INVITRO ACTIVITIES OF MELALEUCA ALTERNIFOLIA(TEA TREE OIL) AGAINST VARIOUS ORAL CANDIDA SPECIES - A PILOT STUDY

Ajay Kumar Nayak, Viraj Patil, Zarir Ruttonji, Vankadara Sivakumar*, Keerthi, Shalini Pandey

Dept.of Prosthodontics,Maratha Mandals Nathaji Halgekar institute of Dental Sciences,Belgaum

***- Sr.Lecturer,Dept.of Prosthodontics,SJM Dental College,Chitradurga**

Context:

Denture stomatitis is an inflammatory reaction occurring in denture wearers and oral yeasts like Candida species were predominantly associated with this condition. This in vitro study intends to investigate the inhibitory effect of natural alternatives like Tea Tree oil(Melaleuca Alternifolia) on growth of different Candida species.

Aims:*The aim of the current pilot study was to investigate the in-vitro activities of Melaleuca Alternifolia against various oral Candida species.*

Settings and Design*:Standard strains of five species of Candida in liophilized form were used to determine the MIC of Melaleuca Alternifolia with incubation period of 48hrs.*

Methods and Material*:*

Microbiological tests were used to perform this study. A total of five oral Candida isolates(C.albicans, C.dubliniansis, C.galbrata, C.Krusei and C.tropicalis) in liophilized form were used and revived in Sabourad's dextrose broth. Fifty tubes each having 100 µl of BHI(Brain Heart Infusion) broth were used. The concentrations of the test solutions were achieved by serial dilution method. After incubation period, by visual inspection of the tubes, the MIC values were determined. We have compared the MIC values of test solution Melaleuca Alternifolia with 0.2% fluconazole.

Results:*The results showed that 30% Melaleuca Alternifolia exhibited antifungal activities against Candida species which were comparable to the antifungal activity of 0.2% fluconazole.*

Conclusions:*The results signify that tea tree oil has a comparable/much better anti-fungal effect than the control(0.2% fluconazole).*

Key-words: *Candida species, Denture stomatitis, Fluconazole, Melaleuca Alternifolia.*

Introduction:

Denture stomatitis is an inflammatory reaction, occurring mostly in the palatal surface of maxilla, in denture wearing patients either partial or complete[4]. Denture stomatitis has been strongly associated with poor hygiene and continuous denture wearing, which

facilitates denture plaque formation in which Candida albicans can be regularly isolated, suggesting a pathogenic association between bacteria and fungi.

Various antifungal agents have been proposed for the treatment of denture stomatitis but because of numerous side effects, recurrence and resistance these have been less popular.[3] Thus, new therapeutic strategies like use of natural products can play an important role in the treatment. Among natural products, essential oils are emerging as promising therapeutic tools for oral infection.

MATERIALS AND METHODS:

The experiment was carried out in the Department of Prosthodontics and crown and bridge & Department of Microbiology at Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research center, Belgaum-590010.

Five standard strains of oral candida isolates(C.albicans, C.dubliniansis, C.galbrata, C.Krusei and C.tropicalis) in liophilised form were used and revived in Sabouraud's dextrose broth (**Fig.1, 2**).

Fig 1

Fig2

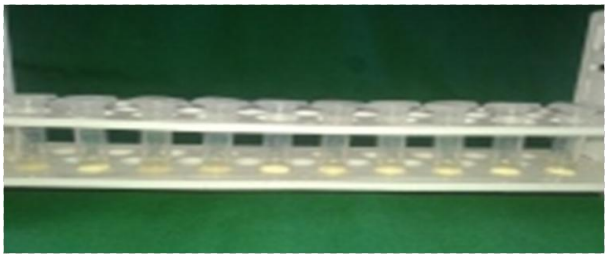

Fig3

Fifty tubes, each having 100 µl of BHI(Brain Heart Infusion) broth were used to which 100µl stock solution was added in the first MIC tube. After mixing well, 100µl solution from this tube was transferred to the second tube. This process was continued till the 10^{th} tube. From the 10^{th} tube which was the last tube 100µl of the final solution was discarded.

