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Original Article

Antifungal effect of sesame medicinal herb on *Candida* Species: original study and mini-review

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The aim of this study was to evaluate the antifungal susceptibility patterns of three antifungals, methanolic extracts and N-hexane oil of sesame seeds on *C. albicans* and *C. glabrata,* isolated from oral cavity of liver transplant recipients. The results were compared with other reports to develop a mini review as well. *Candida* species were isolated from liver transplant recipients. To evaluate the antifungal activity of sesame seed oil and methanolic extract, fluconazole, caspofungin and nystatin, the corresponding minimum inhibitory concentrations were determined by CLSI M27-A3 standard method. Minimum fungicidal concentration was also evaluated. The most prevalent species was *C. albicans*, followed by C. *glabrata*. Findings indicated sensitivity to antifungal agents and resistance to methanolic extract and N-hexane oil for all *C. albicans* and *C. glabrata* isolates. The rate of *Candida* colonization in the oral cavity of liver transplant recipients was high. Our results revealed that the methanolic and N-hexan extracts of sesame seeds are not effective on *C. albicans* and *C. glabrata* species, isolated from the patients. The sesame seed oil pulling and mouthwash cannot effectively cleanse and remove the *Candida* species in the mouth. Investigation of other medicinal plants or other parts of sesame like leaves and roots are suggested.

Keywords: *Candida* species/ antifungal susceptibility. Sesame/study. Liver transplant.

INTRODUCTION

Sesame seed oil is popular edible oil in some parts of the world like North India, Iran, and Japan (Bedigian, 2004). It was cultivated since more than 5000 years ago and the *Sesame radiatum* and *Sesame indicum* are usually cultivated for their seeds and leaves. Antifungal and antibacterial activities were reported for sesame oil (Shittu *et al.*, 2006; Shittu *et al.*, 2007). Oral candidiasis is an opportunistic infection with frequency of 65% in the elderly wearing dentures (Nurdiana, Jusri, 2009) and 16.7% in patients with hematologic disorders (Badiee *et al.*, 2017). *Candida* species are commensal organism in human oral cavity and can transfer to pathogenic form (oral or systemic candidiasis) in immunocompromised patients (Badiee, Alborzi, 2011; Sharon, Fazel, 2010; Tarçın, 2011). During the last two decades, resistance to common antifungal drugs in *Candida* species has increased (Badiee *et al.*, 2011; Badiee *et al.*, 2017b). Nystatin is a routine antifungal agent used in the clinics for the prevention and treatment of oral candidiasis. Fluconazole and caspofungin are important antifungals against human pathogenic yeasts with some side effects and toxicity. The effective and safe antifungal agents against fungi, especially *Candida* species, are needed.

Some investigations have introduced new herbal products with fewer side effects and lower costs. However, there are some controversies about the antifungal properties of such products (Makki, Olama, Holail, 2012; Thaweboon, Nakaparksin, Thaweboon, 2011). Sesame oil can be extracted by a number of methods, depending on the materials and equipment available. The aim of this study was to evaluate antifungal susceptibility patterns of three antifungals, methanolic extracts and N-hexane oil of sesame seeds on *C. albicans* and *C. glabrata,* isolated from oral cavity of liver transplant recipients by micro-

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dilution standard CLSI M27 A3 method. The results could be helpful to the best management of oral candidiasis in such patients. The present study findings were compared with other reports to develop a mini-review as well.

MATERIAL AND METHODS

Test organism

This study was carried out on liver transplant recipients admitted to Nemazee hospital, Shiraz University of Medical Sciences, from January to June 2016. Patients had not received any systemic or local antifungal drugs for one month prior to sampling. A sterile swab was rubbed through the mouth cavity and cultured on Sabouraud Dextrose Agar (Merck, Germany) with chloramphenicol to inhibit the growth of normal oral microflora. Demographic data of the patients were collected from their files. Standard *Candida* species (*C. glabrata* ATCC 2001) was examined along with the test samples processing.

Ethical considerations

This study was approved by Ethics Committee at Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran (Ethic Code 1822).

