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Original Article

Antifungal Effects of Crude Extract and Fractions Of Euphorbia splendida Mobayenagainst Aspergillus Niger, Aspergillus Fumigatus, Candida Albicans and Candida Krusei

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Abstract

The significant decline in infectious diseases was owed to the development of the antibiotic industry. Due to the prevalence of drug-resistant organisms alongside the other problems, scientists have been focused on medicinal plants. Medicinal plants and their antimicrobial components are attractive alternatives to antibiotics. In the present project, the antimicrobial effects of methanolic extract and fractions of aerial parts of Euphorbia splendida Mobayen were evaluated against Aspergillus niger, Aspergillus fumigatus, Candida albicans, and Candida krusei.

Euphorbia splendida samples were transferred to the laboratory after confirmation. The plant samples were then shadow dried before being ground. Different extracts and fractions were prepared by standard methods. The extracts and fractions were kept under standard conditions until used. The antifungal assessment was performed by standard agar diffusion methods.

The concentration of 100 mg/ml of each methanolic, hexanic, chloroformic, and aqueous extract inhibited C. krusei growth (15 mm).The methanolic extracts at 100 mg/ml displayed 15 mm inhibition zone on C. albicans plate. The aqueous extract had inhibition zone of 14 mm, in C. albicans culture at 100 mg/ml. The methanolic extract at 100 mg/ml inhibited A. niger (15 mm). Meanwhile, methanolic extract at the same concentration allowed the growth of A. fumigatus only beyond 13 mm zone. It was concluded that antifungal activities of total extract and fractions of Euphorbia splendida were considerable and more experiments were beneficial for drug development.

Keywords: Euphorbia splendida, A. niger, A. fumigatus C. albicans. C. kruzei.extract

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INTRODUCTION

Various plants, which serve as sources of medicinal compounds, have continued to play a dominant role in the maintenance of humans' health balance. Since ancient times, medicinal plants have continued to be an important therapeutic aid for alleviating the human ailments. Research and developments of medicinal natural products represented an area of great interest in which plants have been the most important source. The medicinal value of these plants lies upon some chemical substances that have physiological activities within the human body. The most important of plant bioactive chemical constituents are alkaloids; tannins, flavonoids, and phenolic compounds (1). Euphorbia is the largest genus in the family Euphorbiaceae, comprising about 2000 known species and ranging from shrub to trees. Over 82 species of this genus have been found in Iran (2), and 80 species in Turkey (4). Euphorbiaceae is one of the largest families of the phylum Anthophyta. The largest genus is Euphorbia, which comprises well over 2000 species, grows in the form of laticiferous herbs, shrubs, and small trees, inhabiting the tropical and temperate zones of Asia and other parts of the world. For centuries, plants and plant materials of Euphorbia have been known to be poisonous. Often, they have been held responsible for the poisoning of livestock and used as arrow poisons. In traditional medicine, they were used for treatment of intestinal parasites, gonorrhea and some of the skin lesions. (3). Some species of Euphorbia have been used as medicinal plants for the treatment of skin diseases, gonorrhea, intestinal parasites, inflammation and wart cures (Singla and Pathak, 1990). Plants of this genus are known for their rich content in secondary metabolites. Indeed, numerous studies undertaken on this genus have revealed the presence of triterpenes, diterpenes, steroids (Singla and Pathak, 1990) and macrocyclic diterpenes (Duarte et al., 2006). Besides the well known skin irritant and tumor promoting tigliane, ingenane and daphnane diterpenes (Evans and Taylor, 1983), some diterpenes of Euphorbia have been found to be cytotoxic, antitumor, antibacterial, (Singla and Pathak, 1990), antinociceptive (Ahmad et al., 2005) and inhibitor of multidrug resistant tumor cells (Hohmann et al., 2000).[10]

Due to low cost, easy accessibility, and ancestral experiences, the medicinal herbs constitute indispensable components of the traditional medicine practices worldwide.

Invasive aspergillosis in immunosuppressed patients is difficult to diagnose, problematic to treat, and results in a high mortality rate. Chronic and allergic pulmonary and sinus aspergillosis are increasingly recognized in numerous clinical settings. Despite progress made in early diagnosis and treatment of the infection, mortality rate with invasive aspergillosis remains high. Treatment with itraconazole, voriconazole, and recently posaconazole is the backbone of therapy for these conditions because azoles are the only licensed class of oral drugs for the treatment of aspergillosis. Amphotericin B and caspofungin are licensed intravenous agents for invasive aspergillosis but have limited utility for chronic and allergic aspergillosis (6). Invasive aspergillosis increased especially within the immunocompromised population. Four classes of antifungal agents are active against Aspergillus: polyenes, triazoles, echinocandins, and allylamines. Primary resistance to Amphotericin B was reported for A. terreus. More recently, it has also been demonstrated for several species including A. lentulusand and A. fumigatus (7). Until recently, species identification was sufficient to guide antifungal therapy, but the emergence of acquired resistance limits the use of species identification for predicting the activity of antifungal agents (8). Resistant Candida and Aspergillus infection are increasingly encountered in the antifungal drug-natıve patientdue to increasing incidence rates of C. glabrata (particularly the northern hemisphere.

C. glabrata and C. parapsilosis represent the two most prevalent species with reduced azole and echinocandin susceptibility respectively (9). The latex of Euphorbia species used in this study is used widely because of different substantial features of this plant species. For instance, these plants are used for the treatment of hypertension, destruction of warts, and skin diseases. It is suggested that Euphorbia species possessed compounds with antimicrobial properties that might be useful for new drug research and developments (4).

The effects of different Euphorbia species on pathogens microorganisms have been investigated at different parts of the world. The antifungal effects of Euphorbia Splendida Mobayen extract against two Aspergillus and two Candida species are reported here.

