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ORIGINAL ARTICLE

Propolis from the northwest of Argentina as a source of antifungal principles

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Abstract

Aims: To determine the antimycotic and cytotoxic activities of partially purified propolis extract on yeasts, xylophagous and phytopathogenic fungi. To compare these activities with pinocembrin and galangin isolated from this propolis and with the synthetic drugs ketoconazole and clortrimazole.

Methods and Results: Ethanolic propolis extract was partially purified by cooling at −20°C. Two of its components were isolated by HPLC and identified as pinocembrin and galangin. The antifungal activity was assayed by bioautography, hyphal radial growth, hyphal extent and microdilution in liquid medium. Cytotoxicity was studied with the lethality assay of *Artemia salina*. The obtained results were compared with the actions of ketoconazole and clortrimazole.The results showed that the antifungal potency of ketoconazole and clortrimazole is higher than pinocembrin, galangin and the partially purified propolis extract in this order. Otherwise, the cytotoxicity of the synthetic drugs is also the highest.

Conclusions: Partially purified propolis extract inhibits fungal growth. The comparison of its relative biocide potency and cytotoxicity with synthetic drugs and two components of this propolis (pinocembrin and galangin) showed that the propolis from 'El Siambón', Tucumán, Argentina, is a suitable source of antifungal products.

Significance and Impact of the Study: The partially purified propolis extract and its isolated compounds, pinocembrin and galangin, have the capacity of being used as antifungals without detriment to the equilibrium of agroecosystems. The impact of this study is that the preparation of agrochemicals with reduced economic costs using a partially purified preparation as the active principle is possible.

Introduction

One of the most serious dangers that plants face are the diseases caused by pathogenic micro-organisms: bacteria, fungi and viruses. Numerous fungal species belonging to the main phylogenetic groups (Ascomycetes, Basidiomycetes and Deuteromycetes) give rise to important plant diseases and considerable crop economic losses. Given these problems, the development of natural or synthetic antifungal agents is an attractive objective. Propolis, a polycomponent product of honeybee activity, is a sticky substance collected by bees from buds, leaf exudates and cracks in the bark of trees and shrubs (Ghisalberti 1979; Cizmarik and Lahitova 1998). It may include different types of bee secretions, such as wax and salivary enzymes, and serves as a protective wall against the bee enemies. They use the resulting material to seal holes in the hives, exclude draught, protect them against external invaders and mummify their carcases. Its main function, however, is to prevent the decomposition of organic matter within the hive by inhibiting microbial growth. Propolis composition and its physico-chemical properties, biological activities and therapeutic uses depend on the vegetation where the hives are placed (Greenaway *et al.* 1991;

Bonvehi *et al.* 1994; Marcucci 1995; Tazawa *et al.* 1998, 1999; Marcucci and Bankova 1999; Bankova *et al.* 2000; Nieva Moreno *et al.* 2000; Salomao *et al.* 2004) and the season of its collection. Propolis has been extensively used in folk medicine in several regions of the world (Burdock 1998). One of the most frequent applications is its antimicrobial activity against many gram-positive and gram-negative bacteria, yeasts and fungi, most of them associated with variying degrees of pathogenicity in man (Burdock 1998; Kujumgiev *et al.* 1999). However, the action of bee glue against xylophagous and phytopathogenic fungi has received little attention.

The aim of the present work was to study the fungitoxic effect, *in vitro*, of propolis extracts from El Siambón, Provincia de Tucumán, Argentina, against yeast, xylophagous and phytopathogenic fungi. Its antimycotic activity and cytotoxicity was compared with that of the isolated flavonoids from the same source (pinocembrin and galangin) and of two antifungal synthetic drugs (ketoconazole and clortrimazole).

Materials and methods

Study site and species

Beehives are situated in the subtropical montane forest of 'El Siambón', Tucumán, Argentina. The vegetation of the area corresponds to the 'Jungas' biogeographic province, District of the Mountain Forest, which is the southernmost extention of the tropical Andean montane forests (Cabrera and Willink 1980). Native vegetation of the area is a semievergreen forest dominated by Lauraceae, Myrtaceae, Fabaceae, Juglandaceae, Salicaceae and Nyctaginaceae. The pristine montane forest was progressively replaced by poplar, eucalyptus and mainly by pinus trees. Consequently, bees living in 'El Siambón' have access to an enormous variety of native and introduced plant species. Nevertheless, preliminary studies on pollen content of propolis of this region showed that the resin-producing trees *Salix humboldiana*, *Pinus* and *Eucalyptus* are the most visited plants.

