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SHORT COMMUNICATION

Flavonoids from *Plinia cauliflora* (Mart.) Kausel (Myrtaceae) with antifungal activity

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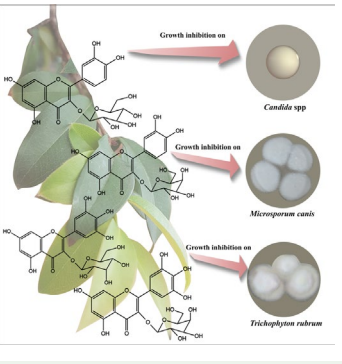
ABSTRACT

Plinia cauliflora is a plant species with edible fruits but its chemical composition is not totally known yet although former studies showed its potential as antifungal agent. This work aimed the chemical analysis of the leaves, the activity against fungi species and evaluated cytotoxicity. Extract was obtained with 70% ethanol. An ethyl acetate fraction was obtained and glycosylated quercetin and myricetin were isolated. Samples were tested against *Candida* species, dermatophytes and entomopathogenic fungi. Cytotoxicity was evaluated against fibroblast cells. Extract showed good activity against *C. albicans* (minimum inhibitory concentration at 156 µg/mL), *C. parapsilosis* (78 µg/mL), *C. krusei* (19 µg/mL), *Trichophyton rubrum* (78 µg/mL) and *Microsporum canis* (156 µg/mL). Isolated compounds were more active against *C. krusei* and *T. rubrum*. The extract, which was the more active sample, demonstrated low cytotoxic effect and encourage more studies against rising non-*albicans* species and dermatophyte *T. rubrum*.



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1. Introduction

Fungal diseases greatly increased their incidence and mortality along of the twentieth century. That increasing is due mainly to the growing number of immunodeficiency caused by AIDS, cancer, diabetes, old age and transplants (Alangaden 2011). Invasive infections are the most aggressive kind of disease which principal pathogens are *Candida albicans* and *Aspergillus* spp (Denning and Hope 2010). Onychomycosis and tinea pedis cannot be forgotten where *Trichophyton rubrum* and the zoophilic *Microsporum canis* play as the most common dermatophytes (Seebacher et al. 2008).

Our group demonstrated the presence of antifungal properties in the extractives from the leaves of *Plinia cauliflora* (Mart.) Kausel (Myrtaceae) (Souza-Moreira et al. 2013), a widespread tree with edible fruits in Brazil (Lorenzi 2000).

Looking for new sources of antifungal compounds, the aim of this study was to determine the activity of extract, fraction and isolated flavonoids from the leaves of *P. cauliflora* against *Candida* and filamentous fungi species and to evaluate their cytotoxicity.

2. Results and discussion

Despite the wide availability of *P. cauliflora* in Brazil and fruit composition description (Reynertson et al. 2006), the flavonoid and tannin presence in the leaves were just recently described (Souza-Moreira et al. 2011, 2013). After analysis of the ethyl acetate fraction (EAF) we could elucidate the presence of quercetin gluco- and galactopyranosides in mixture and isolate myricetin galacto- and myricetin allopyranosides. Compounds quercetin-3-*O*-β-glucopyranoside and quercetin-3-*O*-β-galactopyranoside (represented as compound **1** and **2**, Figure S1 in Supplementary material) were identified from a yellow powder mixture. The ^{1}H NMR spectrum of those compounds showed signals in the region of δ_H 6.19 and δ_H 6.40, which are characteristics of two meta-coupled protons H-6 and H-8 from flavonoidic A ring. Typical signals of flavonoidic B ring were observed in the region of δ_H 6.84 (H-5′), δ_H 7.56 (H-6′), and δ_H 7.55 (H-2′). Furthermore, two anomeric protons were detected at δ_H 5.35 and δ_H 5.43. Based on 2D-NMR (TOCSY, HMQC and HMBC) experiments the linkage and identity of each sugar unity was proposed to be 3-*O*-β-glucopyranoside and 3-*O*-β-galactopyranoside. Additionally, compounds myricetin-3-*O*-β-galactopyranoside and myricetin-3-*O*-β-allopyranoside (compounds **3** and **4** in Figure S1) were isolated. Typical signals of a myricetin nucleus were observed at δ_H 6.37 (H-8), δ_H 6.17 (H-6), δ_H 7.22 (H-2′ and H-6′). The sugar unities for each compound were shown at δ_H 5.27 and δ_H 5.18. 2D-NMR data was compared to literature shifts and allowed us to propose the complete assignments of the compounds (Wu et al. 2013). Reports on leaves composition focus on the essential oil composition and phenolic content (Apel et al. 2006; Duarte et al. 2010). Previous works from our group described the presence of flavonoids (Souza-Moreira et al. 2010, 2011), besides tannin derivatives as casuarinin (Souza-Moreira et al. 2013). After a deeper analysis on the EAF we were able to elucidate the presence of quercetin gluco- and galactopyranosides in mixture and isolate myricetin galacto- and myricetin allopyranosides. The presence of quercetin and myricetin was already described in the fruits of *P. cauliflora* (Reynertson et al. 2006, 2008), but they were not previously described in the leaves.