The concentrations of the test solutions achieved by this serial dilution method were as following- 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2 and 1 mcg/ml[1] (**Fig.3**). Now 100µl standard isolated strains of different species of Candida (C.albicans,

C.dubliniansis, C.galbrata, C.Krusei, C.tropicalis) were added to each of the 10 such prepared MIC tubes with varying concentrations such that the final volume per tube was 200µl. These tubes were then incubated at 37^0C for 24-48hours . After incubation period, by visual inspection of the tubes, the MIC values of different candida species against control and test solutions were determined.

Results:

The comparisons showed that for Candida albicans the MIC value for both control and test was 4, where as for other four candida species MIC values showed wide variations, which were tabulated in (table 1 and 2).

	Concentrations of test solutions achieved by serial dilution method (in mcg/ml).										
Candida species	**Test solutions**	**500**	**250**	**125**	**62.5**	**31.25**	**16**	**08**	**04**	**02**	**01**
Candida albicans	30 % melaleuca alternifolia	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**
	0.2 % Fluconazole	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**
Candida dubliniansis	30 % melaleuca alternifolia	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**
	0.2 % Fluconazole	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Candida galbrata	30 % melaleuca alternifolia	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**
	0.2 % Fluconazole	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**
Candida krusei	30 % melaleuca alternifolia	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**
	0.2 % Fluconazole	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**
Candida tropicalis	30 % melaleuca alternifolia	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**
	0.2 % Fluconazole	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**

Table 1:Comparison of MIC values of Test solutions on Five different Candida species

***Note:** S= Susceptible, R= Resistant.

Table 2: Comparision of MIC values of 30% melaleuca alternifolia with 0.2% fluconazole (in mcg/ml)

Candida species	30 % Melaleuca alternifolia	0.2 % Fluconazole
Candida albicans	4	4
Candida dubliniansis	31.25	125
Candida galbrata	16	62.5
Candida krusei	31.25	62.5
Candida tropicalis	4	62.5

In the graphical representation we can appreciate that the quantity of 30% Melaleuca alternifolia used to inhibit growth of Candida isolates was less compared to the quantity of 0.2% fluconazole (**fig 4**).

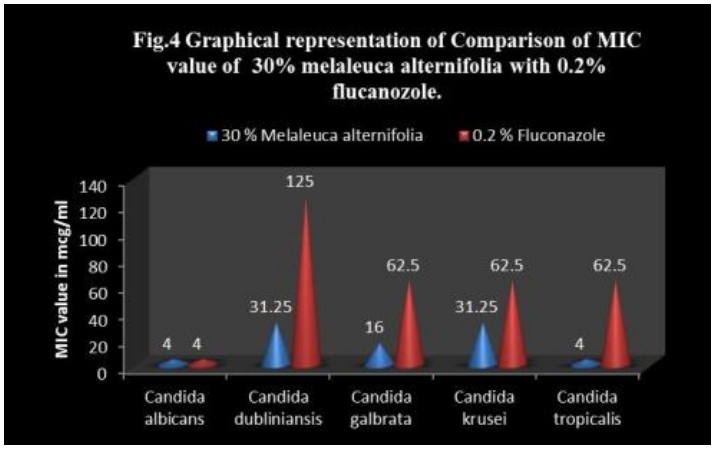

Fig 4

Discussion:

Candida species are considered important opportunistic pathogens due to the increasing frequency of infections they cause in the compromised patient groups and those on cancer chemotherapy, broad spectrum antibiotics[1]. Of the many pathogenic Candida species, C.albicans, C.galbrata, C.tropicalis and C.krusei are the most commonly found in the oral cavity. They frequently inhabit as commensals predominantly within the biofilms, which are spatially organized heterogeneous communities of fungal cells encased in the matrix of extra-cellular polymeric substances (EPS)[2]. Candida biofilms can also develop on surfaces of prosthesis and medical devices, and exhibit resistance to both anti-fungal and host defences compared

with their free-living planktonic counter parts. Melaleuca alternifolia mainly alters the permeability of candida cell, it also inhibits respiration in a dose dependent manner. Earlier studies have also shown that it inhibits formation of germ tubes or mycelial conversion in candida.[6]

In our study we compared the anti-microbial activity of 30% melaleuca alternifolia (tea tree oil) and 0.2% fluconazole against five different candidal strains out of which the tea tree oil showed significant inhibition of various candidal strains at lower concentrations when compared to flucanazole.

