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ANTIFUNGAL ACTION OF THYME AND BASIL EXTRACTS ON DIFFERENT DENTURE BASE MATERIALS

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ABSTRACT

Statement of problem: The use of medical plants has expanded as a result of increased resistance of microbes to chemical medications, as well as their negative effects and excessive costs. Ocimum basilicum (basil) and Origanum Syriacum (Thyme), were studied for their antifungal activities against Candida albicans (C. albicans) species.

Purpose: The goal of this study is to see if two plant extracts, Ocimum basilicum (basil) and Origanum Syriacum (Thyme), have antifungal effects on different denture base materials (long cycle heat-polymerized methyl methacrylate PMMA, short cycle heat-polymerized PMMA, and thermoplastic monomer free microcrystalline polymer karadent).

Material and methods: Plant extracts (basil and thyme) were tested for antifungal activity using the pores method. The effect of plant extracts on various denture base surfaces was determined using a disc diffusion test. Fifty-four discs of 6mm diameter and 2mm thickness were prepared from long cycle heat-polymerized methyl methacrylate PMMA, short cycle heat-polymerized PMMA, and thermoplastic monomer free microcrystalline polymer karadent and distributed into three groups:-ve control, basil, and thyme. The diameter of growth inhibition zones was determined after these discs were placed on a Sabouraud Dextrose agar surface and incubated for 24 hours at 37 C.

Results: Both Origanum syriacum, and Ocimum basilicum were efficient against Candida albicans. *Origanum Syriacum* extracts showed a superior antifungal effect compared to the Ocimum basilicum extract with long and short curing cycle acrylic resin, but in Karadent the values of Ocimum basilicum were higher than those of Origanum syriacum extract.

KEYWORDS: Denture stomatitis, Origanum syriacum, Ocimum basilicum, Candida albicans.

INTRODUCTION

The oral mucosa is an ideal habitat for microbial and fungal colonization, showing a range of ecological niches. (1) The presence of dental devices raises the number of causes for fungal infection. (2) Many adverse effects are caused by the adherence of microorganisms and oral deposits to a dental appliance; unclean dental appliances thus reflect both an esthetic and a health concern for denture wearers. (3)

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Pathogens of the Candida genus are the primary cause of denture stomatitis (DS), a highly prevalent oral mucosal inflammatory disorder in denture wearers. [4] Although it is a chronic, non-life-threatening oral infection, it can become a serious disease, particularly in elderly people and/or people with weakened immune systems. Candida spp. is supported by complex virulence factors. Pathogenesis, including conversion from the yeast to the hyphal form[5], adhesion[6], and biofilm formation [7]. Contemporaneous DS clinical care considers the use as normal treatment choices of local and systemic anti-mycotic drugs miconazole and fluconazole, nystatin, amphotericin B, along with mechanical and chemical agents in the regimen of denture hygiene and avoidance of overnight denture wear. [8] Unfortunately, these chemical solutions can change the physical and mechanical properties.[9] The increase in the resistance to antifungal drugs and potential adverse effects, an alternative way for eliminating *Candida* spp. infections is urgently required.[8]

Herbal medicine is the use of medicinal herbs for the prevention and treatment of diseases that can harm one's overall health. Recently, there has been an increase in interest in combining traditional herbal treatment with synthetic contemporary medications. Natural remedies can be a rational alternative to pharmaceuticals in the treatment of pathologic oro-dental diseases. Herbal treatments are chosen over conventional drugs due to their extensive natural bio-characteristics, lower costs, increased availability, and broad safety margin. [10]

An important source of antimicrobial agents is the plant materials used in conventional medicines. [11] A natural source of antimicrobial agents may be essential oils (EOs) obtained from the leaves, flowers, roots, stems, seeds or fruits of many plant families. They stimulated searchers as a natural alternative therapeutic treatment in dental diseases. [12] A certain number of EOs have been reported to display antibacterial, antifungal or anti-inflammatory properties against oral pathogens. [12, 13]

