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Antifungal Activity of extracts from two *Ouratea* species on *Candida albicans*

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ABSTRACT

Plants are invaluable sources of pharmaceutical products and Brazil, in particular, has yielded an incredible array of plant and animal products that have drawn the attention of ethnopharmacologists from around the world. *Ouratea* is a genus of tropical plants within the Ochnaceae. *Ouratea* is widespread throughtout the tropics, with species in Costa Rica, Brazil, Jamaica, and Kenya. The search for compounds with antimicrobial activity is urgent and indispensable for the treatment of infectious diseases caused by microorganisms resistant to traditional antimicrobial drugs. A screening was conducted with organic extracts from two species, from roots and leaves of *Ouratea hexasperma* var. planchonii Engl. and leaves and branches of *Ouratea parviflora* Engl., to identify plant extracts with antifungal properties against *Candida albicans*. Initially, minimal inhibitory concentrations (MICs) were determined in *C. albicans* cellular suspension in Sabouraud medium, in a range of 0.060-3.0 mg/ml. Fractions OHRMWE (Ethyl acetate fraction) and OHLMWB (Buthanol fraction) obtained by solvents partition of methanol:water extract from *Ouratea hexasperma* with ethyl acetate from the roots and *n*-butanol from the leaves, respectively, showed a higher antifungal activity. The antifungal activity of these extracts was also compared in growth delay of *C. albicans* cultures.

INTRODUCTION

The genus *Ouratea* (Ochnaceae) comprises *ca* 300 tropical species occurring mainly in South America (Heywood, 1978). Some species of this genus have been shown to possess antiviral (*Ouratea lucens*, from Panama) (Roming *et al*., 1992), antimicrobial (*O. parviflora*, from Brazil) (Paulo *et al.*, 1986), as well as pain relief (*O. reticulata*, from Guinea) activities (Vasileva, 1969). Previous phytochemical and pharmacological investigations on *Ouratea* species have shown the presence of terpenoids, isoflavonoids, flavonoid glycosides, and more frequently biflavones which are considered as chemical markers for the genus (Suzart *et al.*, 2007). DNA topoisomerase inhibition, cytotoxic and antitumoral activities of biflavonoids (Carvalho *et al.*, 2002; Grynberg *et al.*, 2002; Daniel *et al*., 2007) have been described as well as other pharmacological activities of extracts from *Ouratea* species (Cortes *et al*., 2002; Gonçalves *et al*., 2001; Sampaio and Oliveira, 1975). Extracts of some plants are toxic to fungi and may be useful for controlling fungal growth and mycotoxin production (Steinhart *et al*., 1996). Substances isolated from plants such as flavonoids, isoflavonoids and biflavonoids, besides other activities, have shown activity against some aspects of fungal metabolism (Weidenborner *et al*., 1990; Weidenborner *et al*., 1989; Mallozzi *et al*., 1996; Norton, 1999). The utilization of extracts and natural compounds have been substituted of conventional treatment against microorganisms in order to prevent contamination has been considered because some flavonoids are biologically active (Gonçalves *et al*., 2001). Oral candidiasis is a significant infection in patients being treated with chemotherapy and radiotherapy for cancer, and in patients who are immunocompromised because of HIV infection and AIDS. *C. albicans* is the most common fungal pathogen and has developed an extensive array of recognized virulent mechanisms that allows successful colonization and infection of the host under suitable predisposing conditions (White *et al*., 2002).

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Antimicrobial resistance is a growing problem that complicates the treatment of important nosocomial and community-acquired infections. In the last years, resistance of *C. albicans* is increasing against traditional antifungal, such as fluconazole (Goldman *et al*., 2004; Briona *et al*., 2007; Ribeiro and Rodrigues, 2007). In the present study, we have evaluated the antifungal activity of different extracts from *O. hexasperma* and *O. parviflora* on *C. albicans*.

MATERIAL AND METHODS

General

Absorption spectra were recorded on a Shimadzu UV-2401PC spectrometer. All the chemicals from Aldrich (Milwaukee, WI, USA) were used without further purification. Solvents (GR grade) from Merck were distilled. Ultrapure water was obtained from Labconco (Kansas, MO, USA) equipment model 90901-01.

Plan Materials

The leaves and roots of *O. hexasperma* var. planchonii Engl. were collected in the sandbank area, Jacunã beach, Conde city, Paraíba state, Brazil. It was identified by Prof. Dra. Maria de Fátima Agra. Voucher specimen (Nº 6747) is deposited at the Prof. Lauro Pires Xavier (JPB) Herbarium, UFPB, João Pessoa-PB, Brazil.

