Revista Brasileira de Farmacognosia 29 (2019) 686–688

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Short communication

Evaluation of Argentinean medicinal plants and isolation of their bioactive compounds as an alternative for the control of postharvest fruits phytopathogenic fungi

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ARTICLE INFO

Article history:
Received 15 March 2019
Accepted 2 May 2019

Keywords:
Bioactive compounds
Fruits
Phytopathogenic fungi
Postharvest

ABSTRACT

One of the main problems that fruit health goes through in recent years is the difficult eradication of their fungal pathogens after harvesting. This concern to the whole world because it represents huge losses of production, fruit export restrictions and consumers distrust. One of the alternatives to solve this problem could be the exploration of plants and their active compounds, which have proven to be antifungal against human pathogens, but now applied to the treatment of fruits health. In this work, eighteen plant species that grow in Argentina were evaluated against four phytopathogenic fungi that greatly affect the postharvest stage of fruits commercially important to our country. All the species studied were at least active against one fungus of the panel, while three of them displayed high antifungal properties inhibiting the growth of selected pathogens. In addition, bio-guided fractionation of these most active extracts, led to the isolation of some compounds which proved to be responsible for their antifungal activity. Although they are known compounds and were previously isolated from other natural sources, this is the first time that they are evaluated for their phytopathogenic activities against this panel of fungi.

Introduction

Plant diseases caused by phytopathogenic fungi are responsible for economic losses arising mainly from crop yield reduction, but also resulting from diminished product quality and safety; sometimes they also represent a risk for human and animal health due to food contami nation and the accumulation of toxic residues in the environment. *Penicillium digitatum* (Pers.) Sacc, *Botrytis cinerea* (Pers.: Fr.), *Monilinia fructicola* (G. Wint.) honey and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, are four of the main phytopathogenic fungi that affect Argentine fruit exports and their producers (Pergomet et al., 2018).

Since regulations on the use of new and existing fungicides are becoming more and more stringent, it urges to identify and develop new chemical entities with antifungal activity. Different non-toxic naturally occurring compounds (Fu et al., 2017), semisynthetic derivatives (Zhang et al., 2015), chitosan-based formulations (Novaes Azevedo et al., 2014) and plant extracts (Gatto et al., 2016) including essential oils (Mohammadi and Aminifard, 2012) have emerged as promising alternatives to synthetic, and many times toxic, fungicides.

This communication provides results, based on *in vitro* studies, about the possible use of Argentine medicinal plants (or their bioactive compounds) as fruit post harvested antifungals. The species have been chosen based on their antifungal activities against human pathogens cited in the literature. All of them were at least active against one fungus of the panel, meanwhile three of them (*Solidago chilensis* Meyen., Asteraceae, *Drimys winteri* J.R.Forst. & G.Forst., Winteraceae and *Polygonum stelligerum* Cham., Polygonaceae) displayed high antifungal properties inhibiting the growth of selected pathogens. The bio-guided fractionation of *S. chilensis* hexane extract led to the isolation of a diterpene-lactone known as solidagenone (**1**) which has been previously isolated from *Solidago canadiensis* L. (Anthonsen, 1966) and recently evaluated by its antiproliferative potential (Gomes et al., 2018). The sesquiterpene-dialdehyde polygodial (**2**) was found to be responsible for *D. winterii* hexane extract activity, in accordance with previous reports (Muñoz-Concha et al., 2007; Derita and Zacchino, 2011). Two flavonoids [a flavanone, pinostrobin (**3**) and a chalcone, flavokawin B (**4**)] were isolated as the bioactive compounds of *P. stelligerum* ethyl acetate extract, which were previously isolated from *Boesenbergia pandurata*, *Myrica pensilvanica*, *Polygonum* spp.

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https://doi.org/10.1016/j.bjp.2019.05.007

Table 1
Antifungal activities of compounds isolated from *Solidago chilensis*, *Drimys winteri* and *Polygonum stelligerum* against four post-harvest phytopathogenics fungi: Minimum Inhibition Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC) are expressed in μg/ml.

