Evaluation of inhibitory effects of cuminum cyminum oil on the fluconazaole resistant and susceptible Candida albicans isolated from HIV patients in Iran

Salari S.1, 3, Khosravi A.R. 1*, katiraee F.1, Ayatollahi Mousavi1 S.A.2, Shokri H3., Nikbakht Borujeni GH4

1- Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2- Faculty of Medicine, Medical University of Kerman, Department of Medical Mycology and Parasitology, Kerman, Iran
3- Faculty of Veterinary Medicine, University of Mazandaran, Amol, Iran.
4- Departemant of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, samirasalari73@yahoo.com

Abstract: Oropharyngeal Candidiasis (OPC) continues to be considered the most common opportunistic fungal disease in HIV/AIDS patients globally. The present study was undertaken to determine the antifungal susceptibility of Candida species isolates were obtained from Iranian PLWH (people living with HIV) and cultured on CHROMagar and Sabouraud's dextrose agar. All isolates were identified according to assimilation profile, germ tube, colony color and other conventional methods. Disk diffusion testing and Broth Micro dilution of Fluconazole according to the methods described in CLSI was performed. In addition, Cuminum cyminum essential oil was used to evaluation in vitro activity its against against fluconazole resistant and susceptible Candida albicans .In our study, C. albicans (50.2%) and C. glabrata (22%) were the most frequent isolated, from these isolates, 25.7% were resistant to fluconazole (MIC ≥ 64 µg/ml. Complement data showed mean MIC, 0.575% ± 0.6810% (range: 0.25%-2%) for Cuminum cyminum essential oil to Fluconazole-resistant Candida albicans isolates and mean MIC, 0.306% ± 0.2640% (Range, 0.125%-0.5%) for susceptible Candida albicans isolates with significantly difference ($p<0.001$) .Based on our result we conclude that screening of resistance candida isolates in clinical laboratory is idealistic for surveillance of antifungal resistance to patient's managements, And Cuminum cyminum essential oil having antifungal properties, can be helpful to treat of candidiasis.

[Salari S, Khosravi A.R, katiraee F. Ayatollahi Mousavi , Shokri H, Nikbakht Borujeni. **Evaluation of inhibitory effects of cuminum cyminum oil on the fluconazaole resistant and susceptible Candida albicans isolated from HIV patients in Iran.** Journal of American Science 2012;8(5):54-60]. (ISSN: 1545-1003). http://www.americanscience.org. 8

Keywords: oral candidiasis; HIV patient, candida species, azoles resistance isolates, Cuminum cyminum essential oil

1. Introduction

Oropharyngeal candidiasis (OPC) is the most common opportunistic fungal infection in human immunodeficiency virus (HIV)-infected patients and other immunocompromised hosts. Oral cavity of PLWH (people living with HIV) usually colonized by candida species and they are the major causes of fungal infections in PLWH (Pelletier et al., 2000; Terai. and Shimahara, 2009; Fidel and Huffnagle, 2005). Anibal Paula, et al mentioned Many predisposing factors have been identified as important in the development of oral candidiasis, including malnourishment, common endocrine disorders, such as diabetes mellitus, antibacterial drug therapy, corticosteroids, radiotherapy and other immunocompromised conditions, such as acquired immunodeficiency syndrome (AIDS) (Anibal., 2010).Antifungal drug resistance is a big problem in the immunocompromised patients (Jabra-Rizk et al., 2004; Morgan, 2005).

The increased reports of antifungal resistance and expanding drug therapy options prompted the determination of antifungal susceptibility profile (White et al., 2002). Despite of presentation of expanded antifungal drugs and HAART, oral candidiasis especially refractory type are common in PLWH. It is obvious that susceptibility profile of Candida spp. isolated from PLWH with and without clinical sign of oral candidiasis is invaluable for prophylaxis and treatment of the exact patients.The increasing amplitude and greatness of antifungal resistance special fluconazole. Despite some difficulties for identifying exactly resistant antifungal clinical isolates on an individual basis, the disk diffusion method may be a useful tool and was developed in order to provide a simple inexpensive method for monitoring antifungal susceptibility in a variety of laboratory settings (Espinel- Ingroff et al., 1998; Pfaller et al., 1992; Barry and Brown.,1996; et al., 2003).