Hydroalcoolic crude extract (CE) from leaves, EAF and isolated flavonoid samples showed antifungal activity (Table S1). CE was more effective than the fraction and isolated

compounds and did not show cytotoxicity against the fibroblast cell line under evaluation in this work (Table S1). Nonetheless, the glycosylated myricetin compounds (**3** and **4**) showed better activity against *Candida* and filamentous fungi species, especially against *C. krusei* and *T. rubrum* than the glycosylated quercetin mixture (not completely separated compounds **1** and **2**). The susceptibility of the filamentous fungi *Aspergillus niger*, *Beauveria bassiana*, *Metarhizium anisopliae* and *Trichoderma reesei* were also evaluated but minimum inhibitory concentration (MIC) was not achieved at the maximum concentration tested (data not shown) demonstrating specificity in the activity against *T. rubrum* the main agent involved in dermatoses.

Activity of hydroalcoolic extract obtained from the leaves of *P. cauliflora* was already reported against *C. albicans*, but there was not active compound isolated (Diniz et al. 2010; Chavasco et al. 2014). The antifungal susceptibility of *C. albicans*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* to CE and EAF was shown in a previous study of our group (Souza-Moreira et al. 2013). EAF activity against *C. krusei* at that time motivated the new analysis in the present work. The activity of the samples studied, mostly for CE, was remarkable against *C. parapsilosis* and *C. krusei*. Some epidemiological studies show that among the increasing number of cases of candidemia due non-*albicans* species, the *C. parapsilosis* species is the most common worldwide and *C. krusei*, an intrinsic azole resistant species, is being frequently detected in infections (Quindós 2014).

Different flavonoids were found to be active against *C. albicans* and *C. krusei* (Orhan et al. 2010), while myricetin-3-*O*-β-galactopyranoside was recently described as promising antibacterial and antifungal activity was reported against *C. parapsilosis* and *Cryptococcus neoformans* (Djouossi et al. 2015). However, it is interesting to note that isolated compounds were less active than the hydroalcoholic extract and ethyl acetate fraction what can be due to a synergistic effect among the secondary metabolites present in the leaves as the flavonoids. It is important to note that combination of some different secondary compounds sometimes are more advantageous than their individual use probably because of the different mechanism of action involved against the microorganism (Wagner and Ulrich-Merzenich 2009).

3. Conclusions

It is interesting to note that this is the first description on the identification of two different quercetin and myricetin derivates in the leaves of *P. cauliflora*. Moreover, it is noteworthy that the crude extract of the leaves had higher activity at non-cytotoxic concentration despite the isolated compounds. This data might be useful to direct the use of the extract of leaves of *P. cauliflora* as antifungal agent due its efficiency and more accessible economically.

Supplemental material

Experimental section of this article is available online as well as Figure S1 and Table S1.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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