Chemicals

All chemicals used were of analytical grade. Pinocembrin was from Sigma and galangin from Fluka (St Louis, MO, USA).

Test micro-organisms

Xylophagous fungi [*Ganoderma applanatum* (IEV 017), *Lenzites elegans* (IEV 012), *Pycnoporus sanguineus* (IEV 006) and *Schizophyllum commune* (IEV 009)] were isolated from local decaying wood in the locality of Anta Muerta, Pcia, de Tucumán, Argentina. Phytopathogenic fungi [*Aspergillus niger* (from citrus), *Fusarium* sp. (isolate 20 from *Glycine max*), *Fusarium* sp. (isolate 21 from *Manguifera indica*), *Fusarium* sp. (isolate 22 from *G. max*), *Macrophomina* sp. (isolated from *G. max*), *Penicillium notatum*, *Phomopsis* sp. (from *G. max*) and *Thichoderma* spp.] were a kind gift from the Instituto Nacional de Tecnología Agropecuaria (INTA) and from the Estación Experimental Agroindustrial 'Obispo Colombres' (EEAOC), Tucumán, Argentina. All phytopatogenic fungi were isolated from crop plants grown in the field. The yeast strains used in this study were *Saccharomyces carlsbergensis* Hansen (IEV 002) and *Rhodotorula* spp. (IEV 001). They were obtained from Laboratorio de Micología, Instituto de Microbiología, UNT, Argentina. Voucher specimens are deposited in the culture collection of the Instituto de Estudios Vegetales 'Dr. A.R. Sampietro', Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina. Strains were maintained on solid culture medium at 4°C [SM: 1·5% w/v malt extract (Britania, Argentina), 0·5% w/v peptone and 1·8% w/v agar]. Other culture media used were semisolid medium (SSM: 1·5% w/v malt extract; 0·5% w/v peptone and 0·6% w/v agar) and liquid medium (LM: 1·5% w/v malt extract and 0·5% w/v peptone).

Preparation of soluble extracts

Natural products

Propolis was gathered from *Apis mellifera* beehives of the locality of 'El Siambón', Pcia, de Tucumán, Argentina, and stored at 4°C in the dark. The sample was cut into small pieces after cooling at −20°C and extracted with 96% ethanol (1 g of propolis per 10 ml of 96% ethanol). This suspension was kept at room temperature for a period of 5 days in the dark with occasional shaking and centrifuged at 105 000 ***g*** for 15 min to eliminate ethanol insoluble substances. Then, the mixture was frozen at −20°C for 2 h and centrifuged at 105 000 ***g*** for 15 min to discard waxes and gums. The solvent of the supernatant was evaporated under reduced pressure at 40°C in a rotary evaporator until constant weight. Residue was dissolved in 96% ethanol up to a concentration of 63·6 mg ml^{-1} (w/v). The obtained brownish but transparent preparation was named partially purified propolis extract (PPPE). It was stored at 4°C in the dark until it was tested for antifungal activity.

Synthetic products

Two kinds of synthetic products with different action modes were used. They were obtained from their manufacturers. Ketoconazole (Parafarm, Buenos Aires,

Argentina) is an antimycotic of systemic action derived from the imidazole structure. Like other imidazole derivatives, ketoconazole interferes with the synthesis of ergosterol and results in alterations in membrane permeability and inhibition of purine transport. Clortrimazole (Orofarm, Buenos Aires, Argentina) is also derived from the imidazole structure and it shows a broad spectrum of antifungal activity. In Europe, this drug is also used as systemic, although most studies indicate that it has limited efficiency by oral administration because of a considerable toxicity on the central nervous system (Georgopapadakou 1998).

