Research Journal of Medical Sciences 10 (5): 461-464, 2016
ISSN: 1815-9346

Comparison of the Anti-Fungal Effect of *Eucalyptus Globulus L.* and *Teucrium Polium L.* Extracts and Nystatin on Candida Albicans: An Experimental Study

[1]Mahsa Poorzamani, [2]Marzieh Saheb Nasagh, [3]Ateke Movaghari poor, [4]Seyed Sajad Hoseini Musa, [5]Roohollah Sharifi, [6]Fatemeh Rezaei, [6]Hamid Reza Mozaffari and [7]Seyed Amir Razavi Satvati
[1]Department of Oral Medicine, School of Dentistry,
North Khorasan University of Medical Sciences, Bojnurd, Iran
[2]Department of Pediatric Dentistry, School of Dentistry,
North Khorasan University of Medical Sciences, Bojnurd, Iran
[3]Department of Oral Medicine, School of dentistry,
Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[4]School of Dentistry, North Khorasan University of Medical Sciences, Bojnurd, Iran
[5]Department of Endodontics, School of Dentistry,
Kermanshah University of Medical Sciences, Kermanshah, Iran
[6]Department of Oral Medicine, School of Dentistry,
Kermanshah University of Medical Sciences, Kermanshah, Iran
[7]Department of Endodontics, School of Dentistry,
North Khorasan University of Medical Sciences, Bojnurd, Iran

Abstract: Medicinal plants have limited side effects and lower cost than pharmaceutical drugs. Thus, the present study was conducted to compare the antifungal effect of *Eucalyptus globules L.* and *Teucrium polium L.* extracts with Nystatin *in-vitro*. In this experimental study, the hydro-alcoholic extract (50, 100 and 250 mg L^{-1}) was obtained through percolation method for *Eucalyptus globulus L.* and *Teucrium polium L.* separately. Candida albicans was cultured in Mueller Hinton Agar and a disk containing each concentration of the plants, a disk containing Nystatin as a positive control and a disk containing distilled water as negative control were placed in each culture plate. After 48 h, the mean diameter of the growth inhibition zone for each extract was compared with the mean diameter of the growth inhibition zone for Nystatin. Data were analyzed by SPSS Software (Version 21). The mean diameter of the growth inhibition zone around the disks containing eucalyptus extract in all concentrations was lower than Nystatin ($p<0.001$). The *Teucrium polium L.* extract did not indicate any effect on the development of Candida albicans in all concentrations. The hydro-alcoholic extract of *Eucalyptus globules L.* showed moderate antifungal effect against Candida albicans compared with Nystatin.

Key words: Eucalyptus, teucrium, nystatin, candida albicans, nystatin

INTRODUCTION

Topical and systemic antimicrobial materials are commonly utilized in dentistry to reduce microorganisms in the oral cavity. These chemical drug have their own side effects as well (Ruddock *et al*., 2005; Gurgan *et al*., 2006). Candida albicans is the most common fungal pathogen causing mucosal and systemic infections. Antifungal drugs have side effects and toxic effects induced on the tissue in other hand the increasing prevalence of drug-resistant Candida have also been posing a challenge to clinicians. Therefore, medicinal plants with antifungal effects can be a choice for treatment this infection *Eucalyptus L.* is a member of Myrtaceae family that involves around 900 species (Brooker and Kleinig, 2004). Inhalation of the vapor containing the extract of Eucalyptus globulus has long been used for the treatment of respiratory diseases such as Pharyngitis, Bronchitis and Sinusitis. Recent studies have confirmed the effect of this plant against the

Corresponding Author: Seyed Amir Razavi Satvati, Department of Endodontics, School of Dentistry,
North Khorasan University of Medical Sciences, Bojnurd, Iran

development of Haemophilus influenza and Streptococcus pneumonia microorganisms (Cermelli *et al*., 2008). The leaves of this plant have widely been used for the treatment of ulcers and fungal skin lesions. Also, the anti-hyperglycemic and anti-oxidant effects have been found in the extract of this plant (Fabio *et al*., 2007).

Teucrium polium L., a member of Lamiaceae L. family, is a plant has been used in traditional medicine because of its anti-diabetic, anti-inflammatory, anti-ulcer and antispasmodic properties. Previous researches has shown that this plant has anti-diabetic, anti-inflammatory, anti-oxidant, anti-pyretic, anti-microbial, analgesic, anti-ulcer and antispasmodic qualities (Esmaeili and Amiri, 2008; Yazdanparast *et al*., 2005).