There are 2600 plant species of which more than 700 are noted for their uses as medicinal herbs. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in

developed as well as in developing countries, because plant-derived drugs have been reported to be safe and without side-effects (Bansod and Rai, 2008).Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, more than yeasts (Sartoratto et al., 2004).

Cumin (Cuminum cyminum L.) is an aromatic plant included in the Apiaceae family and is used to flavor foods, added to fragrances, and used in medical preparations (Iacobellis et al., 2005). Its fruit, known as cumin seed (zire in iran) and possesses numerous medicinal properties. It is an aromatic herb and an astringent that benefits the digestive apparatus. It has been used in the treatment of mild digestive disorders as a carminative and eupeptic, as an astringent in broncopulmonary disorders, and as a cough remedy, as well as an analgesic (De et al., 2003).

Iran has a rich diversity of plants and several studies of new therapies with medicinal plants has been made to test the activity of their extracts, essential oils and active fractions against these microorganisms. Many years in Iranian traditional medicine seeds of the cumin plant (Cuminum cyminum L.) have been used.

The main purpose of this study was to determine the antifungal susceptibility of Candida albicans to fluconazole by using the Disk Diffusion and microdilution broth methods ,as well as evaluation antifungal activity Cuminum cyminum L against fluconazole resistant and susceptible Candida albicans isolated from the oral cavity of HIV patients in Iran.

2. Material and methods:

Isolates and growth of cultures:

The *Candida* species used in this study obtained from 150 People living with HIV in Imam Khomeini Hospital, Tehran, Iran. Oral lesions were clinically diagnosed for each individual and oral specimens were taken by clinician using sterile cotton-stick swab from lesions, tongue or buccal mucosal, and were spread on Sabouraud's dextrose agar plates (Merck) and CHROMagar™ *Candida* (Paris France company) directly. CHROMagar plates were cultured for primary diagnosis and differentiation of *Candida* isolates. A wet mount with 10% KOH was used for microscopic identification of pseudo hyphae and yeast cell forms. The Sabouraud's dextrose agar plates for yeast isolates probably do not have ability of growth on CHROMagar were incubated aerobically at 30°C for 7 days. CHROMagar culture for identification of colony form and color, were incubated at 35°C for 72h in dark condition. After incubation period, the yeasts were identified based on morphological features and growth parameters (Odds and Bernaerts ,1994; Odds and Davidson , 2000).

Germ tube test was performed with fresh Rabbit serum and fresh yeast colony and incubated at 37°C for 3h. For evaluation of Chlamydospore and filamentous forms production, isolates were cultured on dallmau plates (cornmeal -Tween 80 agar) for 48h at 30°C (Kurtezman and Fell, 1998). The ability of the isolates to assimilate carbohydrate sources was determined with Rap ID™ yeast identification system (remel) according to the manufacture, s instruction (Sanguinetti et al., 2007). For differentiation of *C. dubliniensis* from *C. albicans* in addition to colony color on CHROMagar, we used ability of growth on 45°C and Chlamydospore production on Casein agar plates (Mosca et al., 2003)

Susceptibility testing:

Disk diffusion testing (DD) of fluconazole according to the methods described in Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) document M44-A was performed (Wayne, 2004; Hazen et al., 2003) .We used *candida* standard strains including *candida albicans* (ATCC10231) .Muller Hinton Agar (MHA) plates (150-mm diameter) supplemented by 2% Glucose and methylene blue (GMB) at a depth of 4.0 mm were used. The agar surface was inoculated by using a swab moistened in a cell suspension adjusted to the turbidity of a 0.5 McFarland standard. The plates were incubated in air at 35 °C and read at 24 h. Zone diameter endpoints were read at 80% growth inhibition. MIC testing was performed by CLSI Broth Micro dilution (BMD) reference method M27-A2 (Wayne, 2002). The interpretive criteria for disk diffusion test were those published by CLSI (Wayne, 2004).