Measurement of absorption spectra and phenolic compound concentration

The absorption spectra of the PPPE in methanol were observed in a Beckman DU 650 spectrophotometer (wavelengths: 200–600 nm) against a methanol blank. Total phenolic compound concentration was measured with the Folin-Ciocalteu's phenol reagent (Singleton *et al.* 1999). Cumarin (0–10 μg) was used as a standard to produce the calibration curve. The mean of the three readings was used. Total flavonoid content was estimated with the $AlCl_3$ reagent (Woisky and Salatino 1998) using quercetin (0–0·5 μg) as standard. The mean of the three readings was used and expressed as milligram of quercetin equivalents.

Separation of propolis components by TLC

Propolis constituents were separated by thin layer chromatography (TLC) using Silicagel 60 F_{254} plates, 0·2 mm (Merck, Darmstadt, Germany) as adsorbent. The chromatographic system was: toluene-chloroform-acetone (5 : 2 : 3; v/v). After development, the plates were dried and sprayed with natural products–polyethylenglycol reagent (NP/PEG), aluminum chloride reagent and vanillin–sulfuric acid reagent (Wagner *et al.* 1983).

HPLC analysis of propolis

The PPPE was filtered for analysis by HPLC. In all operations, an IB-SIL RP 18 column (5 μm, 250 × 4·6 mm, Phenomenex) equipped with a Gilson 118 UV detector was used. An aliquote was injected via a Rheodyne valve fitted with a 20-μl loop. The mobile phase was water–formic acid (95 : 5, solvent A) and methanol (solvent B). Elution was performed at a flow rate of 1 ml min^{-1} using a linear gradient starting with 30% B for 15 min increasing to levels of 40% B at 20 min, 45% B at 30 min, 60% B at 50 min and 80% B at 60 min to re-equilibrate the column. Detection was achieved at 290 and 340 nm. Reference compounds commercially obtained were co-chromatographed with the experimental sample to confirm the HPLC retention time. UV-visible absorption spectra were compared with literature data and analytical grade standards.

Detection of *in vitro* antifungal activity

Bioautographic assays

Propolis aliquots (19·4–310·4 μg dry principles) were dotted on silica gel plates. After drying in sterile conditions, the plates were covered with a spore suspension (1×10^6 spores ml^{-1}) in SSM. Spores were harvested from a fresh subculture on SM medium to ensure the inoculum's purity and viability. Plates were incubated in a wet chamber at 30°C between 2 and 5 days according to the growth characteristics of each fungus. Inhibitory zones were visualized by observation of fungus growth with the naked eye (Homans and Fuchs 1970).

Hyphal radial growth inhibition

The effects of different concentrations of PPPE, ranging from 0 to 1·164 mg of dry matter per millilitre, on pinicembrin and galangin of the culture medium, were evaluated by radial fungus growth according to the National Committee for Clinical Laboratory Standards (NCCLS 1998) guidelines for antifungal susceptibility testing of yeasts and moulds. Briefly, a 3-mm diameter plug of a growing mycelium of a filamentous fungus harvested from an 8- to 10-day culture or a 10 μl of spore suspensions (2.5×10^4 spores ml^{-1}) was placed onto the centre of petri dishes (60 × 15 mm) containing 5 ml of SM plus increasing extract, purified compound or synthetic drug concentrations. Plates were incubated at 30°C for 4–5 days in a moist chamber and the percentage of growth inhibition was calculated as (Reyes Chilpa *et al.* 1997):

$$\text{Percentage inhibition} = \frac{\text{mycelial growth in control} - \text{mycelial growth in propolis}}{\text{mycelial growth in control}} \times 100$$

Inhibition of hyphal extent

The inhibition of hyphal extent was demonstrated by radial growth inhibition assay (Schlumbaum *et al.* 1986). Briefly, a fungal plug was placed in the centre of a petri dish (100 × 15 mm) containing 10 ml of SM. Dishes were incubated at 30°C in the dark until the mycelium attained 3 cm in diameter. Then, sterile paper discs (4 mm in diameter) with different quantities (0–116·4 μg of dry matter) of filter-sterilized (using a 0·22 μm ultrafree-HC Durapore, Millipore) PPPE, purified compound or synthetic drug were placed at 0·5 cm from the border of the mycelium. A disc with 96% ethanol was used as control.

