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ANTI-CANDIDAL EFFICACY OF OREGANO IN DENTAL PROSTHETICS

Oral Pathology

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ABSTRACT

Introduction: Denture hygiene is considered as an essential part of oral hygiene maintenance for the well-being of oral soft tissue in denture wearers. Denture induced stomatitis is common among denture wearers and that can be due to adherence of candida species.The purpose of the present study is to determine and compare the anti-candidal effect of Oregano against Candida albicans for using it efficiently as a denture cleansing agent. **Materials And Methods:** Swab from the tissue surface of 20 complete acrylic denture wearers were collected and cultured to isolate the Candida albicans. The plant materials were soaked in 99.9 % ethanol and pure extracts were made by the cold maceration method. Various concentrations of Oregano (1 %, 2 %, 4 %, 8 % and 16 %) dilutions were done. Fluconazole was taken as a control to compare with the plant products. The Disc diffusion method was done to find the zone of inhibition by pre-treating the discs with herbal extracts. The zone of inhibition was measured in millimetres. **Results:** The mean value of zone of inhibition of Oregano was less when compared to the fluconazole of higher concentrations. The differences between two groups were analyzed using one way ANOVA test. **Conclusion:** Oregano showed least anti-candidal effect when compared to fluconazole, Where it can have effects on other species of candida.

KEYWORDS

Oregano, zone of inhibition, candida albicans, denture cleanser

INTRODUCTION

Denture hygiene is considered as an essential part of oral hygiene maintenance for the well-being of oral soft tissue in denture wearers. There is an increase in the use of complete dentures is seen in most of the elderly people, of age more than 60 years. Due to the lack of proper motor coordination or skills, they seem to have difficulty in cleansing and maintaining the hygiene of their dentures.As, artificially made dentures are provided with natural contours and the surface of the denture provides more concavityaids in food aggregation and plaque development. (1)The artificial denture surfaces can harbour and promote the growth of oral microorganism's especially fungi and bacteria such as Candida species, streptococci and other microorganisms.

Denture induced stomatitis is common among denture wearers and that can be due to adherence of candida species. Among those, C. albicans adhesion is seen commonly on the dentures intaglio surface than on the palate, signifying that the denture acts as a main source of infection and that adhesion of yeasts to the denture surface leads to colonization and development of pathogenesis.(2)

Commercially available denture cleansers are routinely prescribed in dental practice. They are mostly chemical base which can clean the denture more effectively, but it may have some effects on the surface structure of acrylic denture base materials. Natural organic products can be an alternative to the synthetic chemicals, such as essential oils from medicinal plants, trees, barks and roots are known to contain anti-inflammatory and analgesic properties, good aroma, also have antimicrobial properties. (3,4)

Oregano is known for its aromatic and warm nature and mildly bitter seasoning herb in the mint family. Fresh oregano has a pungent taste that numbs the specialized mucosa of the tongue. Oregano contains high amount of omega-3, Iron, manganese and antioxidants. It is considered to be antibacterial, antiviral, antifungal, antiparasitic and immune stimulator. Externally used in skin infections (including yeast) after dilution. (5) It also helps to promote gingival health. Many studies have been carried out to investigate the antifungal property of Oregano and to find its anti-fungal efficacy. Hence, this study was attempted to evaluate and comparethe anti-candidal effect of Oregano with commercially available anti-fungal drug (Fluconazole 150mg), for being used as a denture cleansing agent.

MATERIALS AND METHODS

Swabs were collected on a random sample of 20 complete denture wearers both males and femalesattending the outpatients department. (Fig 1) Ethical clearance was obtained from the Ethical committee board. Volunteers using Heat cure complete denture for more than 3 months, acrylic complete denture wearer of age group 40 to 60 years were included.Subjects previously using any anti-fungal medication, mouth wash or creams, complete denture user with less than 3 months of placement and any oral pathological lesions or any recently treated lesions are excluded from the study.

The swab samples were inoculated in Saboraud dextrose agar (SDA) and incubated at 4°C. When the primary culture was obtained, it was placed on the chrome agar to isolate C. albicans. To confirm the presence of Candida albicans, germ tube testing and gram staining was done. The colony appears creamy, pasty, smooth and with budding yeast cells. [9]Sub-culturing of the species was done.(Fig 2)

Preparation Of Plant Extract

500 grams of Oregano powder was purchased from the market which were measured and then soaked in 1000 ml of 99.9 % ethanol using cold maceration method. After 72 hours the mixture is filtered using Whatman's filter paper and boiled in the boiling water bath to yield a pure extract until pasty consistency is obtained. The obtained extract appears thick and which is considered as a 100 % concentration.Various concentrations of 1 %, 2 %, 4 %, 8 % and 16 % were prepared using 1 % DMSO solution. (Fig 3, 4)