The review of literature clearly shows that few studies have been carried out to analyze the antifungal effects of different species of eucalyptus and teucrium polium extracts (Orhan *et al*., 2012; Elaissi *et al*., 2012, De *et al*., 2006). This study was conducted to examine the antifungal activity of methanol extracts of 3 plants on the most opportunist oral pathogen, Candida albicans,compering with Nystatin as a positive control.

MATERIALS AND METHODS

In this experimental study, Candida albicans (Id: 5027) was used which was obtained from the industrial and infectious bacteria and fungi of Persian Type Culture Collection (PTCC). To obtain the Hydroalcoholic extract of *Eucalyptus globulus* and *Teucrium polium L.* through percolation method, 500 gr of the aerial organs of each of these plants was grinded and transferred to a Meyer flask (KBG Co., Beijing, China) and 2 L ethanol 70% (Jahan Alcohol Teb, Arak, Iran) was added to it. After 48 h of incubation at 60°C, the extract was filtered by paper filter and the residue was pressed so that the extract was drained completely. The concentrated extract was incubated at 50°, completely dried, cut with a scraper and rubbed in the mortar. From the dried extract, 250 mg L^{-1} solution was prepared in Di-methyl Sulfate (DMSO) (Merck KGaA, Darmstadt, Germany), from which 50, 100 and 250 mg L^{-1} concentrations were obtained in the sterile physiological serum. Then, this yeast was placed in the Tryptic Soy Broth (Merck KGaA, Darmstadt, Germany) at 37°C for 24 h to start the growth process. After the growth started, this microorganism was transferred to Sabouraud dextrose Agar (Merck KGaA, Darmstadt, Germany) containing Chloramphenicol (Merck KGaA, Darmstadt, Germany) to remove the isolated colony (single). After 48 hours incubation at 37°C and removing the single colony from this microorganism, they were transferred to the physiological serum. Next, using Kirby-Bauer method (disk diffusion) a turbidity of the pure microorganism (0.5 McFarland standards) was prepared and this suspension which contained the microorganism was used for the surface culture (Elaissi *et al*., 2012).

First, a sterile cotton swab was dipped in the solution containing the microorganism and the extra liquid was extracted by pressing it against the internal edge of the test tube. Then, the swab dipped in microorganism was placed on the disposable plates (Padtan Teb, Tehran, Iran) containing Mueller Hinton Agar (Merck KGaA, Darmstadt, Germany) using spread plate, so that all the surface was exposed to the microorganism. The culture plates were fixed for 2-5 min until their moisture was totally absorbed.

The blank disks (Nik Farayand, Tehran, Iran) dipped in each of the concentrations of eucalyptus and *Teucrium polium L.* extracts as well as the disks containing 100 units Nystatin (35 μg in each disk) (Nik Farayand, Tehran, Iran) as positive control were placed in the oven and dried, then put cautiously on the medium inside the plate and slowly pressed on the agar surface so that the whole disk was in contact with agar. The blank disks (paper filter) containing distilled water were used as negative control. Thus, a disk containing the extract, a disk containing Nystatin 100 units/mL and a disk containing distilled water were placed in each of the mediums. Also, to increase the analytical accuracy of eucalyptus and *Teucrium polium L.* concentrations, 10 samples were prepared and cultured; therefore, 60 culture samples were obtained. After incubation at 37°C for 48 h, the diameter of growth inhibition zone was measured by a simple caliper (Asim Instruments, Sialkot, Pakistan) with accuracy of 0.001/0.1 mm.

Data were analyzed by SPSS Statistical Software (Version 21). The normal distribution of the data and equality of variance obtained for the diameter of growth inhibition zone were confirmed by Kolmogorov-Smirnov and Levene tests, respectively. The quantitative values were reported as SD±Mean. To compare the mean diameter of the growth inhibition zone (mm) of eucalyptus (50, 100 and 250 mg L^{-1}) and Nystatin 100 units/mL, independent samples t-test was used. Further, to compare the mean diameter of the growth inhibition zone for different concentrations of eucalyptus, one-way ANOVA was applied. The $p<0.05$ was considered significant.