The petri dishes were incubated at 30°C for 48–72 h in the dark. Antifungal activity was observed as a crescent-shaped inhibitory zone at the mycelial front.

Determination of the minimal PPPE concentration necessary to inhibit fungal growth (MIC)

The *in vitro* susceptibility testing was performed using the broth microdilution version of the NCCLS reference method (1997). Briefly, broth dilution assays were performed in a total volume of 0·2 ml in sterile polystyrene 96-well plates containing LM and serial dilutions between 0 and 349 μg of PPPE, 0·1–10 μg of the antifungal drugs or 1–20 μg of the purified compounds. Wells were inoculated with 10 μl of a conidial suspension (1×10^4 conidia ml^{-1}). Controls with fungal inoculum and without the propolis extract were run simultaneously. Dishes were incubated for 48–72 h at 30°C and the minimal inhibitory concentration (MIC) was visually determined as the minimal concentration of the assayed compounds (PPPE, synthetic drugs or flavonoids) that inhibit fungal growth.

Cell cytotoxicity assay

In vitro lethality assay of *A. salina* leach was used to detect cell toxicity (Meyer *et al.* 1982). Brine shrimp eggs were placed in seawater (3·8% w/v sea salt in distilled water) and incubated at 24–28°C in front of a lamp. Eggs hatch and mature within 48 h providing large number of larvae (nauplii). A convenient number of nauplii was placed in vials containing 5 ml of seawater plus increasing concentrations of propolis extract (0·1–500 ppm), synthetic drugs (1–1000 ppm) or each flavonoid (0·05–500 ppm) for comparison purposes (Espinel-Ingroff *et al.* 2001). Controls were made with the same volume of 96% ethanol in seawater without addition of the propolis extract. Alive nauplii were counted after 16 h and the lethal dose 50 (DL_{50}) was calculated. Six replicates were made.

Statistical analysis

Lethality assays were evaluated by Finney computer statistical program to determine the LD_{50} values and 95% confidence intervals. A copy of this program for IBM PC is available from Dr J.L. McLaughlin, Department of Medicinal Chemistry and Pharmacology, School of Pharmacy, Purdue University, West Lafayette, Indiana 47907, USA. All other data were expressed as mean ± SD.

Results

Propolis is a particularly rich source of phenolic compouds and most of its biological activities are attributed to them. Consequently, the assay-guided isolation of bioactive principles was mainly performed by measuring phenolic and flavonoid contents.

Crude propolis preparation (alcoholic extract) was made at room temperature and partially purified by cooling at −20°C and centrifugation at 4°C. This treatment allowed discarding a waxy nature substance, slightly soluble in 96% ethanol but insoluble in this solvent at low temperature (−20°C). The supernatant (PPPE) was selected for this study. The yield of PPPE from the extraction procedure was 47 mg of the total phenolics and 5 mg of flavonoids per gram of crude propolis. The presence of phenolic compounds was evidenced by the intense fluoresecence produced by the dotted sample (on silica gel plates) under UV_{365} light and after spraying it with aluminum chloride and NP/PEG reagents. The TLC analysis of the PPPE allowed the separation of several bands with a strong positive reaction after TLC treatment with the mentioned reagents (not shown). Moreover, the UV–visible extended spectra (200–600 nm) of sample dilutions in methanol presented a maximum in the region around 290 nm that is attributable to a high flavonoid content (Markham 1982). The HPLC profile of PPPE detected at 290 and 340 nm indicated its complex composition with several peaks of varied retention times (Fig. 1a,b). The two majority peaks at 340 nm were identified as pinocembrin and galangin according to their retention times that were coincident with those of standard drugs. Pinocembrin, a flavanone, was the most abundant compound in this sample of propolis and the flavonol galangin is ubiquitous in the plant kingdom and was found in other propolis samples.

