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**COMPARATIVE ASSESSMENT OF EFFECTS OF FLUCONAZOLE AND
ECHINOPHORA PLATYLOBA AGAINST *CANDIDIA DUBLINIENSIS*
ISOLATED FROM ORAL MUCOSA OF PATIENTS
WITH DOWN'S SYNDROME**

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ABSTRACT

Down syndrome occurs when an individual has a full or partial extra copy of chromosome 21. Down syndrome (DS) or Down's syndrome, also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21. Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products. because of the numerous side effects such as nausea, abdominal pain, skin rash, vomiting, and headache, combined with the increasing drug resistance of this fungus led research on herbal drugs without side effects continue. So in current study we compared effect of Fluconazole and *Echinophora platyloba* against *Candidia dubliniensis* Isolated from Oral Mucosa of Patients with Down's syndrome. In this study we used micro-dilution broth method using the 96-well micro-plates. Then for each well, RPMI 1640 were poured. After that, 100µl of Fluconazole and extract of *Echinophora platyloba* were added into the first 8 wells and after mixing, 100 microliter from 1st well were added into the 2nd well, so, serial dilutions were obtained until well 10th. At the end, 100 µl was out. So, drug concentration was 32 and 0.06 µg/ml in the 1st and 10th wells respectively. Then 100 µl of fungal suspension was added to all wells of each row with exception 12th row. Wells of row 11th and 12th were considered as positive control and negative control respectively. Then, micro-plates were incubated for 48 hours at 35°C. At the end, colonies were counted and MIC50 and MIC90 for Fluconazole and extract of *Echinophora platyloba* were calculated. In this study, the age range was 4 to 31 years-old. Of 53 patients, 29 (54.7%) were male and 24 (45.2%) were female. of 53 cases, 46 (86.79%) of them were positive, so that, 26 (56.52%) and 20 (43.47%) of them were male and female respectively. Of 46 cases, 60 candida funguses were isolated. Of that, 23 cases (58.33%) were *C. dubliniensis*. MIC50 of Fluconazole of 3, 9 and 11 cases were 0.25, 0.5 and 2 µg/ml respectively for *C. dubliniensis* isolates and MIC50 of *Echinophora platyloba* of 5, 9 and 9 cases were 1, 1.5 and 2 µg/ml respectively for *C. dubliniensis* isolates.

Keywords: Fluconazole, *Echinophora Platyloba*, *Candidia Dubliniensis*, Down's Syndrome

INTRODUCTION

Down syndrome occurs when an individual has a full or partial extra copy of chromosome 21. Down syndrome (DS) or Down's syndrome, also known as trisomy 21, is a genetic disorder caused by the presence of all or part of a third copy of chromosome 21. In these patients, oral anatomical disturbance is most predisposing factor that causes growth and proliferation of these funguses which is seen most commonly in pre-oral. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (El-Astal et al., 2005). Natural products have served as a major source of drugs for centuries and about half of the pharmaceuticals in use today are derived from natural products (Clark, 1996). Also, another study reports that 25 - 50% of current pharmaceuticals are derived from plants (Cowan, 1999). Microbiologists are combing the earth for phytochemicals which could be developed for treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been reported to have in vitro antimicrobial properties (Cowan,

