International Journal of Molecular and Clinical Microbiology 2 (2012) 206-210

International Journal of
Molecular and Clinical Microbiology

Evaluation of the Effects of Eucalyptus and Lavandula on the Growth of *Candida albicans* Strains resistant and Sensitive to Caspofungin

Leila Fozouni[1,*] (Ph.D), Mansour Bayat[2] (Ph.D), Shahla Roudbar mohammadi[3] (Ph.D), Mohammad Hassan Shahhosseiny[4] (Ph.D)

1- ph.D candidate of mycology ,Islamic azad university science and research ,Tehran,Iran.
2- Associate professor of medical and veterinary mycology, Islamic azad university science and research , Tehran,Iran.
3- Assistant professor of medical mycology, Tarbiatmodares, university of medical science,Tehran,Iran.
4- Associate professor of biothecnology, Microbiology department, Islamic azad university,shahr-e-Qods, Iran.

ARTICLE INFO

Article history:
Received 21 May 2012
Accepted 7 July 2012
Available online 1 November 2012

Keywords:
Candida albicans,
Eucalyptus essence,
Lavandula essence,
MIC

ABSTRACT

Candida albicans is the most common and important factor causing candidiasis infections in human. As fungi are resistant to anti-fungal agents, and also with respect to the restrictions in treatment of fungal diseases such as their high costs and side effects, study of the combination of herbal medicine is truly required. The aim of the present study was to investigate the effects of herbal essences of eucalyptus and lavandula on the growth of clinical *Candida albicans* strains which have been separated from mucosal, cutaneous and visceral infections, and compared to those *Candida albicans* which are resistant and sensitive to caspofungin. The present study has been carried out on 41 *Candida albicans* taken from patients suffering from mucosal, cutaneous and visceral candidiasis. The degree of samples' sensitivity to eucalyptus and lavandula were determined through applying broth microdilution method. In the present study, the MIC (minimum inhibitory concentration) of eucalyptus against *Candida albicans* was determined at 4-1024µl/ml, in which the most growth fluctuations were seen in the densities of 512 and 128 µl/ml. Moreover, the MIC of lavandula was determined at 1-256 µl/ml, and the most fluctuations in its growth were in the density of 32 and 16 µl/ml. Eucalyptus and lavandula essences have a very good anti-fungal effect against *Candida albicans*. In the present study, lavandula essence proved better anti-candidiasis effects while no considerable difference was seen between the strains sensitive and resistant to caspofungin in its MIC. In higher densities, eucalyptus essence prevents strains from growing. Therefore, it is recommended to investigate the effects of these essences on other pathogenic fungi and bacteria. It is also recommended to evaluate the use of combinations of two or more essences for curing infections or preventing their reoccurrence.

1. Introduction

Studies conducted from 1980 onward have shown that the systemic and opportunistic infections resulting from *Candida* have been increased significantly. In spite of the fact that there have been too many pathogenic *Candida* species responsible for candidiasis infections, one of the most important and most pathogen species is albicans (Clarke and Davies, 1980). *Candida albicans* is the natural yeast of Mucosa and skin, which is limited to skin in the form of

[*]Corresponding author. Dr. Lila Fozouni
Tel.: +989111518674 Fax.: +98151-2214990
E-mail address: lili_kia@yahoo.com

saprophyte. The range of this infection varies from mucosal colonization to aggressive and lethal infections. It can easily turn into pathogen and cause mucosal, cutaneous and - in non common cases - systemic diseases in case of having proper environment, weak immunity system, taking systemic corticosteroids and antibiotics, and in malignity (Barchiesi et al., 2000). Echinocandins such as caspofungin is one of the newest anti-fungalagents which has a broad-spectrum and is imported at high costs to be used in treating this type of infections.

Treatment of candidiasis is very complicated as candidiasis strains resistant to common anti-fungal agents are formed. Besides being limited in their amount, most common anti-fungal agents are also very expensive. Recurrence of candidiasis infections is very common, and this adds to the importance of treating this opportunistic infection and consequently has raised the need for developing new anti-fungal agents for expanding the spectrum of anti-candida activities and combating against strains resistant to available anti-fungi (Mora-Duarte et al., 2002).