The MICs for fluconazole was determined by micro dilution method as described by Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) M27-A methodology with RPMI 1640 buffered to pH 7 by using MOPS. The MIC90 was determined as the lowest concentration that produced at least 90% inhibition compared to the growth of the control well. An interpretive susceptibility criterion for fluconazole was those recommended CLSI. For fluconazole, susceptible is, MIC of $\leq$ 8 µg/ml; susceptible dose dependent, MIC of 16 to 32 µg/ml; resistant, MIC of $\geq$ 64 µg/ml. (Rex et al., 1997; Matar et al., (2003).

Preparation Essential oil

The cumin oil used in this study was obtained from Barij Essence Pharmaceutical Company, Kashan, Iran. In Barij Essence Pharmaceutical Company the essential oil of the seeds was produced

by the Clavenger apparatus, using the hydrodistillation method. The dried powdered seeds of cumin (50 g) were placed in a distillation apparatus with 1 L of distilled water and hydrodistilled for three hours. Then its chemical composition was analyzed by gas chromatography (GC) and GC-mass spectrometry (MS) (Davies, 1990). Cumin oils were stored in sealed brown vials in refrigerator temperature at 4 ºC until required.

Isolates for determination of antifungal activity of Cuminum cyminum:

Forty *Candida albicanse* species (fluconazole susceptible and resistant isolates) were selected.

Detetmination of Minimum Inhibitiory Concentration and Minimum fungicidal Concentration of Growth of Candida albicans:

MIC and MFC were determined according to the reference documents M27-A for yeasts with modifications (Wayne, 2002). Briefly, a series of twofold dilutions of cumin, ranging from 8 (%V/V) to 0.015 (% V/V) was prepared in sabouraud dextrose agar (SDA) (Merck) with 2% (V/V) dimethyl sulphoxide (DMSO). These experiments were performed in duplicate as well.

All of the *C. albicans* isolates were grown on Sabouraud glucose agar (Merck, Darmstadt, Germany) at 35 C for 48 hours. Then Yeast cells colonies were suspended in 3 ml of sterile water. The resulting yeast suspension was mixed for 15 seconds with a vortex. The suspension was adjusted to make a yeast concentration of 1×10^6 cell/ml by counting with a hemacytometer. After preparation a suspension of this yeast (described above), the stock solution of these essences prepared with 2% DMSO. Then 1ml of this stock solution combined with Sabouraud glucose broth to prepare diluted compound. In follow step, 1 ml Sabouraud glucose broth added to eleven strill twisted tip tubes and 1 ml of diluted compound added to the first tube and after shaking, 1 ml of this solution added to the second tube. This work continued to ninth tube. At final after mix, 1 ml of mixture of essence diluted compound and culture medium removed from number nine tubes. After this step, 0.5 ml (2000 CFU/ml) of fungal suspension added to first ten tubes and at final these Inoculated plates were incubated at 35°C until in growth control tube observed opaque or turbidity after 24 and 48 hours. At final, the last transparent tube is indicated our Minimum Inhibition Concentration that for confirmation this result. In this test, the eleventh tube contain SDA with DMSO as negative control and tenth tube contain (SDA, with 2% (v/v) DMSO but no oil, was used as positive growth control. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of yeast on the agar plate end of 48 h period incubation. The MFC were determined 0.01 ml from each tube without visible growth in the MIC test on sabouraud dextrose agar (SDA) (Merck) plates. The lowest concentration of the cumin at with negative growth was indicated was the MFC point.

3. Statistical Analysis:

T-test was used to assess the dates.

Table 1: Prevalence of Yeast species in PLWH patients in this study.

