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COMPARATIVE EVALUATION OF EFFECTS OF CLOTRIMAZOLE AND *MYRTUS COMMUNIS* AGAINST *C. KRUSEI* ISOLATED FROM ORAL MUCOSA OF PATIENTS WITH DOWN'S SYNDROME

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ABSTRACT

The genus Candida consist of 200 species that more commonly with C.*albicans, C.dubliniensis, C.tropicalis, C.glabrata, C.parapsilosis* and *C. krusei* are most prevalent and most isolated from lesion of candidiasis. Non-pathogen species of candida such as C.albicans are part of natural microflora of the mouth at the range of 17-50%. Down syndrome is the most common chromosome abnormality in humans. Measurement of the antifungal activity of drugs in vitro using the sensitivity testing can be useful to treat patients with the syndrome. There are many routes for measurement the antifungal activity of drugs in vitro that use of broth medium is one of the most common ways. But 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 this study we compared effect of Clotrimazole and *Myrtus Communis* against *Candidia krusei* 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

Clotrimazole and extract of *Myrtus Communis* 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 12^{th} 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 Clotrimazole and extract of *Myrtus Communis* were calculated. In current 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, 19 cases (23.33%) were *Candidia krusei* MIC50 of Clotrimazole of 2, 4 and 13 cases were 0.25, 0.5 and 2 µg/ml respectively for *Candidia krusei* isolates and MIC50 of *Myrtus Communis* of 1, 7 and 11 cases were 1, 1.5 and 2 µg/ml respectively for *Candidia krusei* isolates.

Keywords: Clotrimazole, *Myrtus Communis, Candidia krusei,* Down's syndrome.

INTRODUCTION

The genus Candida consist of 200 species that more commonly with C.*albicans, C.dubliniensis, C.tropicalis, C.glabrata, C.parapsilosis* and *C. krusei* are most prevalent and most isolated from lesion of candidiasis. Non-pathogen species of candida such as *C.albicans* are part of natural microflora of the mouth at the range of 17-50% **[1]**. Candida is a genus of yeasts. Many species are harmless commensals or endosymbionts of hosts including humans, but other species, or harmless species in the wrong location, can cause disease. *Candida albicans* can cause infections (candidiasis or thrush) in humans and other animals, especially in immunocompromised patients **[2]**. Down syndrome (DS) also known as trisomy 21, is a chromosomal condition caused by the

presence of all or part of a third copy of chromosome 21 **[3]**. Down syndrome is the most common chromosome abnormality in humans. It is typically associated with a delay in cognitive ability and physical growth, and a particular set of facial characteristics **[3]**. 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 **[4]**. So, measurement of the antifungal activity of

drugs in vitro using the sensitivity testing can be useful to treat patients with the syndrome. There are many routes for measurement the antifungal activity of drugs in vitro that use of broth medium is one of the most common ways **[5]**. But 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 this study we compared effect of Clotrimazole and *Myrtus Communis* against *Candidia krusei* Isolated from Oral Mucosa of Patients with Down's syndrome. *Myrtus Communis* is a small genus belonging to the *Myrtaceae* family which includes approximately 100 genera and 3000 species growing in temperate, tropical and subtropical regions **[6]**. It is an evergreen shrub that grows to a height of about 1-5 meter. The oppositely arranged leaves are ovate-lanceolate, 2- 5 cm long, coriaceous, glabrous, punctuate-glandular and entire. When crushed, they have a delicate aromatic odor. White, star-like flowers, which have five petals, five sepals and a mass of tufted stamens, appear from June to September. The leaves of this plant are contained terpinolen, tannin, flavonoid and vitamine C **[7]**.

MATERIALS AND METHODS

For extracting *Myrtus Communis*, 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 Clotrimazole 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) **[8]**. 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 **[8]**.

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 Clotrimazole and extract of *Myrtus Communis* 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 12^{th} 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 Clotrimazole and extract of *Myrtus Communis* were calculated **[8]**.

RESULTS

In current 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, 19 cases (23.33%) were *Candidia krusei* **(Table 1)**.

MIC50 of Clotrimazole of 2, 4 and 13 cases were 0.25, 0.5 and 2 µg/ml respectively for *Candidia krusei* isolates and MIC50 of *Myrtus Communis* of 1, 7 and 11 cases were 1, 1.5 and 2 µg/ml respectively for *Candidia krusei* isolates **(Table 1)**.

Table 1: MIC values obtained from C.albicans strains for Clotrimazole and *Myrtus Communis*

Treatment	Strain	N	MIC_{50} (µ/ml)
Clotrimazole	**C. *krusei***	**4**	**0.5**
		2	**0.25**
		13	**2**
Myrtus Communis	**C. *krusei***	**1**	**1**
		7	**1.5**
		11	**2**

DISCUSSION

Oral candidiasis (also known as oral candidosis, oral thrush, **[9]** oropharyngeal candidiasis, moniliasis, **[10]** candidal stomatitis, muguet) is candidiasis that occurs in the mouth. That is, oral candidiasis is a mycosis (yeast/fungal infection) of Candidaspecies on the mucous membranes of the mouth. *Candida albicans* is the most commonly implicated organism in this condition. *C. albicans* is carried in the mouths of about 50% of the world's population as a normal component of the oral microbiota **[11]**. This candidal carriage state

is not considered a disease, but when *Candida* species become pathogenic and invade host tissues, oral candidiasis can occur. This change usually constitutes anopportunistic infection of normally harmless micro-organisms because of local (i.e., mucosal), or systemic factors altering host immunity. 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 **[12, 13]**. 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 **[14]**. 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, special attention should be focused **[15]**. 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 **[16]**. By the results have shown that *Myrtus Communis* contained antifungal activity against *Candida krusei*. In current study MIC50 of Clotrimazole of 2, 4 and 13 cases were 0.25, 0.5 and 2 µg/ml respectively for *Candidia krusei* isolates and MIC50 of *Myrtus communis* of 1, 7 and 11 cases were 1, 1.5 and 2 µg/ml respectively for *Candidia krusei* isolates, It means that, *C. krusei* are more susceptible for Clotrimazole than *Myrtus Communis*. 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 **[17]**. 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 **[18]**.

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