RESULTS

The mean diameter of growth inhibition zone in different concentrations of eucalyptus extract was compared with Nystatin 100 units/mL by independent

Table 1: Comparison of Antifungal activity of different concentrations of *Eucalyptus globulus L.* extract with Nystatin

Est Material	Quantity	Min.	Max.	Mean±variance	p-value
Eucalyptus globules 50*	10	8.7	9.5	9.180 ±0.26	<0.001
Nystatin 100#	10	25.4	27.3	26.15 ±0.61	
Eucalyptus globules 100	10	11.7	12.7	26.31 ±0.61	<0.001
Nystatin 100	10	25.4	27.3	12.10 ±0.32	
Eucalyptus globules 250	10	15.3	17.0	16.13±0.51	
Nystatin 100	10	25.2	27.3	26.05± 0.62	<0.001

Table 2: Comparison of Antifungal activity of different concentrations of *Teucrium polium L.* extract with Nystatin

Test Material	Quality	Min.	Max.	Mean±variance
Teucrium polium *50	10	0.0	0.0	0
Nystatin 100#	10	25.9	27.3	26.62 ± 0.61
Teucrium polium 100	10	0.0	0.0	0
Nystatin 100	10	25.5	27.3	26.60 ± o.52
Teucrium polium 250	10	0.0	0.0	0
Nystatin 100	10	25.4	26.0	25.77±0.23

*: mg/mL, #, International Unit (IU)

test which was lower than Nystatin 100 units/mL in all concentrations, indicating a significant difference ($p<0.001$) (Table 1).

The results of one-way ANOVA regarding the mean diameter of growth inhibition zone in different concentrations of eucalyptus extract (50, 100 and 250 mg L^{-1}indicated a statistically significant difference ($p<0.001$). The descriptive indices for the diameter of growth inhibition zone of Candida albicans for different concentrations of *Teucrium polium L.* extract and Nystatin 100 units/mL used in each group are presented in Table 2.

Based on the results obtained, none of the concentrations of *Teucrium polium L.* extract could inhibit the growth of Candida albicans. Further, the mean diameter of the growth inhibition zone was zero which was not statistically different in comparison with distilled water.

DISCUSSION

The findings of the present study indicated that all concentrations of the hydro-alcoholic extract of Eucalyptus had antifungal and inhibitory effects against the development of Candida albicans. In separate studies, Carvalhinho *et al.* (2012) investigated the antifungal effect of hydro-alcoholic extract of eucalyptus globulus against Candida albicans and obtained similar results to those of the present study. The results showed the antifungal effect of this extract rose by increasing the concentration and the means for the diameter of growth inhibition zone for 50, 100 and 250 mg L^{-1} concentrations were 9, 12 and 16 mm, respectively.

Moreover, the findings of the study carried out by Ben Hassine on the Hydroalcoholic extract obtained from eucalyptus gilii revealed similar results to those of the present study. However, this value was 30 mm for Nystatin which was probably due to higher dose of Nystatin. In Ben Hassine's study, this amount was 40 microgram per disk and in the present study, it was 35 microgram per disk (equal to 100 units). Also, the results of Ataei's study showed similar antifungal effect of 100 mg L^{-1}. Eucalyptus extract and Nystatin 100.000 mL^{-1} which is routinely prescribed for oral Candidiasis therapy (Atai *et al.*, 2014). However, in the study conducted by Safaei-Ghomi on the antifungal effect of hydro-alcoholic extract of two species of eucalyptus; eucalyptus largiflorens and eucalyptus intertexta, their antifungal effects on Candida albicans were similar and much higher than Nystatin, respectively; which is indicative of the higher antifungal activity of these species compared with Eucalyptus globulus.

All concentrations of the hydro-alcoholic extract of *Teucrium polium L.* used in this study showed no activity against the development of Candida albicans and the growth inhibition zone diameter in all samples was zero. Furthermore, in a similar study, Sarac and Ugur (2007) reported that the hydro-alcoholic extract of *Teucrium polium L.* had no effect on the growth of this fungus even in the highest doses. Ilhami *et al.* (2003) reported that the extracts with acetone and chloroform bases of this plant lack any antifungal activity. Also, in the study performed by Mosadegh as the present study, the hydro-alcoholic extract of this plant was used and the obtained results about Candida albicans were similar, but in Mosadegh's study, this substance inhibited the development of Saccharomyces cerevisiae and Cryptococcosis neoformans fungi which would have no clinical value due to their high sensitivity. However, the findings obtained in the study carried out by Orhan *et al.* (2012) indicated the antifungal effects of the hydro-alcoholic extract of this plant, so that the concentration of 16 μg mL^{-1} inhibited the development of Candida albicans. This can be due to several factors such as higher sensitivity of fungus, higher solubility of possible antifungal compounds of *Teucrium polium L.* in