Species	Frequency(n)	Percent%
C. albicans	103	50.2
C. glabrata	45	22
C. dubliniensis	9	4.4
C .tropicalis	7	3.4
C. kefyr	7	3.4
C. parapsilosis	6	2.9
C. famata	2	1.0
C. guilliermondii	1	0.5
C. krusei	1	0.5
Saccharomyces	2	1.0
Trichosporon	2	1.0
Other yeasts	1	0.5
C. species	19	9.3
Total	205	100.0

4. Results:

Among 150 patients, 60% presented with clinically detectable oropharyngeal candidiasis (based on clinical presentation, direct microscopic examination and culture). Yeasts were isolated from 17.3% of patients without clinical sign. In total, the carriers of yeasts were 77.3% (116/150) and culture negative results were obtained from 22.7% of the patients. Based on Table 1 *C. albicans* (50.2%) was the most frequent of yeast isolated, following *C. glabrata* (22%). Non *Candida albicans* species were isolated from 71 positive cultures (61%). Forty percent of patient with OPC had a history of antifungal therapy. The patients with history of antifungal therapy showed significant increase in OPC ($p < 0.05$).

Two hundred isolates of Candida species were analyzed for susceptibility to fluconazole. The in vitro susceptibilities of 150 oral Candida isolates to fluconazole are summarized in Tables 2. According to disk diffusion method of 105 Candida albicans isolates tested, 25.7% were resistant to fluconazole (MIC $\geq$ 64 µg/ml), respectively. The values of MIC-90% and MIC range with BMD method for the isolates representative of the most frequently isolated species of *Candida* are shown in Table 3. MIC- 90% of fluconazole for *Candida*

albicans isolates was 8µg/ml. Regarding the *candida albicans* (ATCC 10231) it appeared as dose dependent susceptible yeast to azole drugs under study.

Fluconazole-resistant *Candida albicans* isolates showed mean MIC , 0.575% ± 0.6810% (range: 0.25%-2%) and susceptible *Candida albicans* isolates mean MIC, 0.306% ± 0.2640% (Range, 0.125%-0.5%) to *Cuminum cyminum* essential oil.The antifungal activities of cumin oils against fluconazole-resistant and susceptible *Candida albicans* isolated from the oral cavity of HIV patient obtained by the broth macro dilution method are shown in Table 3.

Table 2. Susceptibility and Minimum Inhibitory concentration (MIC) in µg/ml of Fluconazole obtained for Candida species obtained from oral cavity of Iranian PLWH.

Antifungal Drug	Species	MIC Rang	MIC90 µg/ml	Disk Diffusion		
				S%	S-DD%	R%
Fluconazole	C. albicans	1-128	8	55.2	19.1	25.7
	C. glabrata	2-128	128	37.9	4.4	57.7
	C. dubliniensis	1-128	4	77.8	11.1	11.1
	C. tropicalis	2-128	8	56.2	17.7	26.2
	C. parapsilosis	1-128	16	60	16.7	23.3
	C. kefyr	2-128	4	67.1	0	32.9
	C. species	1-128	16	64	25.2	10.8

Table 3: Mean Minimum inhibitory concentration and minimum fungicidal concentration data of fluconazole-resistant and susceptible Candida isolates obtained by the broth macro dilution method.

Isolates	MIC	MFC	Range
fluconazole-resistant Candida species	0.575% ± 0.6810	0.762	0.25%-2%
fluconazole-susceptible Candida species	0.306% ± 0.2640	0.400	0.125%-0.5%)

5. Discussion

The emergence of antifungal resistance *candida* species and immunucompromised patients are more important factors involve in epidemiology of oral candidiasis. Determine of susceptibility profile and emerging resistance is important, because antifungal resistance is a major problem for treatment. In this study , we used disk diffusion and microdilution broth procedure for identification susceptibility to fluconazole in 150 *candida* species, then detection of antifungal activity of cumin oil on 40 fluconazole-resistant and susceptible Candida species isolated from Iranian PLWH. A diversity of non-*albicans* species of *Candida* has been reported, including *Candida tropicalis*, *Candida parapsilosis*, *Candida guillermondii*, *Candida glabrata*, and *Candida dubliniensis*. Among the species *C. dubliniensis* associated with the cause of OPC in HIV infection (De Repentigny et al., 2004).