Compound	MICs/MFCs (μg/ml)			
	Pd	*Bc*	*Mf*	*Rs*
Solidagenone (**1**)	1000/I	1000/I	31.2/125	62.5/250
Polygodial (**2**)	250/1000	250/1000	31.2/125	31.2/125
Pinostrobin (**3**)	1000/1000	1000/1000	62.5/125	62.5/125
Flavokawin B (**4**)	1000/1000	1000/1000	62.5/125	62.5/125
IMZ	3.9/3.9	0.5/0.5	0.12/0.12	7.8/7.8
CBZ	15.6/15.6	0.12/0.25	0.5/0.97	31.2/62.5

Pd: P. digitatum CCC-102; *Bc*: *B. cinerea* CCC-100; *Mf*: *M. fructicola* INTA-SP345; *Rs*: *R. stolonifer* LMFIQ-317.
Commercial antifungals imazalil (IMZ) and carbendazim (CBZ) were used as positive controls. I: inactive (MIC or MFC > 1000 μg/ml).

and *Piper* spp. (Burke and Nair, 1986; Hodgetts, 2001; López et al., 2006; Derita and Zacchino, 2011).

Materials and methods

Plants were collected, mostly, from farms and side of roads in areas surrounding litoral region of Santa Fe province (Argentina), between March 2015 and February 2016. Each vegetal material was identified by Marcos Gabriel Derita and a voucher specimen was deposited at the Herbarium of the FCA-UNL Ärturo Ragonese (SF Herbarium), Kreder 2805-(3080HOF)-Esperanza, Argentina (Supporting information and materials, Table 1). After collected, plants were dried and separated in leaves, flowers, fruits, bark or the whole plant according to the extracts that had to be prepared. Air-dried samples (100 g) were powdered and successively macerated (3 × 24 h each) with hexane, ethyl acetate and methanol or ethanol, using mechanical stirring to obtain the corresponding extracts, after filtration and evaporation.

For the evaluation of the antifungal activity, standardized strains from the Micology Reference Center (CEREMIC, Rosario, Argentina), the National Institute of Agricultural Technology (INTA, San Pedro, Argentina) and the Department of Microbiology of the Chemical Engineering Faculty, National University of Litoral (UNL, Santa Fe, Argentina), were used. The inocula of spore suspensions were obtained according to the Clinical & Laboratory Standards Institute (CLSI, 2008) reported procedures and adjusted to 1×10^4 Colony Forming Units (CFU)/ml. Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC) values were determined by using broth microdilution techniques according to the CLSI guidelines for filamentous fungi (document M38-A2, CLSI 2008). Commercial antifungal agents' imazalil (IMZ) and carbendazim (CBZ) were used as positive controls.

The three most active extracts were submitted to column chromatography and preparative thin layer chromatography in order to obtain the compound/s responsible/s for their antifungal activities.

Results and discussion

Results showed that at least one extract of each selected plant was active against at least one pathogen of the panel. Some of them were selective and displayed activities only against one fungus but some others demonstrated to be active against the four pathogens under study. Most of them proved to be fungistatic (MIC ≤ 1000 μg/ml) and some of them were also fungicides (MFC ≤ 1000 μg/ml).