The fungitoxic activity of the PPPE and the HPLC-isolated flavonoids were tested against an array of yeasts, xylophagous and plant pathogenic fungi. Bioautographic tests revealed that *A. niger* growth was inhibited with quantities as low as 19·4 μg of dry PPPE while *Fusarium* sp., isolate 20 from soy bean, needed, at least, 310·4 μg of dry matter and the growth of *Macrophomina* spp., also isolated from soy bean, was not inhibited up to this quantity. The comparison with the growth inhibitory capacity of azole derivatives and the isolated flavonoids revealed that *Macrophomina* spp. was also the most resistant fungus. The measure of the diameter of the fungal hyphal radial growth on SM containing different concentrations of PPPE, azole derivatives and the HPLC-isolated flavonoids allowed calculating the percentage of growth inhibition (Table 1) (Reyes Chilpa *et al.* 1997). Again *Macrophomina* spp. was the most resistant fungus to the effect of the cited products. These products also affected the hyphal radial extention as was confirmed by the inhibitory action of the extract on hyphal fungal growth as is exemplified for *S. commune* and *P. sanguineus*

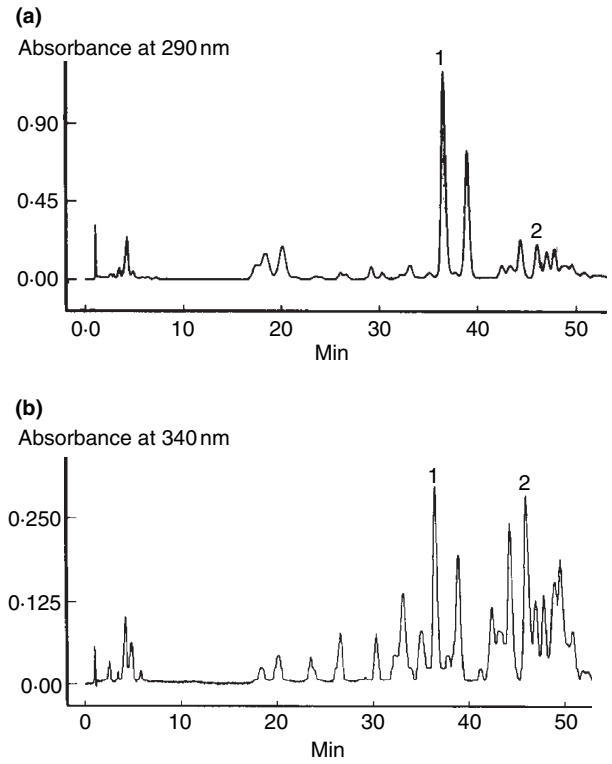

Figure 1 (a) HPLC chromatogram of PPPE recorded at 290 nm and (b) recorded at 340 nm. Pinocembrin: 1 and galangin: 2.

(Fig. 2). The MIC of PPPE on sporulating fungi and yeasts were determined and compared with that of the synthetic drugs (ketoconazole and clortrimazole) and the isolated flavonoids by microdilution in polystyrene 96-well plates. MIC values were 77 μg of PPPE per millilitre for *S. carlsbergensis* and 349 μg of PPPE per millilitre for *Trichoderma* spp., *P. notatum* and *Fusarium* sp. (isolate 21). Whereas the MIC values for ketoconazole, clortrimazole, pinocembrin and galangin were between 1 and 8 μg for the synthetic drugs per millilitre and 14–40 μg of flavonoid per millilitre of LM (Table 2). It is worthwhile to take into account that the cytotoxicity assays carried out with the lethality test of *A. salina* revealed that the LD_{50} of PPPE (965 ppm) was around 13 and 21 times higher than the same values for clortrimazole and ketoconazole (80·5 and 46·1 ppm, respectively). The same values for pinocembrin and galangin were 2010 and 1950 ppm, respectively.

Consequently, although the antifungal potency of the synthetic drugs is higher than that of PPPE and of two of its components (pinocembrin and galangin), the cytotoxicity of our preparation and of the isolated compounds is considerably low suggesting that amongst the several uses of propolis, this natural product would be also used to fight some plant plagues caused by fungi.