Fluconazole resistance is well documented by investigators and resistance is associated with prolonged utilization of azoles (Lewis et al., 1998). Nevertheless it could be expected fluconazole do not show good efficacy against *candida* species especially *C. albicans* isolates, as 25.7% of *C. albicans* isolates demonstrated resistance to fluconazole. This finding are not consist to those reported by other authors, however many studies have reported the incidence of fluconazole resistance, but rate of resistance have reported lower than this study (Badiee et al., 2009; Haberland-Carrodeguas et al.,2002, Bailey et al., 1996). The rank of susceptibility to fluconazole was *C. dubliniensis*> *C. kefyr* > *C. parapsilosis* > *C. tropicalis* >*C. albicans* > *C. Krusei* > *C.glabrata* for the most prevalent species.

Resistance to fluconazole often was observed in patients with history of using of fluconazole for prevention or previous episode. Unfortunately, because it is difficult to obtain the new antifungal drugs so the patients have to use fluconazole therapy. Physicians should be tendency and attention to prescription of new antifungal drugs, and dependent organization should be having approach for entrance or production of new antifungal drugs in Iran.

Because increase of mentioned resistance of candida isolates to antifungal drugs administrated, relapse of Candida infections, the high cost and limited number effective of antifungal drugs., toxicity of the available antifungal drugs and other side effects these drugs and the increasing incidence of systemic fungal infections, with consequent increase in mortality, The management of Candida infections faces is difficult . Thus, it is necessary to search for new compounds from other sources, including medicinal plants to act against these microorganisms with respect to spectrum, potency, safety and pharmacokinetic properties, but in a selective and little or no toxic effect (Anibal et al., 2010; Naeini et al., 2009; Derakhshan et al., 2010).

Agar disc diffusion, agar and broth dilution methods are also commonly used to assess antimicrobial activity to many of different plant extracts and essential oil .However results of these assays due to composition of the plant oils and extracts, local climatic, environmental conditions growth, differences in microbial growth, exposure of micro-organisms to plant oil, the solubility of oil or oil components and the use and quantity of an emulsifier, were much differed (Bansod and Rai, 2008).

The oil tested exhibited different degrees of antifungal activity against fluconazole-resistant and susceptible Candida species isolated from the oral cavity of HIV patients. In this study, 40 *Candida albicans* isolates were tested. Fluconazole-resistant

Candida albicans isolates obtained from oral cavity patients by the broth macro dilution method were shown mean MIC, 0.575% ± 0.681%, range, %0.25-0.2% and susceptible Candida species isolated mean MIC, 0.306% ± 0.2640%, ranges 0.125%-0.5%.In our study dates suggest that the mount of cumin oil for inhibition growth in Fluconazole-suspictible *Candida albicans is higher than* fluconazole-resistant.

Aligiannis et al. proposed a classification for plant materials, based on MIC results as follows:

- Strong inhibitors (MIC up to 500 mg/ml);

-Moderate inhibitors (MIC between 600 and 1500 mg/ml);

- Weak inhibitors (MIC above 1600 mg/ml) (Aligiannis et al., 2001).

Based on Aligiannis's classification, we found that essential oil extracted from Cumin demonstrated strong antifungal activity on Fluconazole- susceptible and Fluconazole-resistant *Candida albicans* isolates in present study.

To date, various publications have documented the antimicrobial activity of essential oils and plant extracts including *Zataria multiflora, Geranium herbarum , Artemisia Siberia, Origanum vulgare L. and* Cinnamomum zeylanicum. They believed to use of this natural products as alternative treatment useful in therapy fluconazole resistant *candida albicans* isolates obtained various from Candidal infection (Katitaee et al., 2008; Akbari, 2007; Quale et al., 1996).

Results of Devkatte study and Derakhshan, et al were shown oils of plant origin have positive effect on Fluconazole-resistant *Candida* (MTCC 227) and *Klebsiella pneumoniae* (Devkatte et al., 2005; Derakhshan et al., 2010).

Similarly, in vitro antimicrobial activity of cumin against human pathogenic fungi such as *Aspergillus parasiticus* and yeasts and Gram-positive and Gram-negative bacteria and commensally bacteria was studied by Iacobellis et al (Iacobellis et al., 2005).The oils of *Cuminum cyminum* , showed comparatively low activity against *A. niger* and *A. fumigates* and toxic effect to Aspergillus and high antifungal activity in Singh investigate (Bansod and Rai, 2008; Iacobellis et al., 2005; Nigam and Rao, 1977; Singh, 2001).

