NMR spectra were recorder in acetone-d_6, in a Bruker spectrometer with the residual solvent peaks as internal references. Compounds were identified by comparison of their spectroscopic and chromatographic data with published information (Vila et al., 1998).

2.5. Microorganisms

The fungal strains used were *Aspergillus flavus* (IEV 018), *Aspergillus parasiticus* NRLL 22, *Aspergillus nomius* VSC23 and *Aspergillus nomius* 13123. Strains were isolated from farm animal feeds and classified by Dr. Dante Bueno from Instituto Nacional de Tecnología Agraria (INTA, Concepción del Uruguay, Entre Rios, Argentina). Voucher specimens are deposited in the mycoteque of the Instituto de Estudios Vegetales “Dr. A.R. Sampietro”, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina. All the strains were maintained in solid culture medium (1.5% (w/v) malt extract, 0.5% (w/v) peptone and 1.5% agar).

2.6. Sterilization

Plant extracts, purified substances, and commercial antifungals were filtered through a 0.22 μm membrane filter before used.

2.7. Fungal cultures

Fungi were grown on solid medium containing 2.0% (w/v) malt-extract, 0.5% (w/v) peptone, 2.0% (w/v) glucose and 1.8 (w/v) % agar (MPGA) and in potato dextrose agar (PDA, Difco Laboratories, Detroit, Mich.), at 30 °C in the dark for 7 days. MIC determinations were made in liquid media (MPGB or PDB). The same media plus 0.6% agar were used for bioautographic assays.

2.8. Antifungal assays

The biological activity of plant extracts and synthetic preservative compounds were assessed by various methods:

a) *Bioautographic assays* were made on Silicagel 60 plates (0.2 mm thick, Merck, Damstadt, Germany) by dot blot with 100 μg of EE or 10 μg of each of the 5′-prenylisoflavanones (Homans and Fuchs,

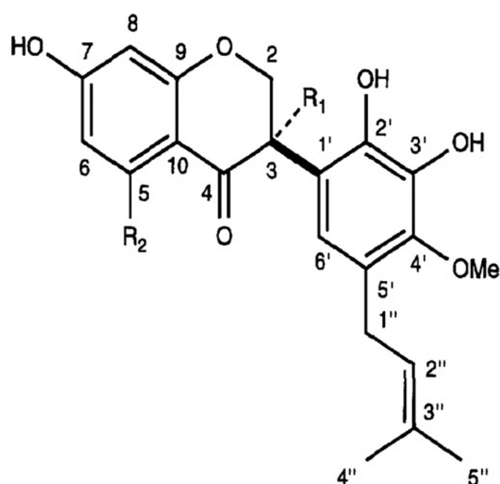

Fig. 1. Chemical structures of the 5′prenylisoflavanones. Compound 1(5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone): $R_1 = H$; $R_2 = OH$. Compound 2 (7-2′-3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone): $R_1 = R_2 = H$.

1970). Plates were dried in sterile conditions to eliminate the solvents. Then, they were covered with 3 ml of semisolid culture medium containing 0.3 ml of spore suspension (10^6 spores/ml) harvested from 7 days old monoconidial subcultures of sporulating fungi to ensure the inoculum's purity and viability. Fungitoxic activity was macroscopically visualized after incubation at 30 °C in a moist chamber for 48–72 h. The same procedure was made for bioautographic assays performed after analytical and preparative TLC development.

b) *Radial growth tests in MPGA and PDA media*: Plant ethanolic extract (EE) and each of the two 5′-prenylisoflavanones were incorporated into 5 ml of solid medium previously liquified and maintained at 45 °C (final concentration: 0.05–0.15 mg of EE or 5–15 μg of each of the two 5′-prenylisoflavanones/ml of culture medium), poured to Petri dishes (60 × 15 mm), and left to stand in a sterile air flow until solidification. The assay was performed by placing a 4-mm diameter plug of fresh mycelium onto the centre of the dish.
Plates containing ethanol without test extracts were included as blanks. Cultures were incubated in the dark at 30 °C. The radial growth of mycelia in all plates. was measured when the growth on the untreated plates neared the edge (approximately five days). Each test was repeated three times and each data point is the mean of at least 4 measurements of a growing colony. The percentage of growth inhibition was calculated by comparing the experimental with the control, which was considered to be 100% growth (Reyes Chilpa et al., 1997).

