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SHORT COMMUNICATION

Antifungal and antimycotoxigenic metabolites from native plants of northwest Argentina: isolation, identification and potential for control of Aspergillus species

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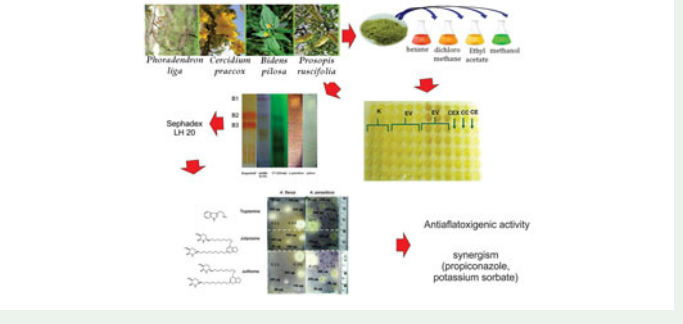
ABSTRACT

Extracts from aerial parts of *Prosopis ruscifolia*, *Bidens pilosa*, *Cercidium praecox* and *Phoradendron liga* were assayed against toxigenic *Aspergillus* species. They were obtained by sequential extraction of the aerial parts with hexane (fHex), dichloromethane (fDCM), ethyl acetate (fEtOAc) and methanol (fMeOH). The fMeOH from *P. ruscifolia* showed the highest antifungal spectrum (MIC = 750–1500 μg mL^{-1}; MID = 50–200 μg; DI = 1.7–3.0 mm). Indolizidine alkaloids (juliflorine and juliprosine) and tryptamine were identified with strong (MIC = 188 μg mL^{-1}) and moderate antifungal activities (MIC = 750 μg mL^{-1}), respectively, towards *A. parasiticus* and *A. flavus*. The fMeOH, the indolizidine alkaloids and tryptamine synergized the fungitoxic effect of potassium sorbate and propiconazole. They completely suppressed the biosynthesis of aflatoxins at concentrations of 47, 94 and 375 μg mL^{-1}, respectively. Our results indicate that fMeOH and its identified alkaloids are promisory additives of commercial antifungals and are antiaflatoxigenic agents at concentrations below of those required for complete suppression of fungal growth.



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1. Introduction

Aspergillus flavus, *A. parasiticus* and *A. nomius* contaminate stored commodities with aflatoxins (Plumridge et al. 2004). The intake of low levels of these mycotoxins generates mutagenic, teratogenic and carcinogenic effects on humans and the livestock (Bueno et al. 2011). Several public institutions have established maximum permissible contents of aflatoxins in food commodities. These contents can be overpassed in years of *Aspergillus* outbreaks restricting the exportation of Argentinian commodities and increasing the mycotoxigenic risk (Bueno et al. 2011). Commercial antifungals are used against the *Aspergillus* species. However, several fungicides (i.e. azole compounds) increase aflatoxin accumulation at the sublethal doses often occurring in the field and damage non-target organisms (Bluma et al. 2008). Food preservatives (i.e. potassium sorbate) are fungistatic, can change organoleptic properties and also promote aflatoxin contamination at non-inhibitory concentrations (Bueno et al. 2011). Plant extracts or their antifungal principles alone or in combination with commercial antifungals against the *Aspergillus* species could overcome these problems. This work reports for the first time the antifungal activity of some plants native from northwest Argentina against *Aspergillus* species. The main antifungal constituents of the most bioactive extract showed novel synergic interactions with commercial antifungals and antiaflatoxigenic activity.

2. Results and discussion

Aerial parts of *P. ruscifolia* yielded the highest contents of dry matter after extraction with hexane (8.0%), dichloromethane (4.1%), ethyl acetate (6.4%) and methanol (11.3%) (Table S1). Dot blot and microdilution assays showed that the methanolic extract of *P. ruscifolia* had the broadest antifungal activity with MIC = 1500–750 μg mL^{-1} and MID values of 50 μg on *A. niger* and 200 μg on the remaining *Aspergillus* strains. Polar compounds likely have a pivotal role in the defense mechanisms of *Prosopis* species against fungi. Seed methanolic extracts of *P. alpataco* and *P. denudans* showed MID values of 100 μg on *Cladosporium cucumerinum* (Mazzuca et al. 2003). Methanolic leaf extract of *P. juliflora* completely suppressed the growth of a banana pathogen (*Colletotrichum musae*) by the disc diffusion method (1000 μg dry matter $disc^{-1}$), 70–89% the growth of seed born pathogens (*Aspergillus candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus and A. tamarii*) at 3000 μg dry matter mL^{-1} in microdilution assays, and 40% the growth of the tobacco pathogen *Alternaria alternata* at 500 μg dry matter mL^{-1} (Raghavendra and Raveesha 2009). TLC analyses and bioautographies of the methanol extract of *P. ruscifolia* indicated inhibition of fungal growth at relation-to-front values of 0.75 (B1), 0.69 (B2) and 0.52 (B3) on all the *Aspergillus* strains (Figure S1). Pools Pp1, Pp2 and Pp3 showing antifungal activity in dot blot assays were isolated from B1, B2 and B3, respectively (Figure S2). ^{1}H NMR spectra indicated the presence, in a high degree of purity, of tryptamine in Pp1 and the indolizidine alkaloids juliflorine and juliprosine in Pp2 and Pp3, respectively (Figure S2). Microdilution assays (Table S3) indicated a strong, moderate and weak antifungal activity on *A. flavus* and *A. parasiticus* for the indolizidine alkaloids (MIC = 188 μg mL^{-1}), tryptamine (MIC = 750 μg mL^{-1}) and the methanolic extract