Discussion

The prolongued use of fungicides provoked problems concerning the increasing public importance in relation to the contamination of perishables with fungicidal residues (Marco *et al.* 1998), and growing resistance in the pathogen populations (Denning *et al.* 1988; Anaissie *et al.* 1988; Walsh *et al.* 1990). Recent research in this area has

Table 1 Inhibition of fungal hyphal radial growth

	Percentage inhibition*				
Fungus	PPPE 1·16 mg ml^{-1}	Ketoconazole 5·10^{-3} mg ml^{-1}	Clortrimazole 5·10^{-3} mg ml^{-1}	Pinocembrin 5·10^{-3} mg ml^{-1}	Galangin 5·10^{-3} mg ml^{-1}
Schizophyllum commune	68·7 ± 0·06	90·1 ± 0·02	87·4 ± 0·02	69·9 ± 0·01	87·8 ± 0·03
Pycnoporus sanguineus	77·1 ± 0·07	95·2 ± 0·02	92·7 ± 0·04	70·9 ± 0·01	72·1 ± 0·04
Ganoderma applanatum	46·1 ± 0·05	62·7 ± 0·05	62·8 ± 0·04	59·9 ± 0·02	55·2 ± 0·02
Lenzites elegans	54·7 ± 0·04	70·6 ± 0·04	73·5 ± 0·04	63·1 ± 0·02	72·4 ± 0·02
Trichoderma spp.	39·4 ± 0·02	47·3 ± 0·06	63·1 ± 0·03	59·9 ± 0·03	65·2 ± 0·01
Penicillium notatum	44·6 ± 0·05	45·2 ± 0·02	60·6 ± 0·04	62·8 ± 0·02	69·9 ± 0·04
Aspergillus niger	60·4 ± 0·04	78·6 ± 0·02	75·9 ± 0·03	71·2 ± 0·04	76·6 ± 0·04
Fusarium oxysporum	55·6 ± 0·03	71·5 ± 0·05	72·0 ± 0·02	63·9 ± 0·03	75·5 ± 0·03
Fusarium sp.(isolate 20)	63·1 ± 0·03	82·6 ± 0·05	79·7 ± 0·02	80·5 ± 0·02	85·6 ± 0·02
Fusarium sp.(isolate 21)	59·9 ± 0·03	81·9 ± 0·02	79·1 ± 0·01	84·1 ± 0·06	86·2 ± 0·03
Fusarium sp.(isolate 22)	59·1 ± 0·04	80·5 ± 0·04	80·4 ± 0·02	83·2 ± 0·05	85·5 ± 0·03
Phomopsis spp.	81·7 ± 0·04	94·9 ± 0·02	92·7 ± 0·01	91·2 ± 0·02	97·6 ± 0·02
Macrophomina spp.	8·0 ± 0·02	21·8 ± 0·02	20·0 ± 0·01	18·1 ± 0·02	19·6 ± 0·02

Mycelial diameters are the mean of 12 determinations ± SD.
PPPE, partially purified propolis extract.
*, The percentage of inhibition was calculated according to Reyes Chilpa *et al*. (1987).

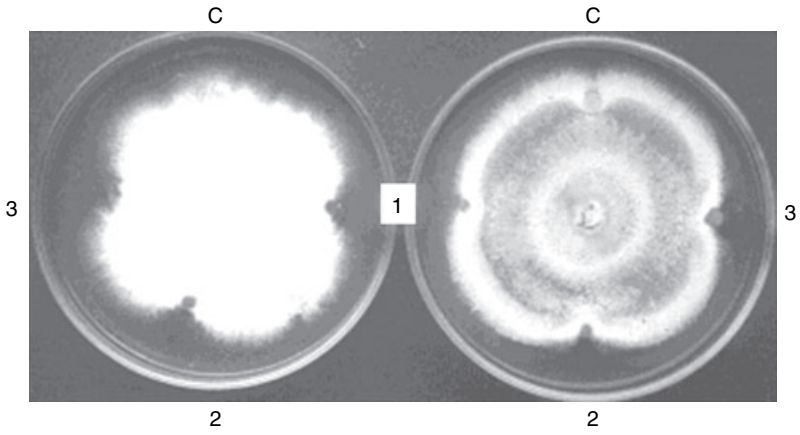

Figure 2 Inhibitory effect of partially purified propolis extract (PPPE) on hyphal radial extent. A fungal plug was placed in the centre of a petri dish containing solid culture medium. Dishes were incubated at 30°C until the mycelium attained 3 cm in diameter. Then, dry sterile paper discs containing: *C*: ethanol as control; *1*: 38·8 μg of PPPE; *2*: 77·6 μg of PPPE and *3*: 116·4 μg of PPPE were placed at 0·5 cm from the border of the mycelium and the dishes were incubated again. The used fungi were *Schizophyllum commune* (*left*) and *Pycnoporus sanguineus* (*right*).