As standard commercially available Fluconazole 150 mg tablet was chosen as a control to compare with Oregano, since it hasa wide range of antifungal property. It was measured, then serially diluted using normal saline and made into various concentrations of 1%, 2%, 4%, 8% and 16 %.(Fig 5)

Assay For Candidal Activity

Antifungal activity was performed using antibody sensitivity testing. Sterilized disc was pre-treated with plant extract of different concentrations. The pure C. albicans culture was equally diluted for each concentration. All experiments were performed in duplicates. The plates were incubated at 37°C for 48 hours. The anti-candidal activity was assessed by measuring the diameter of the inhibition zone formed around the disc and which was measured in millimetres. (Fig 6)

RESULTS

The sensitivities of the test organisms to infusions were indicated by clear zone around the wells. The zone of inhibition around each disk in Mueller-Hinton agar growth medium seeded with Candida albicans were measured to determine the susceptibility of isolates and its resistance to herbal extract.

Mean value ofzone of inhibition was highest for Fluconazoleand least for Oregano(16%). Data obtained from at least three to five independent experiments performed in duplicates, were presented as mean ± SEM. One way ANOVA test was used to compare the reduction of C. albicans in Oregano. P-values of <0.05 were considered as statistically significant, and are indicated by asterisks (* respectively). All data were analyzed by using Graph Pad V7.00 software. (Table 1 and 2, Fig 7)

DISCUSSION

Oregano is obtained from the dried leaves of the oregano plant; the main constituents are 4-terpineol, g-terpinene, thymol, and carvacrol. The phenolic compounds disturb the membrane-embedded proteins, alter the ion transport process of the cell membrane, inhibit cellular respiration and modify the activity of calcium channel. This causesan increases in cell permeability and subsequent release of vital intracellular constituents. (4, 6)

The denture can be cleaned by mechanical method, chemical method and microwave irradiation method. Chemical method includes soaking the denture inside chemical product chiefly composed of sodium hypochlorite and peroxides for overnight. Chemical denture cleaning would aid as aneffective cleaning method. But chemical based cleaning can cause deterioration of the denture base material like bleaching of acrylic heat cure resin, corrosion of Co-Cr denture base metal, and deterioration of tissue conditioner and soft liner if used incorrectly. The research work of Pinto et al. found significant raise in surface roughness after repeated cycles of chemical based disinfection.(5,6,7)

In recent years, there has been a focus of attention on plant-derived herbal products with anti-microbial properties. Oregano was found to posses' wide range of antimicrobial property inherently and has been in use since ancient times for various systemic illnesses.They are relatively cost effective and readily available. Previous studies in the literature showed the effects of oregano in various systemic illness and microbial diseases. However, through a thorough search of the literature, there is a lack of studies having investigated the anticandidal property of Oregano.

Based on the results obtained from the present study, Oregano(16 %) showed least zone of inhibition at the higher concentration, states that it has low or limited activity on C. albicans. This was consistent with the study done by Yotova and Ignatova et al., showed that Oregano had anti-fungal activity on C. glabrata, S. cerevisae but had no activity against C. albicans. (8)

Fresh organic preparation of the plant source and increasing the sample size with different species and strains can provide better outcomes. Further investigation on this with higher sample size and species can provide us a better insight in understanding the mechanism.