c) *Inhibition of hyphal extension*: The inhibition of hyphal extension was demonstrated by radial growth inhibition assays according to Schlumbaum et al. (1986) with some modifications. Briefly: 15 ml of melted (45 °C) MPGA or PDA were poured on 9.1 cm diameter Petri dishes. A fungal plug of 4-mm diameter was placed in the center of the dish. Sterile paper discs (Whatman No. 4 paper, 5 mm diameter) were impregnated with 20 μl of different EE dilutions (0–99 μg of EE) or 5′-prenylisoflavanones (0–10 μg) previously filter-sterilized (using 0.22 μm ultrafree-HC Durapore, Millipore). The discs were allowed to dry and evenly spaced on the agar surface of each plate. A negative control (a disc with 20 μl ethanol) was included. Plates were then inverted and incubated for 4–5 days at 30 °C in the dark. Then, the growth inhibition was observed as a crescent-shaped inhibitory zone at the mycelial front.

d) *Determination of Minimal Inhibitory Concentration (MIC) and minimal fungicidal concentration (MFC)*: MIC and MFC were determined according to the guide-lines of the NCCLS reference method (National Committee for Clinical Laboratory Standards, 1997). The assay was performed in a total volume of 0.1 ml in 96-well sterile polystyrene plates (8 rows × 12 columns). Each column representing one particular treatment and each row representing one replicate. EE was suspended in MPGB to achieve a concentration of 100 to 1000 μg of EE/ml. Synthetic products (ascorbic and sorbic acids) were diluted between 100 and 1000 μg/ml, each of the two 5′-isoflavanones **1**and **2** between 15 and 50 μg/ml, and clotrimazole between 1 and 50 μg/ml. Aliquots (90 μl) of MPGB with or without test serial dilutions (EE, protective synthetic compounds, the 5′-isoflavanones or clotrimazole), were deposited aseptically in each well and 10 μl of a spore suspension (10^6 spores/ml) of test fungi were added to each well. Controls were run simultaneously. For the controls, without fungus, 10 μl sterile water was added. Microplates were incubated at 30 °C for 48–72 h. Each control and each treatment were replicated eight times. After incubation, the MIC was defined as the lowest concentration at which no visible fungal growth was observed.
Aliquots (20 μl) were removed from the wells with no visible fungal growth and streaked onto MPGA or PDA plates and incubated aerobically at 30 °C for 48–72 h. MFC was defined as the lowest concentration at which colonies failed to grow after incubation.

2.9. Cell cytotoxicity assay

Cytotoxicity of the EE, compounds **1** and **2** from *G. decorticans*, sorbic and ascorbic acids and clotrimazole was determined with the *Artemia salina* assay according to Meyer et al. (1982). Briefly, brine shrimp eggs were placed in sea water (3.8% NaCl, w/v, in distilled water) and incubated at 25 °C opposite a lamp (100 W, 2000 Lux). Eggs hatch and mature within 24 h, providing a large number of larvae (nauplii). Ten larvae were placed in vials containing 5 ml of sea water and increasing concentrations of PPE. The dose ranges used were between 1 and 500 ppm. Serial dilutions were prepared in order to reach the chosen concentrations in a final volume of 5 ml. Data were obtained from three to four separate experiments with three plates per concentration. Controls were made in vials containing 5 ml of sea water and a maximum of 230 μl of 96% ethanol. Living nauplii were counted after 16 h at 25 °C (2000 Lux). The results were expressed as micrograms of EE, comp.**1** or **2,** sorbic or ascorbic acids or clotrimazole/ml.