The composions of *C. cyminum* essential oil identified by GC-MS analyses were shown in **Table 4**. the main components of *C. cyminum* oil were *â*-pinene (30%), Limonene (21%), 1, 8 cineol (18.5%), Linalool (10%), Linalil acetate (4%).whereas according Iacobellis's research The antimycotic activity of cumin due to presence of large amount (16.1%) of cumin aldehyde in *Cuminum cyminum L* .They showed antifungal effect this essential oil against Gram-positive and Gram-negative bacterial species and potential and advised use it for the control of bacterial diseases (Iacobellis et al., 2005).

Table 4: The compositions of C. cyminum essential oil identified by GC/MS.

Cuminum cyminum		No.
30%	α- Pinene	1
21%	Limonene	2
18.5%	1,8 cineol	3
10%	Linalool	4
4%	Linalil acetate	5
3%	α - Terpineol	6
1.5%	α - Terpineol acetate	7
1.5%	Geraniol	8
1.2%	Methyl eugenol	9
0.5%	Sabinene	10
0.5%	Terpinene-4 ol	11
0.5%	Terpinolene	12
0.5%	γ- Terpinene	13
0.3%	ρ-Cymene	14
0.2%	α- Thujene	15
0.1%	Myrcene	16
0.08%	γ – Terpineol	17
-	-	18
-	-	19
-	-	20
93.27%	-	Total

In this study, *α-pinene* and *Limonene* were found in high concentration in *Cuminum cyminum* oil and possess antimicrobial activity. High concentrations of *α-pinene* and *Limonene* in the essential oil are probably an explanation for the antimicrobial activity possessed by *Cuminum cyminum* against candida species. α-*pinene* destroys the cellular integrity of Gram positive bacteria and inhibit respiratory activity in yeast mitochondria and had some antifungal activity but Gram negative bacteria were more resistant to it (Andrew et al., 1980).

In summary, we have presented data on species distribution and antifungal susceptibility profiles of *Candida* albicanse isolates to *Cuminum cyminum* oil obtained from PLWH . Based on result we conclude that screening of resistance *candida* isolates by disk diffusion or broth dilution methods in clinical laboratory is idealistic for surveillance of antifungal resistance to patients' managements. Also, our results confirmed the use of cumin can helpful to treatm of some diseases as candidiasis. Further studies are

needed to evolutions of this essential oil against candidiasis in clinical approach.

Corresponding author:
Ali Reza Khosravi
Samira salari
Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
E-mail: samirasalari73@yahoo.com

References:

