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Full Length Research Paper

Antifungal activity of residual medium and biomass of Basidiomycetes species cultivated in coconut water against C*andida albicans*

Ubiraney Dellamura Alencar[1] and Edmar Clemente[2]*

[1]Post-graduate Program in Comparative Biology, Universidade Estadual de Maringá, Av. Colombo, 5.790, Jd. Universitário, Maringá, PR, Brazil. Cep: 87020-900.
[2]Department of Chemistry, Universidade Estadual de Maringá, Av. Colombo, 5.790, Jd. Universitário, Maringá, PR, Brazil. Cep: 87020-900.

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Some species of Basidiomycetes represent a group of organisms that are commonly utilized in natural medicine, especially the families forming macroscopic basidioma. In order to evaluate alternative ways to combat *Candida albicans*, the *in vitro* antifungal effect of residual medium (RM), lyophilized residual medium (LRM) and biomass (B) of *Ganoderma lucidum*, *Lentinula edodes* and *Pleurotus ostreatus* grown on coconut water medium were evaluated. The pH, lipids, carbohydrates and proteins of the extracts were analyzed and the susceptibility of the yeast to extracts was also determined. Chemical analysis showed concentration differences among fungal species. Extracts, such as RM of *L. edodes* and RM and B of *P. ostreatus*, showed respectively, inhibitory activity of 50, 25 and 25%, after counting of cells in a Neubauer chamber. However, the other extracts did not show satisfactory results in the dosage used. Fungal extracts, especially those belonging to edible species, therefore with no side effects, may be ingested in higher concentrations and represent new therapeutic options for the treatment of candidiasis.

Key words: Antifungal activity, *Candida albicans,* Coconut water, *Ganoderma lucidum, Lentinula edodes, Pleurotus ostreatus*.

INTRODUCTION

The important role of fungi in the ecosystems and their applications in biotechnology remain in an incipient level. Estimates point out that only about 5% of the species are known and very little is known about their biology. The therapeutic properties of Basidiomycetes have been recognized in these millennia in China, Korea and Japan. Chinese earlier books on natural medicine products date back 2,000 years ago and listed more than 20 species of medicinal fungi (Kim and Kim, 1999; Mizuno, 1999).

Substances derived from plants and fungi have been extensively studied as source of natural medicine. Most of these researches report phytochemical compounds acting directly or indirectly in the immune system and in biochemical pathways, and altering the cellular response at the molecular level.

The knowledge acquired from the use of natural products, especially higher plants, microorganisms and animal toxins, has been fundamental in the discovery of new drugs in modern therapy. About 40% of medicines are available to treat current diseases which developed from natural sources, 25% being from plants, 13% from microorganisms and 3% from animals. In the case of anticancer drugs and antibiotics, this percentage has reached 70% (Cragg et al., 1997).

The species of the genus *Candida* are part of the normal mouth flora, being present in 30 to 70% of healthy subjects. This genus can also colonize other sites such

*Corresponding autor. E-mail: eclemente@uem.br. Tel: + 00 55 44 3261-3659; Fax: + 00 55 44 3261-5116.

as female genital tract and skin. The species *Candida albicans* is the most common in relation to other species as colonizing agent of dental prostheses (Darwazeh et al., 2001).

With the significant increase in the incidence of invasive fungal infections during the last decade, especially in patients with cancer, SIDA, or hospitalized for a prolonged period in intensive care units, there is a need for research of new antifungal agents with superior quality to those existing ones.

In this sense, the use of fungal extracts is an important therapeutic alternative in terms of economic, safety and pharmacological efficacy. They have many pharmaceutical properties such as anti-inflammatory, antibacterial and antifungal activity, yeasts *Candida albicans* (Burdock, 1998; Imhof et al., 2005).

This study aimed to evaluate the *in vitro* susceptibility of *Candida albicans* to extracts from the residual medium and biomass of *Ganoderma lucidum* (Fr.) Karst*, Lentinula edodes* (Berk.) Pegler and *Pleurotus ostreatus* (Jacq.) Quélet cultivated on coconut water medium.

MATERIALS AND METHODS

Biological material

The antifungal activity of three Basidiomycetes species: *G. lucidum, L. edodes* and *P. ostreatus* was evaluated against yeast *Candida albicans*. Basidiomycetes isolates were acquired from the Mycology Laboratory of the Department of Biology and preserved on PDA medium at the temperature of 6°C.

C. albicans was obtained from the culture bank of the Medical Mycology Sector of the Laboratory of Education and Research in Clinical Analysis (LEPAC), State University of Maringá, Paraná, Brazil. The isolate was cultured on Sabouraud Dextrose Broth (SDB) (Difco, USA) with 10% glycerol and stored under refrigeration at -20°C.