2.10. Statistical analysis

Assays were performed in triplicate and repeated twice. Microdilution assays were conducted eight times for each plant extract, isolated compounds, or commercial antibiotic concentration, and

Table 1
Radial growth inhibition test of filamentous fungi by EE of *Geoffroea decorticans* and of 5′-prenylflavanones **1** and **2**.

	Growth inhibition (%)*			
	Fungi			
	A. Flavus (IEV 018)	*A. parasiticus* NRLL22	*A. nomius* 13137	*A. nomius* VSC23
Ethanolic extract (EE) *G. decorticans*	18.6 ± 0.5	27.7 ± 0.3	39.6 ± 0.2	18.4 ± 0.1
5′prenylisoflavanones				
1	31.2 ± 0.1	40.3 ± 1.5	60.8 ± 1.5	35.4 ± 0.6
2	28.9 ± 0.7	35.8 ± 0.8	57.2 ± 0.6	31.8 ± 0.6

Fungi were grown on MPGA and PDA plates containing 0.4 mg of EE/ml or 50 μg of each 5′-prenylflavanones/ml in the culture medium.
$n = 12$.
*The percentage of radial growth inhibition was calculated according to Reyes Chilpa *et al.* (1987).

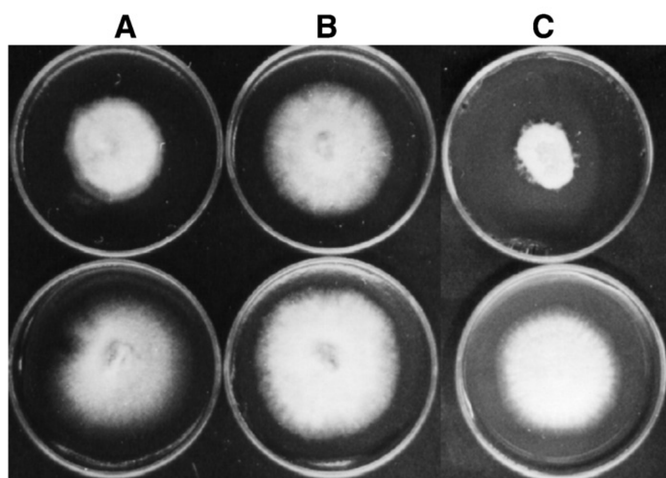

Fig. 2. Radial growth inhibition of the filamentous fungus *Aspergillus nomius* VSC23. Ethanol was added to the culture media as control (lower line). Fungal inhibition of each treatment is shown in the upper line. A: Inhibition produced by 0.4 mg of EE; B: Inhibition produced by 50 µg of compound **2**, and C: Inhibition produced by 50 µg of compound **1**/ml of culture medium. The same percentage of inhibition was observed for fungi grown in PDA and in MPGA media.

repeated twice. The arithmetic means of the MIC and MBC were calculated and reported.

3. Results

Ethanolic extracts (EE, tinctures) of *G. decorticans* were prepared according to Farmacopea Argentina VI Ed. (1978), from the residue of the plant extraction with ethyl ether. The yield of the extraction was 158 ± 5 mg/g of dry material.

The fungitoxic activity of the EE was tested by bioautography against the four strains of *Aspergillus* isolated from animal feeds in Tucumán, Argentina. Aliquots containing 100 µg of EE revealed fungal growth inhibition by bioautography (not shown). Several techniques of fractionation were applied to the EE (solvent extractions and silica gel adsorption column eluted with solvents of growing polarity) allowing the separation of many fractions that were analysed by TLC. Fractions with similar chromatographic profiles were pooled, and their antifungal activity was analysed by bioautography. The bands which showed dark-green fluorescence in $UV_{366\ nm}$ after treatment with natural products reagent showed the same R_f as that with antifungal activity (bioautography). These bands were separated by preparative TLC and analysed by ^1H RMN and ^{13}C RMN. This allowed the identification of three 5′-prenylisoflavanones: (3R)-5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone (compound **1;** yield: 40 µg/g of dry material), (3R)-7-2′-3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone (compound **2;** yield: 70 µg/g of dry material), and a few quantity of (3S)-3-7-2′-3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone (compound **3**) by comparison of their chromatographic and spectroscopic data with those of the literature (Fig. 1, Vila et al., 1998). By bioautography 30 µg of **1** or **2** inhibited the four *Aspergillus* studied (not shown).