For decades, humans have discovered the anti microbial properties of herbs and have used them since then, but it was only after 19th century when chemical combinations replaced herbal medicine. However, scientists nowadays pay particular attention to using herbal and traditional medicine. In countries like Iran remarkable history could be traced about applying herbal medicine for treating diseases. For instance, great Iranian scientist Abu Ali Sina (Avicenna) had considered using herbs like lavandula (Ghisalberti, 1996; Negahban et al., 2007; Takahashit et al., 2004; Sefidkon et al., 2007).

The present study aimed to determine the MIC (minimum inhibitory concentration) of eucalyptus and lavandula essences in preventing the growth of *Candida albicans* separated from patients suffering from mucosal, cutaneous and visceral candidiasis. Also, that *Candida albicans* resistant and sensitive to caspofungin has been studied in terms of their sensitivity to the aforementioned essences to see whether strains resistant to caspofungin are also resistant to these essences or not.

2. Material and Methods

In the present study, 41 *Candida albicans* isolates including 23 isolates were taken from mucosal infections, 16 isolates from cutaneous infections, and 2 isolates from visceral infections in five hospitals in Tehran, Gorgan, Sari, Iran. These strains were studied to find and verify albicans species through direct test, culturing over corn meal agar containing tween 80, germ tube test, colony color on Chromagar, and the test of glucose absorption by API120 kit. In the present study, *Candida albicans* (A90029) was used as the standard strain.

For determining the sensitivity or resistance to caspofungin anti-fungal agent, broth micro dilution method was applied. According to the instructions proposed by CLSI, strains with the MIC≤2 µg/ml are considered sensitive and the MIC≥2 µg/ml are considered as non-sensitive or resistant to caspofungin (NCCLS, 2002). In the present study, 35 strains were reported as sensitive and 6 strains as resistant to caspofungin.

In order to determine the sensitivity of the *Candida albicans* strains to eucalyptus and lavandula essences, a suspension of 48-hour *Candida albicans* strains cultured in saburo dextrose agar with distilled water was prepared. The initial sample containing 106 yeasts per ml of distilled water was then prepared by spectrophotometer in the wavelength of 530 nanometers and with transition of 75-77. After that, the sample was diluted first at the ratio of with distilled water, and finally at the ratio of with RPMI 1640 culture medium (with glutamines, without bicarbonate, and with pH indicator of Sigma Co.) and MOPS buffer (Sigma Co.) so that the final number of yeasts reaches103cfu/ml.

Eucalyptus and lavandula essences were supplied from Barij Essence Pharmaceutical Company. Dimethyl sulfoxide (DMSO) was used for making stocks; therefore, 1.024 milliliters of eucalyptus essence was dissolved in 4 milliliters of DMSO and 0.3 milliliters of lavandula essence was also dissolved in 4.7 milliliters of DMSO to reach the final 5 volume of milliliter.

All the stocks were diluted at the ratio of with RPMI. Then, 0.1 milliliter of each stock was mixed with 9.9 milliliters of RPMI medium in the

tube, and 100 microliters were inseminated in the micro plate wells containing 100 microliters of RPMI medium and were then diluted serially. After that, 100 microliters of the yeast suspension with the density of 10^3 cfu/ml was then inseminated in each well. RPMI combination and yeast were used as the positive control in one well, and RPMI and essence were used as the negative control.

For investigating the anti-candidiasis effects of eucalyptus essence, the densities of 4-1024 µl/ml and densities of 1-256 µl/ml were also used for lavandula essence. Micro plates were incubated for 48 hours in 35ºC, and were then their growth was studied after the incubation. In the cases in which no growth was observed in the well containing negative control, wells were checked with special mirrors to reassure that the process has been done correctly.

3. Results

In the present study, the average MIC of eucalyptus against *Candida albicans* has been determined at 512 µl/ml (from among 4-1024), in which most growth fluctuations were observed in the density of 512 and 128. In the density of 1024 in all organisms, no growth was observed, while growth was seen in all other densities without any change (Table 1). In clinical strains, only cutaneous strains were found to be sensitive to the densities of lower than the essence (128µl/ml). The average MIC of eucalyptus against the standard *Candida albicans* was determined at 128 µl/ml. The average MIC of eucalyptus against those *Candida albicans* resistant to caspofungin was determined to be 640µl/ml and that of *Candida albicans* sensitive to caspofungin was reported to be 64 µl/ml, which proves that there is considerable difference between strains sensitive and resistant to caspofungin (Table 3).