Hexane and ethyl acetate extracts of *S. chilensis* were active against *M. fructícola* and *R. stolonifer* (MIC between 62.5 and 250 μg/ml; MFC between 500 and 1000 μg/ml), slightly active against *B. cinerea* (MIC = 1000 μg/ml) and inactive against *P. digitatum*; while its ethanolic extract and lyophilization product were fungistatic and fungicidal against *P. digitatum* (MIC = 250 μg/ml and MFC = 1000 μg/ml) and less active against the rest of the fungi. Hexane extract of *D. winterii* showed significant activity against *M. fructícola* and *R stolonifer* (MIC and MFC between 62.5 and 250 μg/ml) and displayed moderate growth inhibition of *P. digitatum* and *B. cinerea* (MIC = 500 μg/ml). Regarding *C. officinalis*, all its extracts showed high antifungal potency against *M. fructícola* (MIC between 125 and 500 μg/ml and MFCs between 500 and 1000 μg/ml); the ethyl acetate extract inhibited the growth of *P. digitatum* (MIC = 500 μg/ml) and the hexane extract showed MIC = 125 μg/ml for *R. stolonifer*. Hexane extract of *E. japonica* fruits was active against all the fungi evaluated except *B. cinerea*, displaying MIC and MFC between 250 and 1000 μg/ml, while the rest of the extracts slightly inhibited the growth of *M. fructícola* with MIC = 1000 μg/ml. Among the extracts of *E. japonica* seeds, the most active ones were hexane and ethyl acetate with MIC and MFC between 250 and 1000 μg/ml, while the methanolic extract scarcely inhibited the growth of *R. stolonifer* with MIC = 1000 μg/ml. *M. azadirachta* flowers and fruits extracts were moderately active against *M. fructícola* and *R. stolonifer* (MIC and MFC between 500 and 1000 μg/ml), except for the ethyl acetate extract of flowers that presented a greater spectrum of action, inhibiting the four fungi of the panel with MICs between 500 and 1000 μg/ml. All the extracts of *S. grisebachii* flowers showed important fungistatic activities against the four pathogens of the panel, with MICs between 250 and 1000 μg/ml; although its fungicidal activity was poor, this plant constitutes one of those with the widest spectrum of fungistatic action among all evaluated in this work. Regarding *P. caerulea* fruits, both extracts were selectively active against *B. cinerea* with MIC and MFC between 500 and 1000 μg/ml. Hexane and methanolic extracts of *E. grandiflorum* flowers showed moderate antifungal activity against *B. cinerea* and *M. fructícola* with MIC and MFC between 125 and 1000 μg/ml. Hexane extract of *C. intybus* was active against *B. cinerea* and *M. fructícola* displaying MIC and MFC between 125 and 1000 μg/ml; while the methanolic extract was only fungistatic against the same fungi with MIC between 500 and 1000 μg/ml. *P. madagascariensis* ethyl ether extract, was found to be active against the whole panel inhibiting the growth of the four fungi with MIC between 125 and 1000 μg/ml. Regarding *P. stelligerum*, all the extracts were at least fungistatic against all de fungi tested (MIC between 62.5 and 1000 μg/ml) being the ethyl acetate extract the most active particularly against *M. fructícola* and *R. stolonifer* (MIC = 62.5 and MFC = 125 μg/ml). The hexane and ethyl acetate extracts of *T. aeranthos* showed moderate fungistatic activity with MIC between 500 and 1000 μg/ml against *P. digitatum*, *B. cinerea* and *M. fructícola*, but were inactive against *R. stolonifer*. Finally, *C. bonariensis* hexane extract was inactive against *B. cinerea* and *P. digitatum* but slightly fungicidal against *M. fructícola* and *R. stolonifer* (MFC = 1000 μg/ml).

Antifungal activities of pure compounds isolated through bioguided fractionation of the most active plant extracts are shown in Table 1. Hexane extracts of *S. chilensis* and *D. winterii*, led to the isolation of solidagenone (**1**) and polygodial (**2**), respectively. From *P. stelligerum* ethyl acetate extract, pinostrobin (**3**) and flavokawin B (**4**) were obtained. Compound **1** was not only fungistatic (MIC = 31.2 μg/ml and 62.5 μg/ml for *M. fructícola* and *R. stolonifer* respectively) but also fungicide (MFC = 125 μg/ml and 250 μg/ml for the same fungi); meanwhile it barely inhibited the growth of *P. digitatum* and *B. cinerea* with MIC = 1000 μg/ml. Compound **2** showed the highest antifungal activity among all, with MIC = 250 μg/ml and MFC = 1000 for both fungi *P. digita-*

tum and *B. cinerea*, two of the main pathogens that greatly affect post-harvested citrus and berries. Polygodial (**2**) also showed interesting activities against *M. fructícola* and *R. stolonifer* with MIC = 31.2 μg/ml and MFC = 125 μg/ml, being the most promising compound to go on with *ex vivo* and *in vivo* studies. In addition, both flavonoids **3** and **4** displayed the same activities against the four fungi evaluated, resulting specific on the inhibition of *M. fructícola* and *R. stolonifer* with MIC = 62.5 μg/ml and MFC = 125 μg/ml.

After this evaluation, we can state that plants which have been shown to be antifungal against human pathogens or those that are used in traditional medicine for treatments associated with fungal infections could also have a very promising use as fruit health controllers. In particular, the species that have been most active in this work (*S. chilensis*, *D. winterii* and *P. stelligerum*) are being studied more deeply in terms of their application in *ex vivo* models and formulation technologies.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

MGDL, MIS (PhD students) and MGD contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. LAS and MGD contributed to biological studies, chromatographic analysis and supervised the laboratory work. All the authors contributed to the critical reading of the manuscript and have read the final manuscript and approved the submission.

Conflict of interest

All authors have none to declare.

Acknowledgment

The authors gratefully acknowledge to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) for financial support (PIP N° 2015-0524, PICT N° 2015-2259 and Nº 2016-1833). MGDL and MIS are also thankful to CONICET for their fellowships.

Appendix A Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.bjp.2019.05.007.

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