Identification of the isolated strains

The plates were incubated at 24 °C and monitored for fungal growth every day. *Candida* species isolated from primary cultures, were subcultured to ensure the purity. For DNA extraction, a suspension of the isolated fungi in distilled water (10^5 *Candida*/mL) was prepared in sterile tubes and fungal cell walls were broken using a sonicator (Hielscher, Germany). DNA was extracted by Invisorb (Berlin, Germany) according to the manufacturer's instructions and identified using restriction fragment length polymorphism PCR (RFLP).

PCR was performed using PCR mixture (5 µL of 10x reaction buffer, 1.5 mM $MgCl_2$, 0.4 mM dNTPs, 2.5U of DNA *Taq* polymerase (Cinnagen, Germany), 30 pmol of each ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) primers and 3 µL of the extracted DNA in a final volume of 50 µL. The PCR conditions were as follows: 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min, with a final step at 72 °C for 7 min (Mirhendi *et al.*, 2006). 10 µL of each PCR product was digested with the restriction enzyme Msp I (RR) for two hours at 37°C. Electrophoresis was performed on 2% agarose gel (Sigma, Germany).

Extraction of sesame seeds

Sesame seeds were obtained from market in Shiraz, Iran in July 2016 and sesame seed oil was extracted by Soxhelet extraction method. Crude sesame seeds were air dried for 2 weeks and ground into powder using a grinder. The powders (20 g) were added to 100 mL of absolute N-hexane and 100mL of methanol (80% v/v). The hexane oil extracted with Soxhlet extraction apparatus (Sigma-Aldrich, Germany). Methanol extracts were filtered by Whatman No 1 filter papers (Whatman, USA) and concentrated using a EYELA rotary evaporator (N-1000, Japan), and freeze-dried using the Edwards freeze dryer (Edwards High Vacuum International Crawley, Sussex, England). Stock concentration of 10% in diluted dimethyl sulfoxide in Roswell Park Memorial Institute (RPMI 1640, Sigma, Germany) was prepared for the oil materials (Randhawa, 2008).

Standard antifungal

The standard powder of fluconazole, caspofungin and nystatin were obtained from Sigma-Aldrich (St Louis, MO, USA).

Standardization of micro-organisms

To determine the antifungal activity of agents, the CLSI M27-A3 standard method was used for extracts and three antifungal agents (Wayne, 2008). The *Candid*a species suspension was adjusted spectrophotometrically to 0.5 McFarland (final inoculums concentrations 1.5 x 10^6 CFU/mL) and diluted 1:1000 in RPMI 1640. Ten serial dilutions of each antifungal agent and extract were prepared and mixed with 100 µL of the fungal suspensions. Negative control (well without yeast) and positive control (well without extract, oil or antifungal) were used in each serial of the test. The plates were sealed and incubated at 35°C for 24h and the MIC end-points were determined visually. The MIC is defined as the lowest concentration that causes 50% inhibition of visible fungal growth, compared with positive control. Final concentrations of methanolic and N-hexan solutions of sesame seeds were between 20 and 0.04 µg/mL. Concentration range for fluconazole was from 64 to 0.125 µg/mL, nystatin 18.5 - 0.035 µg/mL and caspofungin from16 to 0.032 µg/mL.

Minimum Fungicidal Concentration (MFC)

To detect MFC, 100 µL of each well and negative and positive controls were cultured on sabouraud dextrose agar plates in triplicate and the rates of yeast growth were counted after 48 hours. The MFC value is the lowest concentration of each extract that inhibits the 99.9% growth of yeast, compared to positive control.

Statistical analysis

Statistical analysis was performed using SPSS 15software (Statistical Product and Services Solutions, Inc, Chicago, IL, USA). P-value ≤ 0.05 was set as the significant threshold for statistical analysis.