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1999). A study demonstrates the antifungal activity of extracts of some Thai medicinal plants which can be excellent candidates for the development of remedy for opportunistic fungal infections in AIDS sufferers (Phongpaichit *et al.*, 2005). Another study reports the activity of traditional medicinal herbs from Balochistan, Pakistan against *C. albicans*, *Bacillus subtilis* and *Bacillus cereus* (Zaidi *et al.*, 2005). In Iran, traditional medicine has a major therapeutic role and for thousands of years, traditional healers have been using different plants to treat patients. *E. platyloba* DC. is one species of *Echinophora* genus (Rechinger, 1987). The 10 different species of this plant has been defined as: *Echinophora tenuifolia*, *E. platyloba* DC. *Echinophora sibthorpiana* Guss, *Echinophora anatolica* Boiss, *Echinophora cinerea*, *Echinophora vadiaus* Boiss, *Echinophora orientalis* Hedge and Lamond, *Echinophora tournefortii* Joub, *Echinophora trichophylla* Sm, *E. spinosa*. Four of these species are native to Iran: *Echinophora orientalis*, *Echinophora sibthorpiana*, *Echinophora cinerea* and *E. platyloba* (Vanden *et al.*, 2002). *E. platyloba* is mainly used for food seasoning in Iran (Chaharmahal va Bakhteyari province) (Sadrai *et al.*, 2002), rather than preventing tomato paste and pickles from mold. The hypothesis of these serial studies was based on the plant's specific characteristic as food preserver which might have been due to its antimicrobial properties. During past 7 years, the authors did several studies on antimicrobial activity of *E. platyloba* ethanolic extract. This article is a review of all previous studies indicating the effectiveness of ethanolic extract of *E. platyloba* against *C. albicans*, dermatophytes and some gram positive bacteria, in addition to its significant synergy with Amphotericin B against *C. albicans*. *Candida dubliniensis* is cosmopolitan (found around the world), and has been described as a separate species in 1995 (Sullivan *et al.*, 1995). Retrospective studies have shown that previously it had been commonly identified as *Candida albicans*, with which *C. dubliniensis* is closely related and shares a number of characteristics. Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections. In a bulk powder form, it appears as a white crystalline powder, and it is very slightly soluble in water and soluble in alcohol. because of the numerous side effects such as nausea, abdominal pain, skin rash, vomiting, and headache, combined with the increasing drug resistance of this fungus led research on herbal drugs without side effects continue. So in current study we compared effect of Fluconazole and *Echinophora platyloba* against *Candida dubliniensis* Isolated from Oral Mucosa of Patients with Down's syndrome.

MATERIALS AND METHODS

For extracting *Echinophora platyloba*, the plant material washed with water for 30 Minutes and was disinfected with 2% sodium hypochlorite solution. Then to remove residual hypochlorite, rinsed with sterile distilled water and dried and plant material powdered. 50 g of dried powder was soaked in 500 ml of methanol and 48 hours was shaken by shaker. Then by two layers of sterile linen filtered after that centrifuged for 10 min at 9000 rpm and filtered whatman paper number 41 again. After that 3.2 mg of Fluconazole was weighted and was poured into the tubes. Then 5 ml of Dimethyl sulfoxide (DMSO) at the 640 µg/ml were added as solvent. This solution was kept at laboratory condition for half hour then was filtered. Then for measurement the minimum inhibitory concentration (MIC), 1 ml of drug dilution was diluted again with 9 ml distilled water, so, final concentration was gained (64 µg/ml) (John *et al.*, 2008). For Preparing the RPMI 1640 Medium, the powder of RPMI 1640 medium (sigma Co.) was solved in the water and sodium bicarbonates was added at the 2g/L. Then was filtered and transferred into the tubes and were kept in the refrigerator at 4°C. At the time using, 1ml glutamine were adding per 100 ml medium (John *et al.*, 2008).

In this study we used micro-dilution broth method using the 96-well micro-plates. Then for each well, RPMI 1640 were poured. After that, 100µl of Fluconazole and extract of *Echinophora platyloba* were added into the first 8 wells and after mixing, 100 microliter from 1st well were added into the 2nd well, so, serial dilutions were obtained until well 10th. At the end, 100 µl was out. So, drug concentration was 32 and 0.06 µg/ml in the 1st and 10th wells respectively. Then 100 µl of fungal suspension was added to all wells of each row with exception 12th row. Wells of row 11th and 12th were considered as positive control and negative control respectively. Then, micro-plates were incubated for 48 hours at 35°C. At the

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end, colonies were counted and MIC50 and MIC90 for Fluconazole and extract of *Echinophora platyloba* were calculated (John et al., 2008).

RESULTS AND DISCUSSION

Results

In this study, the age range was 4 to 31 years-old. Of 53 patients, 29 (54.7%) were male and 24 (45.2%) were female. of 53 cases, 46 (86.79%) of them were positive, so that, 26 (56.52%) and 20 (43.47%) of them were male and female respectively. Of 46 cases, 60 candida funguses were isolated. Of that, 23 cases (28.33%) were *C. dubliniensis* (Table 1).

MIC50 of Fluconazole of 3, 9 and 11 cases were 0.25, 0.5 and 2 µg/ml respectively for *C. dubliniensis* isolates and MIC50 of *Echinophora platyloba* of 5, 9 and 9 cases were 1, 1.5 and 2 µg/ml respectively for *C. dubliniensis* isolates (Table 1).

