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Short Communication

Antimicrobial potential of *Xylaria polymorpha* (Pers.) Grev.

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The ethanolic extracts obtained from the macrofungus *Xylaria polymorpha* (Pers.) Grev. (Ascomycetes) have been investigated for their antimicrobial activity. Growth inhibition using agar disc diffusion assays was determined against *Escherichia coli* ATCC 11230, *S. aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403, *Geotrichum capitatum* ATCC 28576, *Debaryomyces hansenii* DSM 70238, *Kluyveromyces fragilis* ATCC 8608 and *Cryptococcus neoformans* ATCC 90112. As a result of study, we have found that *X. polymorpha* revealed antimicrobial activity against all tested microorganisms. Especially, *G. capitatum* is more susceptible to the extracts.

Key words: *Xylaria polymorpha*, antimicrobial activity.

INTRODUCTION

Large-scaled screening programs from the 1940s for the detection of antimicrobial activity include a variety of fleshy macrofungi (Benedict and Brady, 1972; Dulger et al., 2002). A number of more recent reports recorded additional general observations of microbial antagonism with macrofungi (Conchran, 1978). Unfortunately, the identities of the macrofungal metabolites responsible for the antimicrobial effects are still unknown in most instances.

Xylaria polymorpha (Pers.) Grev. (Ascomycetes), commonly known as dead man's fingers, is a plant pathogen. It is a common inhabitant of forest and woodland areas, usually growing from the bases of rotting or injured tree stumps and decaying wood. In this study, we aimed to determine the antimicrobial potential of the ethanolic extracts obtained from *X. polymorpha*. Extracts were tested for antimicrobial activity against representative the Gram-positive and the Gram-negative bacterial as well as the yeast cultures.

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MATERIALS AND METHODS

The macrofungal materials

X. polymorpha was collected from Trabzon, Turkey on decaying stump in September, 2009. Voucher specimens were deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey.

Microorganisms

Escherichia coli ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403, *Geotrichum capitatum* ATCC 28576, *Debaryomyces hansenii* DSM 70238, *Kluyveromyces fragilis* ATCC 8608 and *Cryptococcus neoformans* ATCC 90112 were used as test microorganisms.

Preparation of extracts

The macrofungal samples were air-dried. Each dry powdered fungal material (50 g) was extracted with 150 mL of 95% ethanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment

Table 1. Summary of antibacterial activity of *X. polymorpha* and some standard antibiotics.

Microorganisms	Inhibition zones (mm)[a]							
	Extract (µg/mL)	Standard antibiotics						
		P	AMP	CTX	VA	OFX	TE	NY
Escherichia coli	14.6	18.2	12.2	10.4	22.0	30.8	28.2	Nt
Staphylococcus aureus	13.8	13.4	16.8	12.6	13.4	24.4	26.4	Nt
Pseudomonas aeruginosa	12.6	8.6	10.8	54.2	10.8	44.0	34.8	Nt
Proteus vulgaris	10.0	10.2	16.2	18.4	20.2	28.6	26.2	Nt
Bacillus cereus	11.8	14.4	12.4	14.6	18.6	30.2	25.4	Nt
Micrococcus luteus	11.2	36.2	32.0	32.2	34.2	28.8	22.4	Nt
Candida albicans	16.8	Nt	Nt	Nt	Nt	Nt	Nt	20.0
Kluyveromyces fragilis	14.4	Nt	Nt	Nt	Nt	Nt	Nt	18.2
Rhodotorula rubra	12.2	Nt	Nt	Nt	Nt	Nt	Nt	18.0
Debaryomyces hansenii	13.6	Nt	Nt	Nt	Nt	Nt	Nt	17.4
Geotrichum capitatum	20.8	Nt	Nt	Nt	Nt	Nt	Nt	16.4
Cryptococcus neoformans	14.2	Nt	Nt	Nt	Nt	Nt	Nt	16.8

[a] includes diameter of disk (6 mm); mean value of three independent experiments; Nt = not tested; P = penicillin (10 µg/disc); AMP = ampicillin (20 µg/disc); CTX = cefotaxime (30 µg/disc); VA = vancomycin (30 µg/disc); OFX = oflaxacin (5 µg/disc); TE = tetracycline (30 µg/disc); NYS = nystatin discs (30 µg/disc)

(Khan et al., 1988). The extract was filtered using Whatman filter paper no. 1, and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extract was stored in labeled sterile screw-capped bottles at -20°C (Dulger and Sener, 2010).