RESULTS AND DISCUSSION

During a six-month period, 65 liver transplant recipients including 25 females (39%) and 40 males (61%) were enrolled. Mean age of the participants was 29.8 years. Forty-eight *Candida* species were isolated from 41 recipients (41/65, 63%), while in some more than one species was isolated. The most prevalent species was *C. albicans* (34, 70.8%), followed by C. *glabrata* (9, 18.8%), and other *Candida* species including *C. kefyr, C. tropicalis*, *C. intermadia* (5, 10.4%) (Table I). The most isolated species (including *C. albicans* and *C. glabrata*) were used in this study. The findings indicated no antifungal effect for either methanolic extract or N-hexane oil against all *C. albicans* and *C. glabrata* isolates. The MIC50 and MIC90 values for both extracts and nystatin were 20 µg/mL and 0.035 µg/mL in all species, respectively. The MIC90 values of fluconazole for *C. albicans* and *C. glabrata* were 0.5 µg/mL and 2 µg/mL, respectively. MIC90 values of caspofungin for *C. albicans* and *C. glabrata* were 0.125 µg/mL and 0.5 µg/mL, respectively (Table II). There was a significant difference ($p>0.05$), observed between antifungal activity of sesame seed extract, oil and the three antifungal agents. No antifungal inhibitory of growth or fungicidal activity was seen and yeast growth was observed on all the treated wells.

The rate of oral *Candida* colonization in this study was 63% and *C. albicans* was the most prevalent isolated species (70.8%). These rates in Nazhvani *et al*. (2016) were reported 67.4% and 75%, respectively. The isolated species in this study were sensitive to all antifungal agents and resistant to the sesame methanolic extracts and hexane oil. In liver transplant recipients, use of antifungal agents with low toxicity and high efficacy is important due to their special conditions (many drugs metabolized in the liver)**.** Studies on medicinal plants have demonstrated their antifungal activities, leading to the development of more potent antifungal drugs (Shittu *et al.*, 2006; Shittu *et al.*, 2007). Sesame is reputed in folk medicine and in this study no antifungal activity of methnolic extract and n-hexane oil of sesame seeds on *C. albicans* and *C. glabrata,* clinically isolated from liver transplant recipients, was observed.

There are many controversies about antifungal activity of sesame extracts. According to the literature, the antifungal activity of sesame is dependent on the type of extraction (oil, ethanol, methanol, hexane and aqueous extract) and part of the plant used in the study (leaves, seeds) (Table III). Also, the methodology used in determining sensitivity in the reported data includes Agar-well diffusion (Shittu *et al.*, 2007; Shittu, Shittu, 2012), disc diffusion or microdilution method (Ogawa, Nishio, Okada, 2014). In a study by Shittu *et al.* (2006), ethanoic leave extract of sesame mildly inhibited *C. albicans* growth while aqueous extract showed no inhibitory effect. In another study, stronger antifungal properties were reported for ethanolic extract of sesame leaves than for its aqueous extract (Shittu, Shittu, 2012). Shittu 2008 reported the methanolic extracts of sesame leaves had no antifungal effect against *C. albicans* while the ethanolic and aqueous extracts exhibited inhibitory growth effect (Thaweboon, Nakaparksin, Thaweboon, 2011).

In the present study, no antifungal activitiy was observed in the oil extract of sesame seed. Similarly, no antifungal activity was reported for sesame oil in the literature (Makki, Olama, Holail, 2012; Thaweboon, Nakaparksin, Thaweboon, 2011). Ogawa, Nishio and Okada (2014) evaluated the effects of sesame oil and some other edible oils on 5 *Candida* species. They reported that sesame oil can inhibit the growth of both yeast and mycelium of fungi. Investigation into the antifungal activity of sesame leaves, stems and roots on filamentus fungi showed that most of the extracts had inhibitory effects on the tested filamentus fungi but root and leaf extracts enhanced the growth of *Alternaria sesami,* and *Fusarium oxysporum,* as compared to the controls (Syed *et al.*, 2015).”The inhibition effects of diethylether, chloroform and hexane fractions were higher than that of ethanol fraction remaining after extraction with the other solvents” (Syed *et al.*, 2015).

There have been some studies about physico-chemical compound of sesame extracts. The chemical compounds of sesame oil by Gas Chromatography/ Mass Spectra system contain protein, carbohydrate, vitamin, ash, unsaturated fatty acids (oleic, linoleic),

saturated fatty acids (palmitic and stearic) and minerals (Ca, P, K, Mg, Na, Fe, low amount of Zn) (Alyemeni, Basahy, Sher, 2011; Borchani *et al.*, 2010; Kanu, 2011). According to the literature, Phenolic ingredients, flavonoid subfractions, punicalin, punicalagin, and tannins are the main ingredients responsible for the plant antimicrobial activity (Anibal *et al.*, 2013; Shahat *et al.*, 2001). However, none of such components has been identified in the analytical studies of sesame extracts. Future studies are needed for the identification of antifungal components in the sesame oil and extracts.