The medium used for submerged culture of the fungi mentioned above was made on the basis of coconut water “in natura”. Coconut water is a natural isotonic existing in the cavity of coconut seed; it is rich in nutrients and very important in seed germination and seedling survival.

This liquid corresponds to approximately 25% of fruit weight, and its basic composition has 93% of water, 5% of sugars, besides proteins, vitamins and minerals, being a soft and refreshing drink and having low calories. The average chemical composition, in the optimal period to harvest the fruit, is as follow: pH (4.8), calories (18.1), acidity (1.3 ml/100 mL), Brix degree (21°C) 7.0, glucose (4.4 g/100 ml), protein (0.37 mg/ml), phosphorus (6.2 mg/100 ml); potassium (175 mg/100 ml), calcium (17.5 mg/100 ml), magnesium (8.5 mg/100 ml), sodium (10.5 mg/100 ml), iron (0.06 mg/100 ml), vitamin C (57 mg/100 g) (Aragão, 2004).

Production of fungal biomass

The coconut from the State of Bahia was purchased from the fruit market of Maringá County, Paraná State. The coconut water was collected and used as medium for basidiomycetes in a submerged culture. This information is confidential and has patent under number PI 1004379-9.

Obtaining of extracts

This information is confidential under patent number PI 1004379-9.

Chemical analysis

The pH of all analyses were measured using a pH meter, model pH 300 (Hanna Instruments). The readings were expressed in pH units according to the method of AOAC (1992). The pH determination of coconut water in natura was made before and after autoclaving the residual liquid medium (MR) and the biomass (B) after 30 days of growth in the culture medium of coconut water.

The total sugars (TS), total lipids (TL) and crude protein (CP) of the residual culture medium and biomass of basidiomycetes were measured following the methods proposed by IAL (2005) and Silva and Queiroz (2002).

Susceptibility assay

The susceptibility tests were performed using microdilution method in Mueller Hinton Broth (MHB) (Difco, BD, USA). These were done in two sterile plastic microplates (TPP Zellkultur TestPlate 96f, Switzerland) containing 96 wells arranged in series labeled A through H, each one with twelve wells numbered 1 to 12. Each row (A-H) corresponded to basidiomycetes extracts as follows: *G. lucidum* - residual medium (A), biomass (B), lyophilized and diluted residual medium (C); *L. edodes* - residual medium (D), biomass (E), lyophilized and diluted residual medium (F); and *P. ostreatus* - residual medium (G), biomass (H).

For testing of extracts, aliquots of 100 µL of MHC were distributed from the 2nd column up to the 10th column. Column number 1 received 200 µL aliquots from each extract and from this a dilution was carried out at a rate of two until the tenth well. Then 100 µL of the measured inoculum were diluted from well one to the tenth well. Thus, the concentrations of tested extracts ranged from 5 to 0.004 mg/ml.

For each sample, negative (only MHC) and positive controls (MHC and inoculum of *C. albicans* without addition of extracts) of growth and possible contamination were included. The plates were incubated in a chamber at 35°C for 48 to 72 h with daily monitoring. After 72 h, the test reading was made by visual comparison through reflection in a mirror.

Table 1. Chemical compositions of biomass of *G. lucidum, L. edodes* and *P. ostreatus*, grown on coconut water medium after 30 days (n=3).

Biomass	Lipids (%) ±δ	Carbohydrates (%) ±δ	Total proteins (%) ±δ
G. lucidum	3.01 ± 0.04	40.41 ± 0.86	28.50 ± 0.71
L. edodes	2.09 ± 0.02	23.91 ± 0.18	24.00 ± 0.00
P. ostreatus	1.96 ± 0.10	26.76 ± 0.30	24.00 ± 1.41

n: number of repetitions; δ = standard deviation.

Table 2. Chemical compositions of residual medium of *G. lucidum, L. edodes* and *P. ostreatus* grown on coconut water after 30 days (n=3).

Residual Medium	Lipids (%) ±δ	Carbohydrates (%) ±δ	Total proteins (%) ±δ
G. lucidum	1.72 ± 0.22	29.21 ± 0.56	11.70 ± 0.71
L. edodes	1.93 ± 0.01	17.66 ± 0.51	13.81 ± 0.27
P. ostreatus	1.90 ± 0.01	13.75 ± 0.46	13.26 ± 0.35

n: number of repetitions; δ = standard deviation.

Table 3. Colony forming unit of *C. albicans* (cfu/mL) treated with basidiomycetes extracts (500 mg/ml).