Screening for antimicrobial activities

The dried macrofungal extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/mL and sterilized by filtration through a 0.45 µm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schull No. 2668, Dassel, Germany) were each impregnated with 50 µL of extract (10 mg/disc) at a concentration of 200 mg/mL. All the bacteria mentioned above were incubated at 35 ± 0.1°C for 24 h by inoculation into Nutrient Broth (Difco Laboratories, MI, USA) and the yeast cultures studied were incubated in Malt Extract Broth (Difco Laboratories, MI, USA) at 25 ± 0.1°C for 48 h.

An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The discs injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes were placed at 4°C for 2 h, plaques injected with the yeast cultures were incubated at 25 ± 0.1°C and bacteria were incubated at 35 ± 0.1°C for 24 h (Collins et al., 1989). At the end of the period, inhibition zones formed on the medium were evaluated in millimeters. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disc was applied, depending on the test microorganisms for comparison.

RESULTS AND DISCUSSION

The inhibition zones formed by the macrofungal extracts and some standard antibiotics are indicated in Table 1. As it can be clearly seen from Table 1, the extracts of the macrofungus have exhibited antimicrobial activity against all tested microorganisms with inhibition zones ranged from 10.0 to 14.6 mm for bacteria, 12.2 to 20.8 mm for the yeast cultures.

E. coli and *S. aureus* are more susceptible to the extracts, as compared to some standard antibacterial antibiotics such as P10, AMP20, CTX30 and VA30. Similarly, in comparison to P10, AMP20 and VA30 standards, it was seen that *P. aeruginosa* is more susceptible. However, the extracts have weak antimicrobial activity against the other microorganisms. These values are far below than the standard antibiotics. In previous study, ethanol was observed as the best solvent for extracting antimicrobial substances (Jonathan and Fasidi, 2003). The results in this study with ethanol are similar to those reported in the mentioned study. It is important to bear in mind that the concentration of extract used in this test may be correlated with a high activity of its chemical components.

The macrofungus differ significantly in their activity against tested microorganism. These differences may be attributed to fact that, the cell wall in the gram-positive bacteria of a single layer, whereas the gram-negative cell wall is multi-layered structure and the yeast well wall is quite complex (Yao and Moellering, 1995).

The polyacetylenes are the most extensively characterized group of antagonistic mushroom constituents. More than 50 of these unsaturated antibiotic substances are known from one or more species of *Aleurodiscus, Clitocybe, Coprinus, Cortinellus, Marasmius, Merulies, Pleurotus, Poria, Psathyrella* and *Tricholoma*. Other known antagonist compounds from the macrofungi include phenolic metabolites (Benedict and Brady, 1972; Conchran, 1978). The activity found in this study may be indicative of the presence of metabolic toxins or the mentioned the macrofungal compounds.

Geotrichosis is an uncommon fungal infection. *Geotrichum capitatum*, an anamorph of *Dipodascus capitatus*, is commonly acknowledge as an etiologic species causing invasive and disseminated geotrichosis in immune compromised patients with hematological malignancies such as acute leukemia and severe neutropenia (Girmenia et al., 2005). It is widely distributed in nature and may be found as part of the normal skin flora. Data on the antifungal susceptibilities of *G. capitatum* are quite limited; however, resistance to fluconazole and decreased susceptibility to amphotericin B have been described. Some investigators believe that *G. capitatum* has decreased susceptibility to amphotericin B (Pfaller and Diekema, 2004). According to our findings, the ethanolic extracts obtained from *X. polymorpha* have strong antifungal activity against *G. capitatum*. So, the macrofungal extracts should be analyzed further, as it might provide a new compound effective against especially *G. capitatum* and the other pathogens.

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