the aqueous solvent, etc. Previous investigations have indicated that all aerial organs of this plant contain tannin, Terpenoids, Saponin, Esterol, Flavonoid and Leucoanthocyanin and possess antimicrobial properties. Moreover, the studies conducted on the antifungal effects of *Teucrium polium L.* compounds have revealed contradictory results. Thus, this antifungal effect cannot be attributed to any definite factor and further studies are required to investigate it (Hashem, 2011).

CONCLUSION

The hydro-alcoholic extract of Eucalyptus indicated a remarkably antifungal activity against Candida albicans. Given the increasing need for new antifungal drugs as well as more acceptable flavor of the extract of this plant than Nystatin, it can be used for the treatment of oral Candidiasis. However, further clinical and experimental studies are required to identify the advantages and possible complications of this substance.

REFERENCES

Atai, Z., M. Ansari, A. Mousavi and A. Mirzaei, 2007. *In-vitro* study of antifungal effects of selected herbal extracts on standard and wild strains of Candida albicans. Mag. Dandanpizishki, 19: 91-97.

Brooker, M.I.H. and D.A. Kleinig, 2004. Field Guide to Eucalyptus. 2nd Edn., Vol. 3, Melbourne Blooming Books, Northern Australia.

Carvalhinho, S., A.M. Costa, A.C. Coelho, E. Martins and A. Sampaio, 2012. Susceptibilities of Candida albicans mouth isolates to antifungal agents, essentials oils and mouth rinses. Mycopathologia, 174: 69-76.

Cermelli, C., A. Fabio, G. Fabio and P. Quaglio, 2008. Effect of eucalyptus essential oil on respiratory bacteria and viruses. Curr. Microbiol., 56: 89-92.

De, M., A. De Krishna A.B. Banerjee, 1999. Antimicrobial activity of some Indian medicinal spices. Phytoter Res., 14: 207-214.

Elaissi, A., Z. Rouis, N.A.B. Salem, S. Mabrouk and Y.B. Salem *et al*., 2012. Chemical composition of 8 eucalyptus species essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC. Complementary Altern. Med., 12: 81-95.

Esmaeili, A. and H. Amiri, 2008. Effects of antimicrobial and identification of composition of essential oil of *Teucrium polium L.* J. Isfahan Univ., 31: 15-22.

Fabio, A., C. Cermelli, G. Fabio, P. Nicoletti and P. Quaglio, 2007. Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections. Phytother. Res., 21: 374-377.

Gurgan, C.A., E. Zaim, I. Bakirsoy and E. Soykan, 2006. Short-term side effects of 2% alcohol-free chlorhexidine mouthrinse used as an adjunct to nonsurgical periodontal treatment: A double-blind clinical study. J. Periodontol., 77: 370-384.

Hashem, M., 2011. Antifungal properties of crude extracts of five Egyptian medicinal plants against dermatophytes and emerging fungi. Mycopathologia, 172: 37-46.

Ilhami, G., U. Metin, O. Munir, B. Suktru and K. Irfan, 2003. Antioxidant and antimicrobial activities of *Teucrium polium L.* J. Food Technol., 1: 9-16.

Orhan, D.D., B. Ozcelik, S. Hosbas and M.Vural, 2012. Assessment of antioxidant, antibacterial, antimycobacterial and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi. Turk. J. Biol., 36: 672-686.

Ruddock, P.S., M. Liao, B.C. Foster, L. Lawson and J.T. Arnason *et al*., 2005. Garlic natural health products exhibit variable constituent levels and antimicrobial activity against *Neisseria gonorrhoeae*, *Staphylococcus aureus* and *Enterococcus faecalis*. Phytother. Res., 19: 327-334.

Sarac, N. and A. Ugur, 2007. Antimicrobial activities and usage in folkloric medicine of some Lamiaceae species growing in Mugla, Turkey. Eur. Asian J. BioSci., 4: 28-37.

Yazdanparast, R., M.A. Esmaeili and J. Ashrafi-Helan, 2005. *Teucrium polium* extract effects pancreatic function of streptozotocin diabetic rats: A histopathological examination. Iran. Biomedical. J., 9: 81-85.