1. Akbari S (2007). Evaluation antifungal activity of *Thymus vulgaris* L. and *Origanum vulgare* L against Fluconazole-resistant and susceptible *Candida albicans*, 53-62.
2. Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB (2001). Composition and antimicrobial activity of the essential oils of two Origanum species. J Agric Food Chem; 40:4168-70.
3. Andrew RE, Parks LW, Spence KD. Some effects of *Douglas fir* on certain microorganisms. Appl Environ Microbiol. 1980;40:301–4.
4. Anibal Paula Cristina, de Cássia Orlandi Sardi Janaina, Peixoto Iza Teixeira Alves, de Carvalho Moraes Julianna Joanna, Höfling José Francisco (2010). conventional and alternative antifungal therapies to oral candidiasis, brazilian journal of microbiology 41: 824-831).
5. Badiee P, Alborzi A, Shakiba E, Ziyaeyan M, Rasuli M., (2009). Molecular Identification and In-Vitro Susceptibility of *Candida albicans* and *C. dubliniensis* Isolated from Immunocompromised Patients. Iranian Red Crescent Medical Journal, 11(4):391-397
6. Bailey DA, Feldmann PJ, Bovey M, Gow NA, Brown AJ. (1996). The Candida albicans HYR I gene, which is activated in response to kyhal development, belongs to a gene family of yeast cell wall proteins. J Bacteriol, 178: 5353 - 60.
7. Bansod S, Rai M (2008) Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic Aspergillus fumigatus and A. niger, World Journal of Medical Sciences 3 (2): 81-88.
8. Barry AL Brown SD (1996). Fluconazole disk diffusion procedure for determining susceptibility of *Candida* species. J Clin Microbiol, 34(9):2154-7.
9. Davies, N.W. (1990) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon, and Carbowax 20m phases. *J. Chromatogr. 503: 1-24*.
10. De M, De AK, Mukhopadhvay R, Banerjee AB, Micro M (2003). Antimicrobial activity of *Cuminum cyminum* L. Ars Pharmaceutica; 44:257-69.
11. Derakhshan Safoura, Sattari Morteza, Bigdeli Mohsen (2010). Effect of cumin (*Cuminum cyminum*) seed essential oil on biofilm formation and plasmid Integrity of *Klebsiella pneumonia*. pharmacy magazine: 6 (21) p: 57-61.
12. De Repentigny L, Lewandowski D, Jolicoeur P. (2004). Immunopathogenesis of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infection, Clin Microbiol Rew, Oct; 729–59
13. Devkatte, A.N., Zore, G.B., Karuppayil, S.M.(2005).Potential of plant oils as inhibitors of *Candida albicans* growth, FEMS Yeast Research Volume 5, Issue 9, P: 867-873.
14. Espinel-Ingroff A, Barchiesi F, Hazen KC, Martinez-Suarez JV, Scalise G., (1998). Standardization of antifungal susceptibility testing and clinical relevance. Med Mycol. 1998;36 Suppl 1:68-78.
15. Fidel PL and Huffnagle GB (2005). Fungal immunology from an organ perspective. New York, Springer Science. pp 59-83.
16. Haberland-Carrodeguas C, Allen CM, Beck FM, Buesching WJ, Koletar SL, Sundstrom P. (2002). Prevalence of fluconazole resistant strains of *Candida albicans* in otherwise healthy outpatients. J Oral Pathol Med, 31: 99-105.
17. Hazen KC, Baron EJ, Colombo AL, Girmenia C, Sanchez-Sousa A, del Palacio A, de Bedout C, Gibbs DL; Global Antifungal Surveillance Group (2003). Comparison of the susceptibilities of *Candida* spp. to fluconazole and voriconazole in a 4-year global evaluation using disk diffusion. J Clin Microbiol, 41:5623–32.
18. Iacobellis NS, Lo Cantore P, Capasso F, Senatore F (2005) Antibacterial activity of Cuminum cyminum L. and Carum carvi L. essential oils. J Agric Food Chem;53:57-61
19. Jabra-Rizk M.A., Falker W.A., Meiller T.F (2004). Fungal Biofilms and Drug Resistance. Emerging Infectious Diseases, 10 no 1 www.cdc.gov/eid
20. Katitaee F ,Eidi S, Bahonar A, Zarin Far H ,Khosravi A.R, (2008). Comparison the minimum inhibitory concentration (MIC) Some native plant essential oils on growth against azole susceptible and resistant Candida species, Journal Of Medicinal Plants, 37-44.
21. Kurtezman, C.P., and J. W. Fell (1998). The Yeast, a taxonomic study, 4Th ed. Elsevier science, BV, Amsterdam, The Netherlands.
22. Lewis RE, Kepser ME, Pfaller MA., (1998). Update on clinical antifungal susceptibility testing for *Candida* species. Pharmacotherapy, May – June; 18 (3): 509 – 15.
23. Matar MJ, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH

