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Original Article

Evaluation of Antimicrobial Activity of *Oudemansiella sp* (Basidiomycetes)

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Abstract

Antimicrobial activity of different culture extract of *Oudemansiella sp* grown on liquid medium (MYGP) were tested. Different fungi *(C. albicans, C. lipolytica, Saccharomyces cervisiea, Cladosporium herbarum, and Aspergillus niger)* and bacteria *(Microccocus luteus, E. coli, Staphylococcus aureus, and Staphylococcus epidermidis)* were used as test organisms.

Various antimicrobial assay methods including paper disc agar diffusion and microdilution method, were employed to determine possible activity of the extracts. Relative MIC values showed strong activity of the ethyl acetate extract of the fungus against tested bacteria and fungi, especially filamentous fungi.

Key word: *Oudemansiella sp*; Antimicrobial; Basidiomycetes.

Introduction

The need for less toxic, more potent and non anti - infectives antibiotics, as well as the evolving resistance of microorganisms are some of the medical areas that have posed a challenge to therapeutics since 1990s. A corresponding situation exists in the agricultural sectors. These factors have the combined effect of injecting a sense of urgency into the search for new bioactive compounds (1). The basidiomycetes (mushrooms) are valuable as gene pool sources, which have not yet been the subject of extensive screening for any possible antifungal and antibacterial activity (2).

Oudemansiella (Fam. *Tricholomatacea*) is a saprophytic fungus, found mostly growing on wood. Different antifungal agents have been isolated from the *Oudemansiella* genus. Oudemansin and mucidin were isolated from the *Oudemansiella radicata*, showing an antifungal activity (3). Antifungal activity of *Oudemansiella platyphylla* has also been reported. The fungus has shown activity against *C. albicans*, *C. tropicalis* and *A. fumigatus* (4).

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Experimental

Fungus material

The fungus was collected in November 1997 from the northern parts of Iran. Next, the fungus was identified as *Oudemansiella sp* and stored in the Department of Pharmacognosy at the School of Pharmacy, Shaheed Beheshti University of Medical Sciences (Voucher number 01- Ram 97).

Growth and preparation of extracts

Tissue culture was carried out, using the cap of the fungus (5). Mycelium of the fungus was cultured in a liquid medium containing 10 g/l glucose, 10 g/l malt extract, 3 g/l Yeast extract and mycological peptone. After 10 days the culture was filtered and filterate was separately extracted with different solvents including ethyl acetate, chloroform and petroleum ether respectively. Each extract was concentrated under reduced pressure. The condensed products

Table 1. The preliminary antimicrobial activity of *Oudemansiella Sp* (1mg/disc) culture extract

Strain	Ethyl acetate	Chloroform	Petroleum ether	Chloramphenicol	Nystatin
S. Aureus	8.6*	-	-	17	nt
S. epidermidis	12.3	9.2	-	14	nt
E. coli	9.2	-	-	15	nt
M. luteus	18.4	10.1	-	19	nt
C. albicans	10.5	8.8	9.1	nt	12
S. cervisiae	12.1	9.4	8.6	nt	18
C. lipolytica	11.1	9.1	8.7	nt	15

*mm zone of inhibition
nt: not tested

were weighed and kept at 4°C perior to testing.

Test microorganisms

Microorganisms including fungi and bacteria were obtained from the Persian Type Culture Collection (PTCC) as well as the American type culture collection (ATCC).

Candida albicans (PTCC 5027), *Candida lipolytica* (ATCC 825), *Saccharomyces cervisiea* (ATCC 9783), *Cladosporium herbarum* (School of Health, Tehran University), and *Aspergillus niger* (PTCC 5010), were used as the fungal tested organisms and *Micrococcus lutes* (ATCC 9273), *E. coli* (PTCC1330), *Staphylococcus aureus* (PTCC1112), and *Staphylococcus epidermidis* (PTCC1114) used as the bacterial tested organisms. For the bacterial suspension, 3×10^7 cell /ml was prepared, compared to that of the Mc Farland standard tube number 1. For the filamentous fungi and yeast-like fungi, a suspension containing 1×10^6 spores or cells per ml was prepared by using the Neubauer Counting Chamber.