Radial growth inhibition tests of filamentous fungi grown on MPGA or PDA plates containing 0.4 mg of EE/ml of culture medium varied between 18.4 ± 0.1 and 39.6 ± 0.2% (Reyes Chilpa et al., 1997) and confirmed the above result. Otherwise, the fungal growth inhibition produced by 50 µg of **1** or **2**/ml of culture medium was between 31 ± 0.1–60 ± 0.1 and 28 ± 0.1–57 ± 0.2%, respectively (Table 1, Fig. 2). The fungal hyphal extension was also affected by EE and the two 5′-prenylated isoflavanones (not shown).

The comparison of the antifungal spectrum of EE, the isolated 5′-prenylisoflavanones, clotrimazole, and synthetic products was performed by their MIC and MFC determinations (Table 2). As can be seen clotrimazole was the best antifungal against the four *Aspergillus* species being *A. flavus* (IEV 018) the most sensible. Compound **1** showed higher antifungal activity than compound **2,** and both 5′-prenylisoflavanones are better antifungals than the weak organic acids assayed, that are usually employed to preserve manufactured foods and beverages from yeasts and mould spoilage.

The cytotoxicity assays showed that the LD_{50} obtained for *G. decorticans* EE, compounds 1 and 2, sorbic and ascorbic acids, and clotrimazole were 2150 > 250 > 290 > 5000 > 5000 and 80 µg/ml, respectively. These values are high enough to assure the absence of cytotoxicity. Note that high LD_{50} means low cytotoxicity.

4. Discussion

A cornerstone of the search of new sources of antifungal substances is the growing resistance of pathogen population to commercial antifungals (Anaissie et al., 1988; Denning et al., 1988; Walsh et al., 1990). The phytochemical analysis of components of aerial parts of *G. decorticans* have been mainly directed towards compounds active against plant pathogenic fungi belonging to *Aspergillus* section Flavi (*A. flavus* (IEV 018), *A. nomius* VSC23, *A. nomius* 13137 and *A. parasiticus* NRLL 22), because research along these lines is of scientific and economical interest. It has been demonstrated that plant-derived constituents may offer potential leads for novel agents against systemic mycoses (Hufford and Clark 1988).

The isolated compounds, isoflavonoids, have the same general skeleton of the flavonoids (C_6–C_3–C_6) but are characterized by a rearrangement to give a 1,2-diphenylpropane that is common to all molecules in this group and can be related to the skeleton of 3-phenylchromane. Isoflavanones are among the Dicotyledonous, mainly or almost exclusively in the Fabaceae family. This plant specificity is attributed to the presence of the enzymes responsible of the rearrangement of 2-

Table 2
MICs and MFCs of ethanolic plant extract (EE), 5′-prenylisoflavanones **1** and **2**, clotrimazole, and food protective substances (ascorbic and sorbic acids).

Fungi				
Substance/s	*A. flavus* (IEV 018)	*A. parasiticus* NRLL22	*A. nomius* 13137	*A. nomius* VSC23
	MIC/MFC (µg/ml)			
EE	256 ± 5.0/520 ± 6.0	256 ± 3.0/520 ± 7.0	307 ± 3.0/720 ± 9.0	128 ± 3.0/310 ± 9.0
Comp **1**	9.00 ± 0.7/20 ± 0.1	18 ± 0.1/30 ± 2.0	12 ± 0.5/24 ± 1.0	9 ± 0.5/24 ± 1.0
Comp **2**	10 ± 0.6/21 ± 0.8	21 ± 0.2/50 ± 4.0	15 ± 0.1/45 ± 2.0	15 ± 0.1/35 ± 1.0
Ascorbic acid	100 ± 0.1/250 ± 0.4	150 ± 2.0/320 ± 4.0	200 ± 0.8/340 ± 4.0	150 ± 1.0/320 ± 8.0
Sorbic acid	200 ± 2.0/260 ± 5.0	400 ± 4.0/840 ± 6.0	100 ± 1.0/240 ± 1.0	400 ± 0.8/840 ± 6.0
Clotrimazole	3 ± 0.1/9 ± 0.1	10 ± 0.1/30 ± 1.0	5 ± 0.1/15 ± 0.5	4 ± 0.1/12 ± 0.8