The leaves and branchs of *O. parviflora* Engl. were collected in the Ouro Preto city, Minas Gerais state, Brazil by Profª Drª Alceni Augusta Werle, Dptº de Química, ICEB, UFOP. It was identified by Profº Dr. Elsie F. Guimarães. Voucher specimen (Nº RB 44,334) is deposited at the JBRJ Herbarium, Jardim Botânico, Rio de Janeiro-RJ, Brazil.

Extracts

The dried and powder leaves (2.127 kg) and roots (3.133 kg) of *O. hexasperma* were extracted with methanol and methanol:water (80:20) at room temperature and the solvents was removed under vacuum to afford 377.8 g of leaves methanol extract and 209.8 g of roots methanol extracts, besides afforded 170.0 g of leaves methanol:water extract and 160.5 g of roots methanol:water extract.

The leaves methanol extract was partitioned into dichlorometane, ethyl acetate and methanol:water (90:10) to afford the dichlorometane fraction (OHLMD), the ethyl acetate fraction (OHLME) and the methanol fraction (OHLMM). The leaves methanol:water (80:20) extract was partitioned into dichlorometane, ethyl acetate, n-buthanol and methanol:water (90:10) to afford the dichlorometane fraction (OHLMWD), the ethyl acetate fraction (OHLMWE), the buthanol fraction (OHLMWB) and the methanol fraction (OHLMWM). The roots methanol extract was partitioned into ethyl acetate e methanol:water (90:10) to afford the ethyl acetate fraction (OHRME) and methanol fraction (OHRMM). The roots methanol:water (80:20) was partitioned into ethyl acetate, n-buthanol and methanol:water (90:10) to afford the ethyl acetate fraction (OHRMWE), the buthanol fraction (OHRMWB) and the methanol fraction (OHRMWM). The dried and powder leaves (821.0 g) and branches (293.8 g) of *O. parviflora* were extracted with methanol at room temperature and the solvent was removed under vacuum to afford 326.0 g of leaves methanol extract (OPLM) and 37.5 g of branches methanol extract (OPBM).

Microorganism and growth conditions

C. albicans strain PC31, recovered from human skin lesion, was previously characterized and identified according to conventional procedures (Cormick *et al*., 2008; Harrigan and Mccance, 1986). Primary classification of colonies from plates was based on colony characteristic (pigmentation and shape), mode of vegetative reproduction, formation of pseudohyphae and ascopore production.

Identification of the yeast isolates to species level was done using the API 20C AUX (BioMérieux, Marcy l´Etoile, France) system of carbohydrate assimilation profiles. Strain of *C. albicans* was grown aerobically overnight in Sabouraud (Britania, Buenos Aires, Argentina) broth (4 ml) at 37 ºC to stationary phase. Viable *C. albicans* cells were monitored and the number of colony forming units (CFU) was determined on Sabouraud agar plates and ~48 h incubation at 37 ºC. This procedure produces ~10^7 CFU/mL after an overnight incubation.

Antimicrobial activity of extracts

After overnight incubation, the cells were appropriately diluted to obtain ~10^4 CFU/ml in Sabouraud broth. In all the experiments, 2 ml of the cell suspensions in Pirex brand culture tubes (13x100 mm) were used and the extracts were added from a stock solution ~10 mg/mL in *N*,*N*-dimethylformamide:water (1:1). The minimal inhibitory concentration (MIC) values were considered to be the lowest concentration of sample able to totally inhibit microbial growth (range 0.064-3.0 mg/ml). The MICs were determined after 48 h incubation at 37 ºC. Prior to the assays, it was verified that the loading solvent, *N*,*N*-dimethylformamide:water (1:1), was completely inactive against the test organisms under the assay conditions. Also, control experiments were carried out under the same conditions in the absence of extracts. Each experiment was repeated separately three times.

Growth delay of C. albicans

Cultures of *C. albicans* cells were grown overnight as described above. A portion (1 ml) of this culture was transfer to 20 ml of fresh Sabouraud broth medium. The suspension was homogenized and aliquots of 2 ml were incubated with different concentration of the extracts at 37 ºC. The culture grown was measured by turbidity at 660 nm using a Tuner SP-830 spectrophotometer. In all cases, control experiments were carried out in the absence of the extracts. Each experiment was repeated separately three times.

STATISTICAL ANALYSIS

All data were presented as the mean±standard deviation of each group. Variation between groups was evaluated using the Students *t*-test, with a confidence level of 95% ($p<0.05$) considered statistically significant.