(MIC = 1500 μg mL^{-1}), respectively (Sartoratto et al. 2004). Dot blot bioassays (Figure S3) showed MID values of 50 μg (fMeOH on *A. parasiticus*; tryptamine on *A. flavus*), 100 μg (juliflorine and juliprosine on both *Aspergillus* strains) and 250 μg (tryptamine on *A. niger*; methanolic extract on *A. flavus*). The commercial fungicide propiconazole was several folds more active on the *Aspergillus* strains than fMeOH and its alkaloids. Nevertheless, the brine shrimp assay indicated that propiconazole was more toxic than juliflorine, juliprosine and tryptamine while fMeOH was not toxic (Table S4). The selectivity index, which is a measure of how specific is the toxicity of an antifungal compound (Bueno et al. 2011), showed that fMeOH and tryptamine should be fungitoxic (LC_{50}/MIC < 1) while the indolizidine alkaloids and propiconazole should be also hazardous to other organisms (LC_{50}/MIC > 1). Table S5 shows that the alkaloids potentiated (synergized) the mechanisms of action of propiconazole (FICI = 0.17–0.37) and potassium sorbate (FICI = 0.31–0.41). However, the reasons of these joint effects are hard to unravel. Sorbates accumulate in the cytosol of the fungal cells and generate a disbalance in cellular homeostasis with inactivation of several endogenous enzymes (Plumridge et al. 2004) while azole fungicides inhibit ergosterol biosynthesis (Bueno et al. 2011). In the case of the identified alkaloids, they are amphoteric compounds which likely interact with membrane-embedded proteins. Juliflorine blocked calcium channels when added to rabbit jejunum preparations (Soreq and Seidman 2001). Blocking of calcium channels inhibited aflatoxin biosynthesis in *A. parasiticus* (Praveen Rao and Subramanyan,1999) and could be the reason of the antiaflatoxigenic activity (Table S6) observed at a one-half concentration of the MIC for the alkaloids (94 μg mL^{-1}, juliflorine and juliprosine; 375 μg mL^{-1}, tryptamine).

3. Conclusions

A screening of extracts from four native species of northwest Argentina (*P. ruscifolia*, *B. pilosa*, *C. praecox* and *P. liga*) was performed for antifungal activity against foodstuff spoilage *Aspergillus* strains. The methanolic leaf extract of *P. ruscifolia* was the most active. Tryptamine and indolizidine alkaloids (juliflorine and juliprosine) were isolated and identified as the main antifungals involved. The methanolic extract and its identified alkaloids were antiaflatoxigenic agents at subinhibitory concentrations of fungal growth and could be used as synergic additives of the xenobiotics currently applied in the chemical control of toxigenic *Aspergillus* species.

Disclosure statement

No conflict of interest was reported by the authors.

References

Bluma R, Amaiden MR, Daghero J, Etcheverry M. 2008. Control of *Aspergillus* section Flavi growth and aflatoxin accumulation by plant essential oils. J Appl Microbiol. 105(1):203–214.

Bueno DJ, Soria MC, González HHL, Sampietro DA, Federico FJ, Silva JO. 2011. Fungi and mycotoxins in food. In: Filip R, ed. Multidisciplinary approaches on food science and nutrition for the 21st century. Kerala (India): Research Signpost; pp.215–243.

Mazzuca M, Kraus W, Balzaretti V. 2003. Evaluation of the biological activities of crude extracts from Patagonian *Prosopis* seeds and some of their active principles. J Herb Pharmacother. 3(2):31–37.

Plumridge A, Hesse JA, Watson AJ, Lowe KC, Stratford M, Archer DB. 2004. The weak acid preservative sorbic acid inhibits conidial germination and mycelial growth of *Aspergillus niger* through intracellular acidification. Appl Env Microbiol. 70(6):3506–3511.

Raghavendra MP, Raveesha KA. 2009. Alkaloid extracts of *Prosopis juliflora* (Sw.) DC. (Mimosaceae) against *Alternaria alternata*. J Biopesticides. 2:56–59.

Sartoratto A, Machado AL, Delarmelina C, Figueira CM, Duarte CM, Rehder VL. 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol. 35(4):275–280.

Soreq H, Seidman S. 2001. Acetylcholinesterase-new roles for an old actor. Nat Rev Neurosci. 2(4):294–302.