MATERIALS AND METHODS

The aerial parts of E. splendida were collected from Arak, (Markazi Province of Iran), and reconfirmed by a botanist among the faculty members. The plant samples were then dried at the shaded conditions and were ground. All solvents and chemical reagents were purchased from Merk (Darmshtot, Germany). Extractions were obtained using Maciration standard methods. Briefly, 2/3 volume of percolator were filled with nearly 600 g of dried and powdered of plant sample. The whole volume of the container was then filled with methanol solvent and left for three days. The above procedures were repeated three times. Finally, approximately, 50 g methanolic extract were obtained. The different fractions were obtained using the standard method of solvent/solvent. Briefly, 30 g of crude extract were put within decanter coil followed by 2500 cc of hexane. The outflow was collected and concentrated by a standard rotary (40°C, 50 rpm) and was kept under standard condition until used. The procedure was repeated two times with 1500 cc chloroform and 2000 cc n-butanol, respectively. Finally, the procedure was done by distilled water. As a result, hexane fraction 2 g, chloroform fraction 1.6 g, and H2O fraction 5g were obtained that were kept at standard condition until used.

The standard methods of agar diffusion were used for antifungal activity assays. The standard species of C. Albicans (ATCC 10231), C. krusei (ATCC 6258), A. niger (ATCC 16404), and A. fumigates (ATCC 5009) were donated by Pasteur in Tehran, Iran. All fungal species were grown on YGC (Yeast Extract Glucose Chloramphenicol) Agar, and half Mac Farland standard was used as standard turbidity of each spp. Fluconazole 10 μg/ml and amphotericin B 5 μg/ml were used as positive controls for Aspergillus and Candida species respectively. Three concentrations of 1000, 500, 100, 250, 125, 62.5, 31.25, 15.6 μg/ml of extract and different fractions were used for antifungal activity assay. The standard microplate methods and PRMI 1640 (GIBCO LTD) were also used for MIC determination. All statistical analysis were done using SPSS statistical software.

RESULTS

All data are the mean of triplicate sets of independent experiments. According to Table 1, anti-fungi activities of E. splendida extracts at 500 mg/ml concentration were not desirable enough. According to the data obtained, antifungal activities of all extracts at this concentration were about 11 mm. Meanwhile, Amphotericin B inhibited aspergillus with 20 mm inhibition zone. Candida species, on the other hand, showed more sensitivity to the plant extract at the same concentration. Compared with Fluconazole with 19 mm, the maximum antifungal activities was for methanolic extracts with 13 mm (Table 1). Neither of fungal spp. at concentration of 1000 mg/ml, Crude extracts and fractions inhibited the growth of showed the highest

anti-fungal activities with a zone of inhibition 15 mm. The methanolic extract inhibited the growth of both C. Albicans and C. kruzei at a concentration of 15 mm. While the water crude extract had slightly less (14 mm) activates at the same concentration. A. niger and A. fumigatus growth were inhibited (15mm and 13 mm respectively) by methanolic extract at 100 mg/ml (Table 1). The MIC of aspergillus and Candida species calculated 39 mg/ml for methanolic extract.

Anti-microbial activities of plant extracts (at 1000 mg/ml) are seen in Table 2. Anti-fungi activities of E. splendida extracts at 1000 mg/ml concentration were higher than lower concentration, but not as high as expected. According to the data shown, antifungal activities of all extracts at this concentration were 11- 15 mm. Compared with Amphotericin B and Fluconazole, as a positive control, all extracts demonstrated more activities than 500 mg/ml concentration. Candida species showed more sensitivity to the plant extract at the same concentration. Compared with Fluconazole with 19 mm, the maximum antifungal activities were for methanolic extracts with 13 mm (Table 2).

The comparison of effects of different extracts on Aspergillus species (Figure 1) and Candida species (Figure 2) showed no considerable differences between E. splendida extracts against Aspergillus spp. Although, there was more and less difference between different extract effects against Candida species.

The comparison of effects of different extracts on Aspergillus species (Figure. 3) and Candida species (Figure. 4) had been demonstrated. Again, the data showed no considerable differences between E. splendida extracts against Aspergillus spp . Although, there was more and less difference between plant extract effects against Candida species at 1000 mg/ml concentration.

DISCUSSION

At present study, no anti-fungal activities were observed in none of less than 500 mg/ml extracts. Different extracts of E. splendida including Methanolic extract, Hexanic extract, Chloroformic extract, Aqueous extract were tested against four Aspergillus and Candida species. The antimicrobial contents of all fractions were low because the first activities were observed at 500 concentration. The polar fraction of the methanolic extract from the plant Euphorbia peplis L. exhibited interesting antifungal and antitubercular activity. A complex mixture of four glucocerebrosides was responsible for this activity. The isolation and structure elucidation of four cerebrosides with antifungal and antitubercular activity from Euphorbia peplis L. was reported (11). The leaves of Euphorbia hirta were found to contain triterpenoids, sterols, alkaloids, glycosides and tannin (10). The plant has a reputation for being a remedy for bronchitis, asthma, eczema, laryngeal spasm and cough (in liquid extract or tincture form). Other uses include lactation, as tonic, anthelmintic, anticonvulsant, mild sedative and antimicrobial agent and in the treatment of wounds and tumors. In this study, the interaction between nystatin and methanol extract of E. hirta leaves has been investigated using Checkerboard method. The results of this research could provide a rational basis for the use of standardized herbal drugs in combination therapy of prevailing diseases (5). The antifungal activities were not increased by concentration, clearly because the agar diffusion ability of extracts had decreased considerably at higher concentration. More photochemistry and Pharmacognosy analysis are required before the final conclusion.

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