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Original article

Antifungal activity of *Camellia sinensis* crude extracts against four species of *Candida* and *Microsporum persicolor*

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ABSTRACT

Objective of the study. – Candidiasis and dermatophytoses are benign infections in humans and animals, but they are very dreaded diseases in immunocompromised individuals. These infections become resistant to different treatments which make them more dangerous. In this work, we tried to find a new way for treating them. So we were interested in the antifungal activity of *Camellia sinensis* (tea); this plant is known to have many health benefits.
Materials and methods. – We tested the ability of the acetone and aqueous crude extracts of the plant to inhibit in vitro the growth of *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei* and *Microsporum persicolor*. Then, the antifungal activity against these species was tested in vivo in mice.
Results. – The results showed that the acetone crude extract had the most important in vitro activity against all the fungi. But in vivo it was only the most active against *Candida albicans, Candida glabrata*, *Candida tropicalis* and *Microsporum persicolor*. *Candida krusei* was more sensitive to the aqueous crude extract.
Conclusion. – These results indicated that tea could be considered to treat infections caused by the five tested species.

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1. Introduction

Candidiasis and dermatophytoses are the most common human infections in the world [1]. They are generally treated with a wide variety of antifungal drugs [2], but they became increasingly resistant to different treatments, especially when they are caused by *Candida*, *Microsporum* and *Trichophyton* species [3,4]. These infections can be acute or chronic and in some cases they require a combination of several drugs [4,5]. But the treatments can show significant liver toxicity which limits their use [6]. Also, mycosis are very dangerous in immunocompromised people. In fact, they may take on dramatic proportions from patient to patient [7].

Camellia sinensis (tea) is one of the most popular beverages in the world [8]. The traditional medicine recommends this plant for several health benefits because it prevents and treats many human diseases: cancers, infections, cardiovascular and neurological diseases [9].

In this work, we tested the antifungal activity of tea against four *Candida* species known to be pathogenic to humans and animals (*Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Candida krusei*) and also against *Microsporum persicolor*. The activity was tested in vitro and in vivo for the acetone and the aqueous crude extracts of the plant.

2. Materials and methods

Camellia sinensis was bought from herbalists and identified at the Department of Botany, University of Jijel (Algeria).

The yeasts provided from the hospital of Jijel (Algeria) and the university hospital center of Constantine (Algeria). They were isolated from different patients.

2.1. Preparation of the crude extracts

The acetone crude extract was prepared by maceration of 10 g of plant in 100 mL of acetone/water (70/30, V/V) and the aqueous crude extract was obtained using 100 mL of sterile water. The maceration was done in an electric shaker during 2 hours. Then, the solvents were recovered by filtration using the Whatman No.1 paper and evaporated with the rotary evaporator. The obtained crude extracts were diluted in sterile water to obtain suspensions of 1 mg/mL [10,11].

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2.2. Preparation of the fungal suspensions

Four yeasts were tested: *Candida albicans*, *Candida glabrata*, *Candida tropicalis* and *Candida krusei*. Each one was cultured in Sabouraud's dextrose broth and incubated at 30 °C for 24 hours. Then, cells were filtered and rinsed with a normal phosphate-buffered saline suspension (PBS). The fungal suspensions were prepared and diluted with PBS to obtain 10^7 CFU/mL [3]. For the in vivo tests against the yeasts, the inoculum size sufficient to cause the death of untreated mice was 1.5×10^4 CFU/mL; it was determined in a preliminary experiment.

Microsporum persicolor was cultured in Sabouraud agar and incubated at 28 °C for 14 days. After this, a conidial suspension was prepared by submerging the mycelium surface with PBS and filtering conidia. The concentration applied was 10^7 conidia/mL [12].

2.3. In vitro antifungal activity

The crude extracts were diluted in distilled water to obtain different concentrations from 0.005 to 0.200 mg/mL. The fungal suspensions were inoculated on Petri dishes containing Sabouraud agar and different doses of the crude extracts. An incubation time of 24 hours at 30 °C was applied for yeasts. *M. persicolor* required 48 hours at 28 °C. The antifungal activity was expressed with growth or no growth and the minimum inhibitory concentration (MIC) was determined for each crude extract [11].