Antimicrobial assay

Paper disc agar diffusion method

A series of 90 mm Petri dishes containing the Muller Hinton agar for the growth of bacteria and the malt extract agar for the growth of yeast-like fungi were prepared and each plate was separately inoculated with different cultures of the yeast like fungi and bacteria by swabbing aseptically on the whole surface of the agar with cotton wool. A 6 mm diameter filter paper disc was impregnated with 20 µl of each extract in absolute ethanol (50 mg/ml). The discs were air dried and placed aseptically at the center of the plates. The plates were left undisturbed for 1 h to allow the extract to diffuse into the agar. Chloramphenicol (0.050 mg) and Nystatin (0.050 mg), dissolved in absolute ethanol, were also impregnated onto the discs, air dried, and used as a positive control. The plates were incubated at the desired temperature. The growth inhibition, indicated by the areas of clear zone, was measured. Evaluation of the inhibitory properties was carried out in duplicates.

Microwell plate techniques

For the filamentous fungi, a 96 microwell plate was used. 20 µl of the spore suspension and 100 µl liquid culture medium (malt extract broth) were added to each well. 100 µl of the extracts in 10 % DMSO (100 mg/ml) was also added and plates were stored at 25°C for 48 h. Nystatin (100 mg/ml) was used as the positive control and DMSO served as a negative control. After microscopic examination, results were reported as the percentage of inhibition of spore germination (100%, 80%, 50%, 20% and 0%) (6, 7).

Minimum Inhibitory Concentration (MIC) measurement

The MIC value of extracts showing a strong activity was determined, using serial dilution method. Serial dilutions of each extract were individually placed in tubes labeled 1 to 6. Tube 1 was filled with 100 µl of the extract stock solution (200 mg/ml, 10% DMSO) . Only 50 µl of the stock solution in tube 1 was transferred to

Table 2. Inhibition of spore germination of the tested filamentous fungi (45µg/well)

Strain	Ethyl acetate	Chloroform	Petroleum ether	Nystatin
A. niger	80#	50	20	80
C. herbarum	100	50	20	100

percentage of inhibition of spore germination

tube 2 and diluted with 50 µl of a 10% DMSO solution. This procedure was repeated for solutions in tubes 2 to 6. Each tube was then filled with 100 µl muller hinton broth for bacteria and malt extract broth for the fungi and also 100 µl spore or bacterial suspension to obtain a serial dilution of the test materials (40, 20, 10, 5, 2.5 1.25 and 0.63 µg/µl). The resulting mixtures were then stirred thoroughly and incubated at 25°C for the fungi and 37°C for the bacteria, overnight. Nystatin (200 mg/ml) and chloramphenicol (200 mg/ml) were used as positive controls and DMSO served as a negative control. Turbidity was taken as an indication of growth and the lowest concentration which remained clear after macroscopic evaluation was recorded as the relative minimum inhibitory concentration. The MIC value was recorded as the mean concentration of duplicates.

Results and discussion

Extraction of 500 ml fungus culture filtrate gave the following yields: ethyl acetate extract (0.05% W/V), chloroform (0.02% W/V) and petroleum ether (0.01% W/V).

Results obtained from the controls indicate that solvents had no effect on the fungal and bacterial strains. Amongst the different extracts studied, only the ethyl acetate extract showed a strong activity. The extract showed high selectivity against M. luteus, exhibiting a relatively high inhibition zone diameter (18.4 mm) (Table 1). On the contrary, the efficacy of the extracts towards the various yeast like fungi was non- selective, as the inhibition zones showed little variation. The extracts also showed strong activity against the filamentous fungi (Table 2), by giving the lowest relative minimum inhibitory concentration values of 10 µg/µl with *C. herbarum* (Table 3).

The chloroformed extract of the fungus was particularly active against the tested microorganisms, especially filamentous fungi. A relative minimum inhibitory concentration values of 40 µg/µl with both *Cladosporium herbarum* and *A. niger* was observed. No strong activity was noted when petroleum ether used as the solvent for extraction. However, it is interesting to note that the extract showed activity against the tested fungi. Therefore, the possibility for presence of specificly active compounds within the petroleum extract of the fungus is rather high. The ethyl acetate extract has shown strong activity against spore germination of *C. herbarum* and *A. niger* with 100 % and 80 % inhibition, respectively. It is interesting to note that different extracts showed strong activity against spore germination of the tested microorganisms.

Table 3. MIC values of the active extracts against test organisms (µg/µl)

Strain	Ethyl acetate	Chloroform
S. epidermidis	40	na
M. luteus	10	na
C. albicans	40	na
C. cervisiae	20	na
C. lipolytica	40	na
A. niger	20	40
C. herbarum	10	40

na: no activity

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