Table 2 Determination of the minimal partially purified propolis extract (PPPE), synthetic drugs, pinocembrin and galangin concentrations necessary to inhibit fungal growth (minimal inhibitory concentration, MIC). MIC determinations were performed in polystyrene 96-well plates containing liquid medium and serial dilutions of PPPE or the antifungal drug

	MIC values (μg ml^{-1})				
Fungus	PPPE	Ketoconazole	Clortrimazole	Pinocembrin	Galangin
Aspergillus niger	232 ± 0·12	8 ± 0·02	3 ± 0·02	32 ± 0·02	28 ± 0·01
Trichoderma spp.	349 ± 0·23	2 ± 0·02	6 ± 0·04	50 ± 0·04	40 ± 0·02
Penicillium notatum	349 ± 0·14	1 ± 0·01	2 ± 0·02	25 ± 0·02	30 ± 0·03
Fusarium sp. (20)	349 ± 0·15	2 ± 0·02	6 ± 0·02	45 ± 0·02	35 ± 0·02
Fusarium sp. (21)	310 ± 0·10	3 ± 0·02	3 ± 0·01	30 ± 0·01	25 ± 0·02
Fusarium sp. (22)	194 ± 0·10	3 ± 0·01	3 ± 0·02	30 ± 0·02	27 ± 0·01
Phomopsis spp.	125 ± 0·10	3 ± 0·02	3 ± 0·01	32 ± 0·01	30 ± 0·03
Saccharomyces carlsbergensis	77 ± 0·09	3 ± 0·01	1 ± 0·01	20 ± 0·01	18 ± 0·02
Rhodotorula spp	116 ± 0·12	1 ± 0·01	1 ± 0·02	20 ± 0·02	15 ± 0·02

Values are the mean of 16 determinations ± SD.

been directed to the development and evaluation of diverse alternative control strategies to reduce the dependence on synthetic drugs. Moreover, it was demonstrated that biologically active natural products have the potential to replace synthetic drugs (Holderna and Kedzia 1987). The PPPE has an elevated content of phenolic compounds. These propolis constituents are considered to be responsible in keeping the interior of the beehives free of micro-organisms and are used in therapeutic applications (Marcucci 1995; Burdock 1998; de Castro 2001). Nevertheless, its use for the control of phytopathogens was poorly assayed. The micro-organisms used for the bioassays were selected among the most economically important fungus to control in Tucumán, Argentina. Variation between the results of the tests is a problem (Praxton 1991); consequently several methodologies were applied to assay the antifungal activity. The bioautographic method allowed the easy and rapid demonstration that PPPE avoids spore germination in a suitable culture medium. Otherwise, PPPE had inhibitory effect on filamentous fungal growth in a newly established fungal culture and on a growing culture confirming its antifungal action when fungi are in different states of growth. Pinocembrin, a flavanone that was the most abundant compound in this sample of propolis and the flavonol, galangin were partially responsible for the observed fungitoxic activity. Furthermore, as the PPPE was not equally active against closely related fungi, its biocidal activity and of the isolated flavonoids were compared with that of two synthetic antimycotic drugs, ketoconazole and clortrimazole. The MIC values showed that the antifungal action of PPPE, pinocembrin and galangin are lower than that of the synthetic drugs. However, their cytotoxicity is several times higher than PPPE.

However, the cytotoxicity of our preparation and of the isolated compounds is considerably low suggesting that among the several uses of propolis, this natural product would be also used to fight some plant plagues caused by fungi. Furthermore, our results suggest that products that behave as biocides in natural ecosystems would contain substances suitable for the development of antimicrobials applicable for health care in humans and also in agriculture, veterinary and in food and feed protection.

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