2.4. In vivo antifungal activity against yeasts

C57BL6 mice were immunosuppressed with 2 mg of cyclophosphamide given intraperitoneally during 4 days before infection, then on the third day post infection. The animals were kept in clean cages and served autoclaved food and water in order to maintain a sterile environment throughout the experiment [13]. The infections were induced intravenously and after 24 hours, the crude extracts were administered intravenously during 5 days at a concentration of 20 μg/mouse/day. We used groups of animals containing five mice each. The mortality was monitored daily [14]. As controls we used two groups of animals: the first was immunosuppressed, infected and not administered with the extracts. And the second one was not immunosuppressed, but infected and administered with 100 μg of the tested extract. The first group gave 100% of mortality and the second 0% of mortality.

2.5. In vivo antifungal activity against M. persicolor

C57BL6 mice were immunosuppressed by a subcutaneous injection of 500 μg of estradiol valerate 4 days before the infection. For each mouse, we shaved a little surface of skin and sprayed on it 10 μL of the fungal suspension. When the mycosis appeared, the crude extracts were orally administered during 5 days at a concentration of 20 μg/mouse/day diluted into 250 μL of distilled sterile water. As with the previous activity, the animals were kept in clean cages and served autoclaved food and water. Each group contained five animals. The skin lesions were scored on a scale from 0 (no visible lesions) to 3 (significant crusting and erythema) [12]. As controls we used two groups of animals: the first was immunosuppressed, infected and not administered with the extracts. And the second one was infected and administered with 100 μg of the tested crude extract, but it was not immunosuppressed. The first group showed significant crusting and erythema and in the second group, the infection did not appear.

The experiments were conducted according to the Algerian Association of Experimental Animal Sciences. Permission to use the animals was approved by the Department of Applied Microbiology and Food Sciences, Faculty of Natural and Life Sciences, University Mohammed Seddik Ben Yahia.

3. Statistical analysis

The mean values of the in vitro and in vivo antifungal activities were calculated using Microsoft Office Excel 2007. The in vitro antifungal activity experiments were repeated twice and the results were expressed as MICs mean value ± standard error of the mean (SEM). For the in vivo antifungal activity, we used groups of animals which contained five mice each. The results were obtained by calculating the percentage of mortality.

4. Results

4.1. In vitro antifungal activity

The lowest concentrations of the crude extracts that gave no growth were registered to all the species (Fig. 1). The results showed that the acetone crude extract was active against all the fungi. *Candida albicans* was the most sensitive species towards this extract, followed by *Candida glabrata* and *M. persicolor*. The aqueous crude extract had a good activity only against *Candida albicans*; all the other species required greater MICs (Table 1).

4.2. In vivo antifungal activity against yeasts

The in vivo activity against yeasts showed that the acetone crude extract was more active against *Candida albicans*, *Candida glabrata* and *Candida tropicalis* (Figs. 2–4). *Candida albicans* required the lowest dose to give 100% of viability, so it was the most sensitive species. But, the infection caused by *Candida krusei* was resistant even the administration of 100 μg of the acetone crude extract (Table 2).

On the other hand, the aqueous crude extract was only active against *Candida albicans* and *Candida krusei*: it gave 100% of viability with 60 μg and 100 μg respectively (Fig. 5). These experiments were repeated twice and gave exactly the same results.

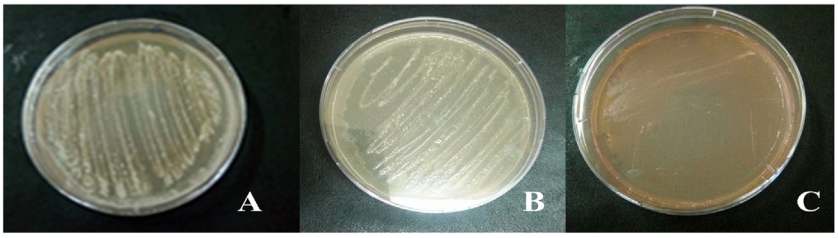

Fig. 1. Determination of the CMI for aqueous crude extract against *Candida albicans*. A. Medium with 0.005 mg/mL of the extract. B. Medium with 0.010 mg/mL. C. Medium with 0.020 mg/mL. The MIC was determined at 0.020 mg/mL.

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