Other authors have also observed the antifungal and fungicidal effects of α – terpineol and terpinen-4-ol. Mondallo et al(2006) reported that terpinen-4-ol (main component of malaleuca alternifolia –tee tree oil) was fungistatic (MIC_{90} of 0.06%) and fungicidal (MFC_{90} of 0.125%) against fluconazole susceptible and resistant candidal isolates. These authors suggested that this compound could be a mediator of the in vivo activity of tea tree oil in a rat model of vulvovaginal candidiasis.

(Mondello F, De Bernardis F, Girolamo A, Cassone A, Salvatore G: Invivo activity of terpenin-4-ol. The main bioactive component of melaleuca alternifolia cheel (tea tree) oil against azole-susceptible and resistant human pathogenic candida species. BMC Infect Dis 2006, 6:158.)

Our study demonstrates anti microbial activity in vitro only. However since tea tree oil is known to have immune modulating activity(Cox SG, MannCM, MarkhamJL, BellHC, GustafsonJE, WarmingtonJR, WyllieSG:the mode of antimicrobial action of essential oil of melaleuca alternifolia (tea tree oil). J ApplMicrobiol 2000,88:170-175.

Its effectiveness clinically could be much better and in vivo studies would probably demonstrates better control of infections due to synergistic actions many active substances are present in tea tree oil and these individually contribute to bioactivity observed invitro some roles of individual constituents are known whereas some still unknown.

To conclude tea tree oil with its multipotential constituents may play an important role as an adjunct in the treatment of infectious and inflammatory diseases with candidal etiology. Since our sample size is less and in vitro results cannot be

extrapolated in vivo, further investigation is needed by launching in vivo clinical trials.

CONCLUSION:

There is an increasing trend of resistance shown by various Candida species. So there is an increasing demand to introduce natural materials. Tea tree oil with its proven antifungal activity can be an alternative to these antifungal agents. These in vitro results cannot be extrapolated in vivo and so further research is needed by launching in vivo clinical trials to assess whether any adverse effects exists or not.

REFERENCES:

1. Catalan A, Pacheco JG, Martinez A, Mondaca MA. Invitro and Invivo activity of Melaleuca alternifolia mixes with tissue conditioner on Candida albicans. Oral Surg Oral Med Oral Pathol Oral RAdiolEndod 2008;105:327-32.
2. Ye Jin, Lakshman P. Samaranayake, YuthikaSamaranayake. Biofilm formation of Candida albicans is affected by saliva and dietary sugars. Archives of Oral Biology (2004) 49, 789—798.
3. Cristina Marcos-Arias, Elena Eraso, LucilaMadariaga and Guillermo Quindós. In vitro activities of natural products against oral Candida isolates from denture wearers. BMC Complementary and Alternative Medicine 2011, 11:119.
4. Jean Barbeau, PhD,aJacyntheSe´guin, ART,b et el. Reassessing the presence of *Candida albicans* in denture-related stomatitis.
5. Hammer KA, Carson CF, Riley TV: in vitro activity of essential oils in particular Melaleuca alternifolia oil and tea tree oil products, against Candida spp. J AntimicrobChemother 1998;43:591-595.
6. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (tea tree oil): a review of antimicrobial and other medicinal properties. Clinical Microbiol Rev.2006;19(1):50-62.
7. Sharma S et al. Comparative Evaluation of Antifungal Activity of Melaleuca Oil and Fluconazole When Incorporated in Tissue Conditioner: An In vitro Study. J Prosthodont 2014;Jul23(5):37-73.