The average MIC of lavandula against *Candida albicans* was reported at 64 µl/ml (from among 1-256), in which the most growth fluctuations were seen in densities of 32 and 16. No growth was observed in the densities of 256, 128 while growth was observed in all other densities with no change (Table 2). The average MIC of lavandula against the standard strain of *Candida albicans* was determined at 16µl/ml. Mucosal and cutaneous strains showed most sensitivity in the density of 32 µl/ml.

The average MIC of lavandula for the strains sensitive to caspofungin was determined to be 32 and for the strains resistant to caspofungin was determined at 64, which showed no considerable difference between the strains sensitive and resistant to the antibiotic (table 3).

4. Discussion

Echinocandins are a new class of anti-fungal agents with an expanded operational spectrum. They prevent the function of 1, 3–β–Dglucan synthetase, which is needed for the formation of the 1,3–β–Dglucan polymer in the fungal cell walls. Caspofungin is the first anti-fungal antibiotic of this class which is imported to and used in Iran at very high prices. The medicine is used to treat stable and systemic infections resulting from *Candida albicans*. It is also an effective medicine for treating all types of candidiasis in special patients, including patients suffering from various types of cancers or immunodeficiency diseases such as AIDS (Chandrasekar and Manavathu, 2002; Sergey et al., 2006; Lesage et al., 2004).

Eucalyptus is a tree belonging to Myrtaceae family, which originally found in Australia but has also been spread all over the world. Its scientific name has derived from two Greek words of “eu” meaning “good” and “kalypto” which means “hidden”. This tree entered in Iran about fifty years ago. Eucalyptus species mostly grow in semi-arid and sub-humid climates (Gilles et al., 2010; Bakkali et al., 2008). The essence of eucalyptus is comprised of cineol (70%-85%); monoterpenes (such as linalool and borneol), and also sesquiterpenes (such as beta-caryophyllene). Anti-microbial properties of eucalyptus against microorganisms have been shown (EL-Ghorab et al., 2003). Results obtained from their study

Table 1. Comparison of growth of *Candida albicans* strains in the densities of 512,128 eucalyptus essence.

essence /Density µl/ml	strain 1×103cfu/ml	no growth		growth		comparison
		number	percent	number	percent	
512	*C. albicans*	29	70/7	12	29/3	x2=0 NS
128	*C. albicans*	5	12/2	36	87/8	x2=0 NS

NS: Not significant

Table 2. Comparison of growth of *Candida albicans* strains in the densities of 32, 16 µl/ml Lavandula essence.

essence /Density µl/ml	strain 1×10^3cfu/ml	no growth		growth		comparison
		number	percent	number	percent	
32	*C.albicans*	37	90/2	4	9/76	x^2=0 NS
16	*C.albicans*	32	78	9	22	x^2=0 NS

NS: Not significant

Table 3. Quantative distribution of Minimum Inhibitory Concentration in two herbal essences according to sensitive to caspofungin

MIS/ essence	C.albicans Resistant or sensitive to caspofungin	average µl/ml	standard deviation	comparison
Eucalyptus	Resistant	640	12.1	S
	Sensitive	64	5.7	S
Lavandula	Resistant	64	7.2	NS
	Sensitive	32	6.5	NS

S: Significant
NS: Not significant

showed that using eucalyptus in lower densities is not proper for treating candidiasis infections, and also a considerable difference was also observed in their sensitivity to eucalyptus between the strains resistant and sensitive to caspofungin.

Lavandula usually grows wildly in most parts of the world. This short-lived plant has thin long green leaves, and its essence which is taken through distillation of its flower and the flowering branches, is a yellow or a greenish yellow with a pleasant odor (Pinto et al., 2006; Costa-de-diveria, 2011). Its essence contains about 40% linalyl acetate, as well as butyric acid, propionic acid, valeric acid, free linalool, and geraniol. The plant is also known as naardin. The results of the study showed that lavandulahas desirable anti-candidiasis effects. With respect to the results gained through comparing MICs of the strains sensitive and resistant to caspofungin, it could be noted that this herb can be used to treat candidiasis infections and the cases resistant to treatment.