The antifungal activity of sesame hairy roots due to

TABLE I - *Candida* species isolated from oral specimens of liver transplant patients

***Candida* Species**	**Number/percent**
C. albicans	34 (70.8)
C. glabrata	9 (18.8)
C. kefyr	2 (4.1)
C. parapsilosis	1 (2.1)
C. tropicalis	1 (2.1)
C. intermedia	1 (2.1)
Total	48 (100)

TABLE II - MIC50 and MIC90 values of methanolic and N-hexane extracts of sesame seeds and antifungals for the isolated *Candida* species

***Candida* species**	**MIC**	**Methanolic extracts**	**N-hexane oil**	**Nystatin**	**Fluconazole**	**Caspofungin**
Candida albicans	MIC50 (µg/mL)	25	25	0.035	0.5	0.063
	MIC90 (µg/mL)	25	25	0.035	0.5	0.125
Candida glabrata	MIC50 (µg/mL)	25	25	0.035	2	0.250
	MIC90 (µg/mL)	25	25	0.035	2	0.500

TABLE III - The comparative antifungal activity of different parts and extracts of sesame seed

Reference	**Plant**	**plant part**	**microorganism**	**Type of extract**	**Inhibitory activity**
(Shittu *et al.*, 2006)	*Sesame radiatum*	leaves	*Candida albicans*	Ethanolic Aqueous	Mildly Negative
(Shittu *et al.*, 2007)	*Sesame* radiatum	leaves	*Candida albicans*	Methanolic Ethanolic Aqueous	Negative Positive^{+} Positive $^{4+}$
(Thaweboon Nakaparksin, Thaweboon, 2011)	*Sesame* sp.	oil	*Candida albicans*		Negative
(Makki, Olama, Holail, 2012)	*Sesame* sp.	oil	*Candida albicans*		Negative
(Shittu, Shittu, 2012)	*Sesamum radiatum*	leaves	*Candida albicans*	Methanolic Ethanolic Aqueous	Negative Positive^{2+} Positive^{+}
(Ogawa, Nishio, Okada, 2014)		oil	*Candida albicans*		Positive
(Bankole *et al.*, 2007)	Combined *Sesame radiatum* & *Sesame indicum*	leaves	*Candida albicans*	Methanolic Ethanolic Aqueous	Positive^{3+} Positive^{+} Positive^{4+}
(Tabassum, Vidyasagar, 2014)	*Sesame* sp.	oil	*Candida albicans*	Hexane Methanolic	Positive Positive
(Uniyal *et al.*, 2012)	*Sesame* sp.	oil	*Aspergillus niger* *Aspergillus fumigatus*		Moderately
(Shirurkar, Wahegaonkar, 2012)	*Seasame* sp.	oil	*Aspergillus Filamentus fungi*		Negative

$^{+}$ Full concentration presents anti- *Candida* activity $^{4+}$ Diluted concentration presents anti- *C andida* activity

three anthraquinones, anthrasesamones A, B and C and chlorinated red naphthoquinone pigment were reported (Begum, Furumoto, Fukui, 2000; Furumoto *et al*., 2003). The sesame flowers are purple to whitish and have been used to prepare perfumes in Africa (Morris *et al.*, 2002). The antifungal activities of sesame roots and leaves were investigated against some the fungi like *Macrophomina phaseolina*, *Alternaria sesame* and *Fusarium oxysporum*. However, the results of this study demonstrated that compared to the control, root and leaf extracts can enhance the growth of *A. sesame* by and *F. oxysporum,* respectively (Syed *et al.*, 2015).

CONCLUSION

Taking into account the results, we can conclude that the rate of *Candida* colonization in the oral cavity of liver transplant recipients is high. Use of a mouth wash with high efficacy and low toxicity is essential. Our results revealed that the methanolic and N-hexan extracts of sesame seeds are not effective on *C. albicans* and *C. glabrata* species, isolated from the patients. The sesame seeds oil pulling or mouthwash which contains sesame extract cannot effectively cleanse and remove the *Candida* species in the mouth.

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