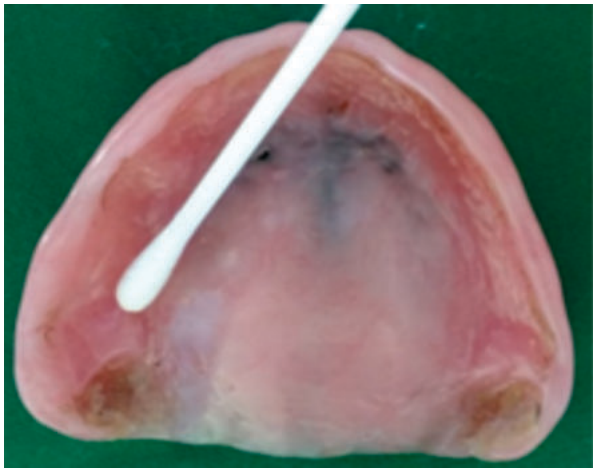

Fig 1: Swab Taken From Tissue Surface Of Complete Denture

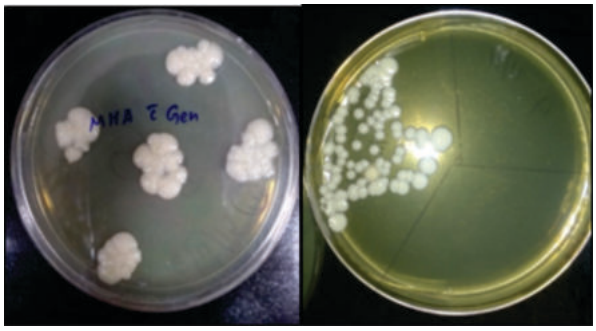

Fig 2:primary Culture In Sda Agar Plate And Incubated

Fig 3:pure Extract Of Oregano

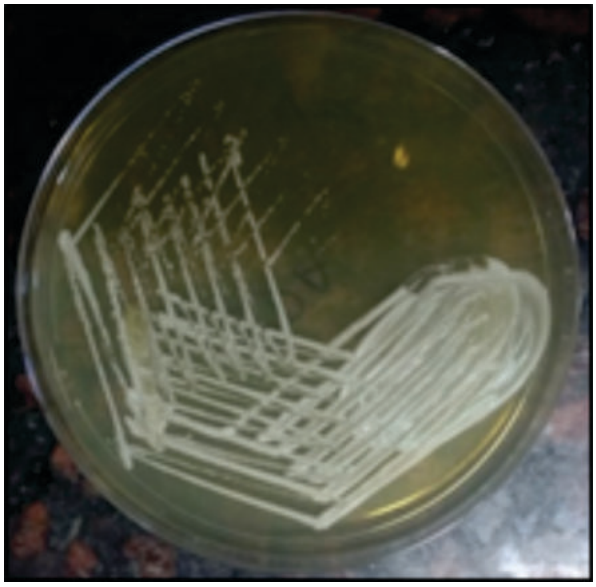

Fig 4: Subculture Of Isolated C. Albicans

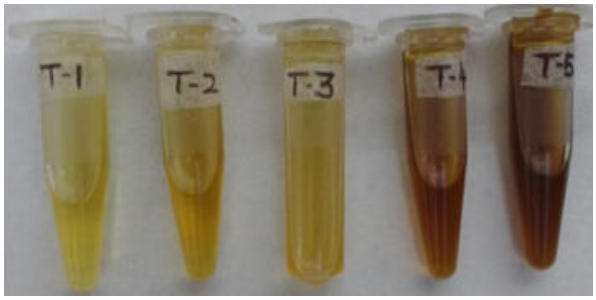

Fig 5: Various Concentrations Of Oregano Diluted With 1% Dmso

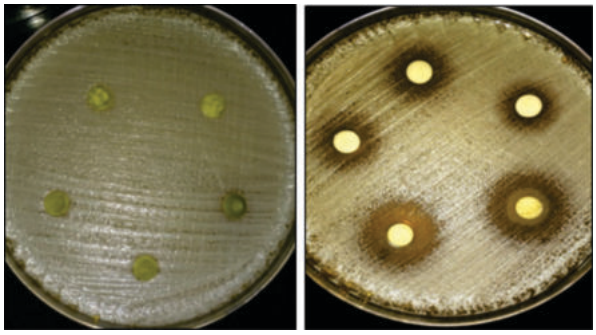

Fig 6: Zone Of Inhibition At 1%, 2%, 4%, 8%, 16% (a) Oregano (b) Fluconazole

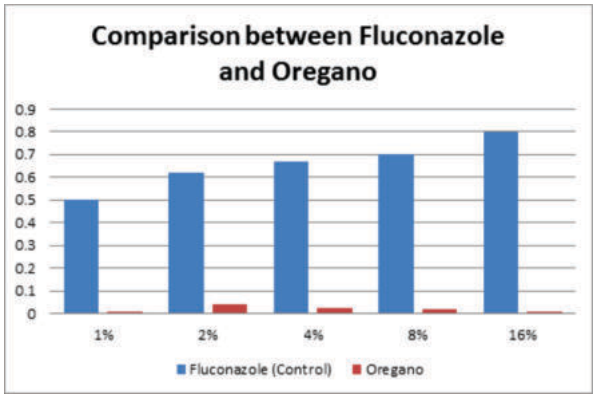

Fig 7: Comparison Between Oregano And Fluconazole

Table 1: Descriptive Analysis

Groups	N	Mean				
		1%	2%	4%	8%	16%
Fluconazole (Control)	20	0.5	0.62	0.67	0.7	0.8
Oregano	20	0.009	0.043	0.023	0.018	0.009

Table 2: Significance Rate Of Fluconazole (control), Oregano

	Df 1%	P value	Df 2%	P value	Df 4%	P value	Df 8%	P value	Df 16%	P value
Oregano	38	0.54	38	0.90	38	0.89	38	1.1	38	1.4
Fluconazole										

CONCLUSION

In this present study,Oregano showed the only least anticandidal effect.Therefore, further studies with increasing the sample size can provide a better outcome and can also be used as an alternative to chemical based denture cleanser.

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Conflicts Of Interest: There are no conflicts of interest.

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