Basidiomycetes	UFC/mL
Positive control	1.5×10^8
Negative control	0
G. lucidum- RM*	1.3×10^8
G. lucidum- B**	1.2×10^8
G. lucidum- MLR***	1.5×10^8
L. edodes- RM	5.0×10^4
L. edodes- B	1.5×10^8
L. edodes- MLR	2.0×10^6
P. ostreatus- RM	1.2×10^6
P. ostreatus B	1.3×10^6

RM*: residual medium; MLR***: middle lyophilized residual; B**: biomass.

The minimum inhibitory concentration (MIC) of the fungal extracts was determined by considering the lowest concentration which is able to inhibit visible growth of yeast, taking as reference its respective positive control.

RESULTS

Chemical compositions

The pH of the coconut water "in natura" (4.67) and after sterilization (4.74) did not significantly differed from each other. After 30 days of mycelial growth, the residual medium of *L. edodes* turned to slightly acidic (4.33), while *G. lucidum* and *P. ostreatus* had pH of 6.06 and 6.05, respectively.

The amount of lipids, carbohydrates and total proteins found in fungal biomass showed dependent species. *G. lucidum* contained higher levels of the substances analyzed. The levels of lipids and proteins in the biomass of *L. edodes* and *P. ostreatus* were similar, differing in carbohydrate contents (Table 1). The contents of lipids and proteins were lower in the growing residual medium of *G. lucidum*. In relation to carbohydrate content, differences occurred in the residual medium of the three species, being higher in the cultivation of *G. lucidum*, followed by *L. edodes* and *P. ostreatus*. These two species showed similar levels of protein and lipids (Table 2).

Susceptibility of *Candida albicans* to extracts

The extracts of the residual medium (RM) of *L. edodes* in the concentration used (500 µg/mL) inhibited the growth of *C. albicans* by 1,000 times, that is, had the minimum inhibitory concentration (MIC_{50}) capable of inhibiting over 50% of the isolates. However, extracts of lyophilized residual medium (LRM) of *L. edodes* and of residual medium (RM) and biomass (B) of *P. ostreatus* inhibited 100 times MIC_{50} which was able to inhibit 25% of the isolates. Extracts of *G. lucidum* (RM, B and LRM) and of *L. edodes* (B) in maximum concentration of 500 µg/mL were unable to cause any inhibition of yeast. The antifungal activity of extracts of the species studied on the yeast *C. albicans* was performed in microdilution plates (Table 3).

DISCUSSION

The pH values of coconut water found in this study do not differ with the value (4.8) obtained by Assis et al. (1995). Sterilization did not alter the pH. Although the acidic pH of coconut water was not altered, the three fungal

species still developed. The increase in pH observed in the culture media of *G. lucidum* and *P. ostreatus* is due to the rapid growth of biomass, leading to death of the mycelium, which incorporated organic matter in the culture medium.

In relation to the lipids, carbohydrates and proteins, differences on the amount of contents of the three species were noted. This shows different physiological activity for each species, despite the three species being lignin eaters. These findings do not differ from other studies in the literature (Herrera and Domingues, 2001).

According to Yagiela (1999), there was no optimal antifungal therapy, that is, the agents applied were not 100% effective or the patients experienced side effects (toxicity, bad taste, etc.). The use of antibiotic such as Amphotericin B and other antifungal such as Nystatin, for example, was effective in controlling the yeast in *in vitro* studies; however, in *in vivo* studies, this efficacy has not been verified due to non-acceptance by patients (nausea and vomiting).

The alternative culture medium consisting of coconut water “in natura”, besides having low cost when compared to other industrial facilities, was effective in the production of biomass and in the modification of the medium, incorporating nutraceutical substances.

There is no consensus on the acceptable level of inhibition for natural products when compared to standard antibiotics; thus, some authors consider only results similar to antibiotics, while others consider them as good potential even those with higher levels of inhibition. Aligiannis et al. (2001) proposed a classification for natural products based on MIC results, as given: strong inhibition - MIC up to 500 µg/mL, moderate inhibition - MIC between 600 and 1,500 µg/mL, and weak inhibition - MIC greater than 1,600 µg/mL.

In this study, extracts of basidiomycetes had significant antifungal activity against the yeast of *C. albicans*, and the species of *L. edodes* presented MIC of 50% and *P. ostreatus* MIC of 25% at concentrations of 500 µg/mL.

Conclusion

Coconut water has proven to be an excellent alternative medium for submerged cultivation of fungi, and the extracts of *L. edodes* and *P. ostreatus* presented good performance for *in vitro* tests against the yeast of *C. albicans*, and may be included among the new options for treatment of candidiasis.

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