(2003).Correlation between ETest, Disk Diffusion, and Microdilution methods for antifungal susceptibility testing of fluconazole and voriconazole. Antimicrob Agents Chemother, 47:1647-51.
24. Morgan J., (2005). Global trends in candidemia: review of reports from 1995–2005. Curr Infect Dis, Rep 7:429–439.
25. Mosca CO, María D. Moragues, José Llovo, Asmaa Al Mosaid, David C. Coleman, and José Pontón (2003). Casein Agar: a Useful Medium for Differentiating Candida dubliniensis from Candida. J Clin Microbiol. March; 41(3): 1259–1262.
26. Naeini, A.R. Khosravi, M. Chitsaz, H. Shokri, M. Kamlnejad, (2009). Anti-Candida albicans activity of some Iranian plants used in traditional medicine, Journal de Mycologie Médicale 19, 168-172.
27. Nigam, S.S., T.S.T. Rao, (1977). Antimicrobial efficacy of some Indian essential oils. In L.D. Kapoor and Ramakrishnan, (Eds), Advance in essential oil Industry, New Delhi: Today's and Tomorrow's printer and publishers, pp: 177-180.
28. Odds FC, Bernaerts R (1994) CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J Clin Microbiol, 32 :1923–9.
29. Odds FC, Davidson A. (2000). "Room temperature" use of CHROMagar *Candida*. Diagn Microbiol Infect Dis, Nov; 38(3):147-50.
30. Pelletier R, Peter J, Antin C, Gonzalez C, Wood L, Walsh TJ.. (2000). Emergence of Resistance of *Candida albicans* to Clotrimazole in Human Immunodeficiency Virus-Infected Children: In Vitro and Clinical Correlations. J Clin Microbiol., Apr: 1563–1568.
31. Pfaller MA, Dupont B, Kobayashi GS, Müller J, Rinaldi MG, Espinel-Ingroff A, Shadomy S, Troke PF, Walsh TJ, Warnock DW (1992). Standardized susceptibility testing of fluconazole: an international collaborative study. Antimicrob Agents Chemother, 36(9):1805-9.
32. Pfaller MA, Diekema DJ, Boyken L, Messer SA, Tendolkar S, Hollis RJ. (2003). Evaluation of the Etest and disk diffusion methods for determining susceptibilities of 235 bloodstream isolates of *Candida glabrata* to fluconazole and voriconazole. J Clin Microbiol, 41:1875–80.
33. Quale JM, Landman D, Zaman MM, Burney S, Sathe SS, (1996).In vitro activity of Cinnamomum zeylanicum against azole resistant and sensitive Candida species and a pilot study of cinnamon for oral candidiasis. Am J Chin Med.; 24(2):103-9.
34. Rex JH, Pfaller MA, Galgiani JN, Bartlett MS, Espinel-Ingroff A, Ghannoum MA, Lancaster M, Odds FC, Rinaldi MG, Walsh TJ, Barry AL. (1997). Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro–in vivo correlation data for fluconazole, Itraconazole, and candida infections. Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards. Clin Infect Dis, 24: 235–47.
35. Sanguinetti, M., Porta, R., Sali, M., La Sorda, M., Pecorini, G., Fadda, G., Posteraro, B. (2007). Evaluation of VITEK 2 and RapID Yeast Plus Systems for Yeast Species Identification: Experience at a Large Clinical Microbiology Laboratory. J. Clin. Microbiol. 45: 1343-1346.
36. Sartoratto A; Machado Ana Lúcia M.; Delarmelina Camila; Figueira Glyn Mara; Duarte Marta Cristina T.; Rehder Vera Lúcia G (2004). composition and antimicrobial activity of essential oils from aromatic plants used in brazil, brazilian journal of microbiology 35:275-280.
37. Singh, N., (2001). Trends in the epidemiology of opportunistic fungal infections: predisposing factors and the impact of antimicrobial use practices. Clinical Infect Diseases, 33(10): 1692-1696.
38. Terai H. , Shimahara M., (2009). Usefulness of culture test and direct examination for the diagnosis of oral atrophic candidiasis. International Journal of Dermatology, 48: 371–373.
39. Wayne P (2002). National Committee for Clinical Laboratory Standards. Reference method for broth dilution testing of yeasts. Approved standard, 2nd ed., M27-A2. National Committee for Clinical Laboratory Standards.
40. Wayne P (2004). National Committee for Clinical Laboratory Standards. Method for antifungal disk diffusion susceptibility testing of yeasts: approved guideline M44-A. National Committee for Clinical Laboratory Standards.
41. White TC, Hollemann S, Dy F, Mirels LF, Stevens DA. (2002). Resistance mechanisms in clinical isolates of *Candida albicans*. Antimicrobial Agents Chemother, 46: 1704 - 13.

04/23/2012