Table 1: MIC values obtained from *C. dubliniensis* strains for Fluconazole and *Echinophora platyloba*.

Treatment	Strain	N	MIC ₅₀ (µ/ml)
Fluconazole	<i>C. dubliniensis</i>	9	0.5
		3	0.25
		11	2
<i>Echinophora platyloba</i>	<i>C. dubliniensis</i>	5	1
		9	1.5
		9	2

Discussion

Oral candidiasis is an infection of yeast fungi of the genus *Candida* on the mucous membranes of the mouth. The incidence of candidemia due to *C. dubliniensis* is not known, largely because of the difficulty in readily distinguishing this species from the morphologically similar *C. albicans*. However, in laboratory-based surveillance conducted in 1992-93 in two sites in the United States (population 5.8 million), we did not find *C. dubliniensis* as an agent of candidemia, even with the DNA-based identification method used in this study (Elie et al., 1999). More recently, three cases of *C. dubliniensis* fungemia have been reported from Europe in patients with chemotherapy-induced immunosuppression and bone marrow transplantation (Meis et al., 1999). The four cases described here are the first reported in the United States. Down's syndrome favours alterations of the buccal cavity of the children whose bear this chromosomal alteration. Such alterations allow the tissue of the mouth to be populated by *Candida* yeasts as colonizing and/or pathogenic microorganisms, as in the case of angular cheilitis, in an incidence of 16% of the children (Mustacchi et al., 1990) and (Roncari et al., 2002). Periodontal disease can be related with microbiological alelobiosis, which includes *Candida* isolates, due to the formation of dental plaque, low buccal hygiene, neutropenic compromising and repair capacity deficiency present in children with this chromosomal mutation (fast bone loss) (Mustacchi et al., 1990). Another factor that would favour the high carriage of *Candida* in the mouth of children with Down's syndrome is the verification of the physical chemical alterations of saliva secretion. Variation of salivary pH and sodium, calcium and bicarbonate ions concentration, among other substances, seem to affect *Candida* mouth survival, as it keeps pH oscillation between acidity and alkalinity (Roncari et al., 2002). It is also added to this chromosomal alteration, the situation of the immune system of children with Down's syndrome. In a study by Carlstedt et al., (1996) which carried out on 55 cases, it has been revealed that patients with Down's syndrome are more susceptible than normal people (Carlstedt et al., 1996). They showed that colonization of *Candida* yeasts in 41 cases (74.54%) was more than normal people (25.46%).

Considering this problem that Down's syndrome is one of the most prevalent disorders from buccal point of view, and because of its risk in developing secondary tumors to the mouth, pharynx and esophagus as well as systemic infection, candida infection in order to diagnose and treat patients with this syndrome,

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special attention should be focused (Amano *et al.*, 2008). However there are so many antifungal drugs, but, because of unknown mechanism of action of fungal diseases and also resistance of some fungus to specific agents from other hands yield to extend the fungal diseases and hard to control (Dominique 2008). Achieved results have shown that *Echinophora platyloba* contained antifungal activity against *Candida dubliniensis*. In present study MIC₅₀ of Fluconazole of 3, 9 and 11 cases were 0.25, 0.5 and 2 µg/ml respectively for *C. dubliniensis* isolates and MIC₅₀ of *Echinophora platyloba* of 5, 9 and 9 cases were 1, 1.5 and 2 µg/ml respectively for *C. dubliniensis* isolates and the results showed that mean value of MIC for Fluconazole is less than *Echinophora platyloba* against *C. dubliniensis*. It means that, *C. dubliniensis* are more susceptible for Fluconazole than *Echinophora platyloba*. In a research by Hanan *et al.*, (2004) it has been showed that susceptibility of *C. albicans* isolated from oral cavity of patients with cancer to azolic antifungal agents such as ketoconazole and fluconazole was 1, 0.125 and 1-8 µg/ml and was 1-2 and 2-8 µg/ml about *C. glabrata*, it shows more susceptibility of candida species to ketoconazole than fluconazole, which is compatible with our research's results (Hanan *et al.*, 2004). In another research by Hamza *et al.*, (2008) it has been declared that candida species isolated from oral cavity of patients with HIV have more susceptibility to azolic agents which is compatible with our research results (Hamza *et al.*, 2008).

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