Considering the conducted study and by investigating the effects of the essences on candidiasis infections and the growing anti-medicine trend among Candidas, it seems reasonable to use herbs to treat all kinds of

infections. Therefore, it is recommended to investigate the effects of these essences on other pathogenic fungi and bacteria. It is also suggested to evaluate the use of combinations of two or more essences for curing infections or preventing their reoccurrence.

Acknowledgement

This work was supported by the Research Council of the university Science and Research, Tehran branch

References

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils –A review. Food Chem Toxicol. 46, 446–75.

Barchiesi, F., Schimizzi, A.M., Fothergill, A.W., Scalise, G., and Rinaldi, M. G., 2000. In vitro activity common and uncommon clinical isolates of Candida species. Microbiol. Infect. Dis. 18, 302–304.

Chandrasekar, P.H., and Manavathu, E.K., 2002. Caspofungin. Drugs Today (Barcelona).38, 829-846.

Clarke, M., Davies, D.P., 1980. Neonatal systemic candidiasis treated with miconazole. Br Med J. 281 (6236), 354–354.

El-Ghorab, A.H., El-Massry, K.F., Marx, F., Fadel, H.M., 2003. Antioxidant activity of Egyptian Eucalyptus camaldulensis var. brevirostris leaf extracts. Nahrung. 47, 41-5.

Ghisalberti, E.L., 1996. Bioactive acylphloroglucinol derivatives from Eucalyptusspecies. 41,7–22.

Gilles, M., Zhao, J., An, M., Agboola, S., 2010. Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. Food Chem.119, 731–7.

Lesage, G., Sdicu, A.M., Menard, P., Shapiro, J., Hussein, S., and Bussey, H., 2004. Analysis of β-1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. Genetics. 167, 35-49.

Costa-de-Oliveira M.J., Cavaleiro, S., Palmeira, S., Zohra, M., and Atik, F., 2011. Antibacterial activity of essential oils from Cistusladaniferus L. and Lavandulastoechas L., International Journal of Pharm Tech Research. 3(1), 484-487.

Mora-Duarte, J., Betts, R., Rotstein, C., Colombo, A.L., Thompson-Moya, L., Smietana, J., Lupinacci, R., Sable, C., Kartsonis, N., Perfect, J., 2002. Caspofungin Invasive Candidiasis Study Group N. Engl J Med. 347 (25), 2020.

National Committee for Clinical Laboratory Standards 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 2nd ed. NCCLS document M27-A2. Wayne, Pa: National Committee for Clinical Laboratory Standards.

Negahban, M., Moharramipour, S., 2007. Fumigant toxicity of Eucalyptus intertexta, Eucalyptus sargentii and Eucalyptus camaldulensis against stored–product beetles. J Appl Entomol.131, 256–61.

Pinto, E., Pina-Vaz, C., Salgueiro, L., Goncalves, A., Rodrigues, A., and Martinez-de-Oliveira, J., 2006. Antifungal activity of the essential oil of Thymus pulegioideson *Candida*, *Aspergilus* and dermatophyte species, J. Med. Microbiology. 55, 1367-73.

Sefidkon, F., Assareh, M.H., Abravesh, Z., Barazandeh, M.M., 2007. Chemical composition of the essential oils of four cultivated Eucalyptus species in Iran as medicinal plants (E-microtheca, E-spathulata, E-largiflorens and E-torquata) Iran J Pharm Res.6, 135–4.

Sergey, V., Balashov, S.P., and Perlin, D.S., 2006. Assessing Resistance to the Echinocandin Drug Caspofungin in *Candida albicans* by Profilling Mutations in FKS1. Antimicrobial Agents and Chemotherapy. 2058-2063.

Takahashi, T., Kokubo, R., Sakaino, M., 2004. Antimicrobial activities of Eucalyptus leaf extracts and flavonoids from Eucalyptus maculate. Let Appl Microbiol.39,60.