RESULT AND DISCUSSION

Antimicrobial activity of extracts on C. albicans

The biological activity of extracts form various plant parts of two species *O. hexasperma* and *O. parviflora* were investigated on *C. albicans* cellular suspension in Sabouraud medium. The extracts were evaluated in a range of 0.060-3.0 mg/ml. The results of MICs are summarized in figure 1. As can be observed, OHRMWE (Ethyl acetate fraction) and OHLMWB (Buthanol fraction) showed a higher antifungal activity. Only this extracts from roots and leaves of *O. hexasperma* exhibited activity against the yeast *C. albicans*. Although the root wood extract with ethyl acetate from this species was more active than that from leaves with *n*-buthanol. In contrast, the other extracts were less effective to inactivate *C. albicans*.

Table. 1: Antifungal activity of *Ouratea hexasperma* (OH) and *Ouratea parviflora* (OP) extracts on *Candida albicans*.

Extracts*	Species	Plant part assayed	MIC (mg/ml)
OHLME	*Ouratea hexasperma*	leaves	2.500
OHLMD		leaves	1.250
OHRMWB		roots	1.250
OHLMM		leaves	1.250
OHLMWE		leaves	1.250
OHLMWM		leaves	1.250
OHRMWE		roots	0.500
OHLMWD		leaves	1.250
OHLMWB		leaves	0.625
OHRMWM		roots	2.500
OHRMM		roots	2.500
OHRME		roots	2.500
OPBM	*Ouratea parviflora*	branches	2.500
OPLM		leaves	2.500

*OHLME (Ethyl acetate fraction), OHLMD (Dichlorometane fraction), OHRMWB (Buthanol fraction), OHLMM (Methanol fraction), OHLMWE (Ethyl acetate fraction), OHLMWM (Methanol fraction), OHRMWE (Ethyl acetate fraction), OHLMWD (Dichlorometane fraction), OHLMWB (Buthanol fraction), OHRMWM (Methanol fraction), OHRMM (Methanol fraction), OHRME (Ethyl acetate fraction), OPBM (Methanol extract), OPLM (Methanol extract).

Taking into account these results, growth delay of *C. albicans* cultures produced by extracts OHRMWE and OHLMWB was carried out in Sabouraud medium. Thus, different amounts of extracts were added to fresh cultures of *C. albicans* reaching the log phase and the flasks were incubated at 37 ºC. Three concentrations of extracts, under and over MIC value, were analyzed under these conditions. As can be observed in Figure 1, growth was suppressed when *C. albicans* cultures were treated with both extracts using concentration identical or up than MIC. After 30 min of incubation in the presence of 0.4 or 0.5 mg/ml of OHRMWE, the cells no longer appeared to be growing as measured by turbidity at 660 nm. Under these conditions, the effect of OHRMWE is faster than that of OHLMWB. On the other hand, *C. albicans* cells incubated with a lower concentration of extracts (under MIC value) showed only a small growth delay compared with controls. Therefore, the data illustrate that the observed growth delay is due to the antimicrobial effect of these two extracts on the cells.

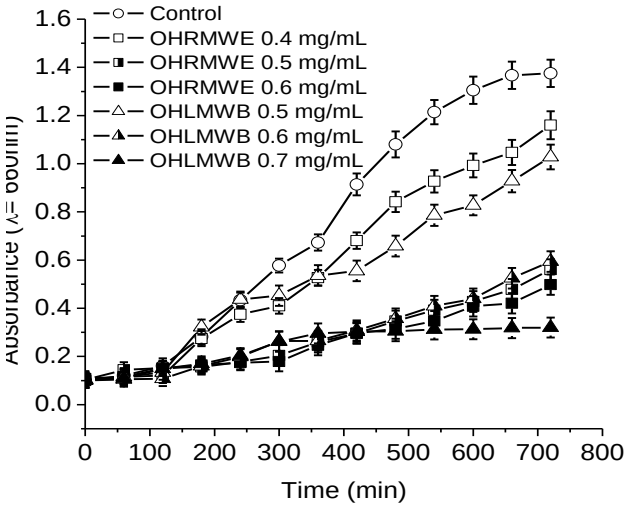

Fig. 1: Growth delay curves of *C. albicans* cells incubated with different concentrations of fractions OHRMWE (squares) and OHLMWB (triangles) in Sabouraud broth at 37 ºC. Control cultures: cells without extracts (○). Values represent mean±standard deviation of three separate experiments.

CONCLUSION

This preliminary screening is an interesting evaluation of the potential antifungal of *O. hexasperma* var. *planchonii* Engl. and *O. parviflora* Engl. against the strains of *Candida albicans*. Further studies need to be carried out to define active principle(s) of fractions and to study the relation between chemical structure and antifungal activity.

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