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Evaluation of antifungal activity of hydroalcoholic extracts of *Citrullus colocynthis* fruit

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ABSTRACT

Due to the development of resistance to antimicrobial drugs of several multi-drug resistance pathogens, the discovery of new active agents has become a major public health concern. The purpose of this study was to evaluate the antifungal activity of hydroalcoholic extract of *Citrullus colocynthis* fruits against different *Candida* and *Aspergillus* strains. Anti-*Candida* and anti-*Aspergillus* effects of the extract were determined by disk diffusion and broth macrodilution methods. The study revealed that all tested fungal strains were sensitive to the extract. The growth inhibition value of the extract showed high antifungal activity against *Aspergillus fumigatus* and *Aspergillus niger* and a lesser effect against *Candida guilliermondii* and *Candida kreusei*. The minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values ranged from 1.56 to 12.5 mg/ml and 3.125 to 25 mg/ml, respectively. The results obtained in the present study suggest that *C. colocynthis* may be a valuable plant source of medicinally useful active compounds that can be used for the treatment of some fungal infections.

1. Introduction

The increase in the rising incidence of fungal infections is due to the pathogens resistant to multiple antifungal agents and their nosocomial dissemination of their resistance.

Scientific efforts to discover new potential antifungal drugs are principally guided toward synthetic and natural products of plant origin (Marzouk et al., 2011). The extracts of many plants/herbs have been shown to exert biological activity *in vitro* and *in vivo*, justifying research on traditional medicine that focuses on the characterization of antifungal activity of these plants. Iran, India, Pakistan, Turkey, Jordan, Brazil and Mexico are examples of countries that have a diverse flora and a rich tradition in the use of medicinal plants for both antibacterial and antifungal applications (Ghasemi Pirbalouti et al., 2009).

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Since plants produce a variety of compounds with antimicrobial properties, it is expected that screening programs for some under-represented targets, such as antifungal activity, may yield candidate compounds for developing new antimicrobial remedies (Ahmad and Beg, 2001).

Egusi (*Citrullus colocynthis* L.) which belongs to the melon family of cucurbitaceae, is native to Asia, especially in Turkey and Iran (Hadizadeh et al., 2009) and produces bitter flavored fruits about the size of a cantaloupe melon with seeds rich in oil and protein.

It is a long lived perennial and grows wild in sandy shore and exerophitic conditions. The fruits are smooth, spherical and mottled green when young, yellow when ripe and are known as "bitter apple", "colocynth", "vine-o-Sodom" or "tumba" (Mehta et al., 2013). The fruit of *C. colocynthis* has been used medicinally since ancient times. Traditionally, fruit of *C. colocynthis* was used for the treatment of diabetes, microbial diseases, ulcer, inflammation, jaundice, amenorrhea, ascites, bilious disorders, cancer, fever, leukemia, rheumatism, snakebite, tumors and urogenital disorders in Asian and African countries (Hadizadeh et al., 2009; Gurudeeban et al., 2011).

The purpose of this study was to evaluate the possible antifungal activity of hydroalcoholic extract of *C. colocynthis* fruits against some selected fungal pathogens.

2. Materials and methods

2.1. Collection and identification of plant samples

The fresh fruits of *C. colocynthis* were purchased from a traditional drug store during May–November, 2012 in Mashhad, Khorasan Razavi Province, Iran. Their identity was confirmed by The Iranian Research Institute of Forests and Rangelands, Mashhad, Iran and voucher specimens were deposited at the Research Center of Medicinal Plants, School of Pharmacy University of Mashhad, Iran.

2.1.1. Preparation of plant extract

Fruits were freed from foreign materials, dried in the shade at room temperature and carefully rubbed between soft cloths to remove dust before grinding to a fine powder using a mixer (Molinex®). The fruit powder obtained was soaked in 80% ethanol (W/V) at a ratio of 1:3(100 g of powder in 300 ml of ethanol 80%), kept in a refrigerator for 24 h and then the supernatant subsequently decanted. The remaining powdered sediment was extracted with 80% ethanol a further three times as above and the combined supernatants filtered twice, first under vacuum through a double layer of Whatman filter paper (Nos. 3 and 1) and then by gravity through a single sheet of Whatman No. 1 filter paper. The solvent was removed by using a Rotary Evaporator (R214B2L). The final yield was 10.43 g of a dark green to brown extract referred to as "the crude extract" that was stored at 4 °C in airtight bottles (Tegegne et al., 2008).

2.2. Fungal strains and growth media

Nine reference strains, including *Candida albicans* ATCC 76615, *Candida glabrata* ATCC 90030, *Candida kreusei* DSM 11957, *Candida parapsilosis* ATCC 90018, *Candida guilliermondii* ATCC 90877, *Candida tropicalis* ATCC 90874, *Candida dubliniensis* DSM 13268, *Aspergillus fumigatus* DSM 790, *Aspergillus niger* DSM 1988 and *Aspergillus flavus* obtained from the Medical Department, Division of Hematology, Oncology and Tumor Immunology, Charité-Universitätsmedizin Berlin, Germany were chosen for antifungal investigation. All strains were grown on Sabouraud dextrose agar (Merck Co., Darmstadt, Germany) supplemented with chloramphenicol (0.005%; to prevent possible bacterial contamination) at 35 °C for 48–72 h.

2.3. Synthetic antifungal drug

Amphotericin B, used as a control, was purchased from Sigma Chemical Co., St. Louis, MO, USA, dissolved in dimethyl sulfoxide (10% DMSO) and stored as 0.25 ml aliquots at −20 °C.

2.4. Preparation of fungal suspensions

Fungal suspensions including 1×10^6 *Candida* cells and 2×10^4 *Aspergillus* conidia in sterile 0.85% saline were prepared by counting with a haemocytometer. To avoid spore propagations a drop of Tween 80 was added into the suspensions.

2.5. In vitro susceptibility tests

2.5.1. Disk diffusion method

The agar disk diffusion method was employed for the determination of antifungal activity of hydroalcoholic extract of *C. colocynthis* (Wayne, 1997; Tepe et al., 2006). Prior to being tested for antifungal activity the crude plant extract was dissolved in dimethyl sulfoxid (10% DMSO) as stock 500 mg/ml and sterilized by passing through a 0.22 μm Millipore GV filter (Millipore, USA). Fungal inoculums (0.1 ml) of a 48–72 h old culture were distributed uniformly on the surface of solid media plates by using a sterile cotton swab. Disks (8 mm in diameter) impregnated with 15 mg plant extract prepared by adding 30 μl of stock solution to each blank disk were placed on the surface of the agar plates and then incubated at 35 °C for 48 h. The diameters of the inhibition zones were measured in millimeters. All tests were performed in duplicate. The test was also performed with a standard antifungal drug, amphotericin B, which was dissolved in DMSO 10% (6 μg/disk) and served as a positive control.

2.5.2. Broth macrodilution method

Antifungal analysis and determination of the minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract and amphotericine B against different fungal strains were performed by broth macrodilution method. 1 ml of Sabouraud broth in a capped tube was used as the substrate. 1 ml of the crude plant extract in DMSO (50 mg/ml) was added into this substrate and mixed thoroughly. This dilution process was then repeated sequentially until the 6th tube so that twofold serial dilutions ranging from 25 to 0.78 mg/ml were obtained for testing