MIC determinations were performed in polystyrene 96-well plates containing liquid medium (MPGB) and dilutions of the assayed substances. MFCs were determined in plates with solid media (MPGA or PDA).
Values are the mean of 8 determinations ± SD.
MICs and MFCs were calculated as µg of ethanolic extract (EE)/ml of plant extracts.
MICs and MFCs for compounds **1**; **2**, clortrimazole, ascorbic and sorbic acids were calculated as µg/ml.

phenylchromane to 3-phenylchromane. Many other flavonoids are present in the Fabaceae family but it is noticeable the high frequency of the isoprenylated derivatives as occurs in *G. decorticans*. When isoflavonoids have a hydroxyl group in the position 2′, a cyclization can occur with the generation of a new ring, as happen with the formation of pterocarpans. Consequently, the isoflavanones are intermediaries among the isoflavones and the pterocarpans. Besides, in plants, many phytoalexins have isoflavonoid related structures. Phytoalexins serve as a natural defence against infections by pathogens that frequently are of fungal nature because they accumulate when plants are attacked by these microorganisms (Dewick, 1994; Galefi et al., 1997).

The results of an antifungal test can be varied according to the applied methodology (Praxton, 1991). Consequently, various methodologies, which act on different stages of fungal growth were employed. Bioautography was selected as the first stage for detecting antifungal activity because this technique assays the effect of extracts on spore germination and is the basis for carrying out the purification steps. The radial growth test was used to study the action of EE and the isolated substances on the first stages of filamentous fungal growth, and hyphal extension permitted the analysis of the same fractions on fungi in active growth. Although, MIC and MFC are the most effective assays for the detection of antimicrobial compounds, the methodologies previously mentioned are necessary to establish the experimental conditions of analysis. Another point that was taken into account was the presence of compounds of known antifungal activity such as lupeol and lupenone in the stem bark of the plant (Vila et al., 1998). Consequently, measures were taken to demonstrate their absence in the plant EE to be sure that the measured antifungal activity was the consequence of the presence of other components from leaves and twigs of *G. decorticans.*

The effect of EE and the 5′-prenylisoflavanones **1** and **2** were compared with antifungal commercial drugs of known molecular mechanism. Among them, benzoic acid derivatives (methyl-benzoate) are usually employed as fungicides because they inhibit fungal enzymatic activity and mycotoxin production while ascorbic and sorbic acids are able to affect the sporulation process and mycelial growth. Even though ascorbic and sorbic acids do not eliminate mycotoxin production they are often used in foodstuff conservation. This is the first study on the antifungal activity of *G. decorticans* EE and the 5′-prenylisoflavanones isolated from this extract and the comparison of their antifungal strength with commercial drugs. The absence of cytotoxicity indicates that the use of *G. decorticans* as a natural fungicide could be made with safety for consumption and manipulation.

Our data showed that the growth of the assayed species of *Aspergillus* can be inhibited *in vitro* by alcoholic extracts, and two 5′-prenyl isoflavanones isolated from aerial parts of *G. decorticans*, a plant species that spontaneously grows in the Northwestern of Argentina.

Consequently, this plant constitutes an important source of biological compounds with biopesticidal activity against fungi, and especially to the mycotoxin producing fungi. In the future, this plant and its derivatives may be used as agricultural fungicides, with a reasonable margin of safety.

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