Origanum syriacum EO (Os-O) is obtained from Oregano (Origanum spp, Lamiacae family), a plant characterized by a wide variety of morphology and chemistry. [14] When many EOs (cloves, cilantro, cinnamon, thyme, mint, rosemary, mustard or sage) are compared, the EO of oregano is one of the strongest antibacterial efficiencies. [15] There are two major chemo-types of Os-O: thymol and carvacrol, which have a powerful and potent antimicrobial capacity. [16, 17]

Recent studies have shown that both C. albicans growth and activity have been inhibited by Ocimum basilicum BEO. are more effective than clotrimazole, an antifungal medication, and may therefore serve as a possible promising alternative to common antimicrobials. [18]

MATERIALS AND METHODS

Preparation of the samples

Fifty four dices were fabricated, from the following materials long cycle heat-polymerized methyl methacrylate PMMA, short cycle heat-polymerized PMMA, and thermoplastic monomer free microcrystalline polymer (Karadent) with the measurements 6 mm diameter and 2 mm thickness.

Materials that have been used are:

1- Conventional heat cured polymethyl methacrylate (PMMA) (Acrostone, manf. &co, Egypt)

2- Thermoplastic monomer free microcrystalline polymer (Karadent) (TCS, Inc., USA)

Eighteen long cycle heat–cured denture base specimens were made in a hot water bath at 72°C for 6.5 hours, while eighteen short cycle heat–cured denture base specimens were made in a hot water bath at 92°C for 1.5 hours.[19]

Injection of injecting cartridge was carried by (Sabilex, Microinjection machine, Argentina), the temperature was kept at 280°C and the pressure was kept at seven bar for 20 minutes, according to the manufacturer's instructions for fabricating eighteen Karadent samples.

Plant extract

Ocimum Basilicum (basil) and Origanum Syriacum (thyme) were selected for this study*. Extraction of plant extract was carried in Nawah Scientific Center, Cairo, Egypt. Thyme and basil were collected, dried and grinded, using experimental distillation at room temperature and ethyl alcohol as solvent, steamed and extracted volatiles are collected in condenser. Evaporator is then used for evaporation of excess solvent.

Microorganism growth condition

Candida albicans** (ATCC10231) was obtained and cultivated* on Dextrose agar, as well as germ tube tests and microscopy exams. To obtain 1x108 cells per ml, a loop of Candida albicans was transferred to 4 ml of BHIB and cultivated for 24 hours at 37°C before being placed to a haemocytometer slide.

To evaluate basil and thyme antifungal properties, Dextrose agar was prepared to aid Candida albicans growth, pores on the surface of the agar were created with a sterile Pasteur pipette, 0.1 ml of Candida suspension was transferred with a micropipette, and the plant extracts were streaked with a L–shape spreader. The widths of inhibitory zones were measured after 0.1 ml of each plant extract was applied to each pore and the plates were incubated at 37°C for 24 hours. [20]

To investigate the antifungal effect of plant extracts on the surface of various denture base materials, specimens were divided into three groups. Each of the three groups had 18 specimens:(-ve) control (Ethyl Alcohol), thyme, and basil. Each group will be divided into three subgroups, each with six discs of long cycle PMMA, short cycle PMMA, and Karadent.

Candida albicans broth was streaked on Dextrose agar with a L–shape spreader; after 15 minutes, discs of denture base materials were added to the surface of the agar with a sterile forceps using 6 replicate plates, and the plates were incubated at 37°C for 24 hours, then the diameters of growth inhibition zones were measured to see if any of the denture base materials had an antifungal effect before application of plant extracts and ethyl alcohol. [21]

The discs were dried then coating materials were applied with a smooth brush to the whole surface of discs, this was repeated until three coats have been added, and the denture base discs were ready to undergo disc diffusion test after application of plant extracts and ethyl alcohol. Figure (1)

Statistical analysis

All statistical analyses were performed with SPSS Statistics for Windows software (version 26.0; IBM Armonk, NY). All collected data was calculated, tabulated and statistically analyzed using paired t-test to compare before and after treatment within thyme and basil, independent T-test to compare between thyme and basil in each groups and one way ANOVAs to compare among groups in each extract. The analysis was followed by the post hoc test with the Duncan's correction. P value < 0.05 is considered be statistically significant.

* The studied plants were identified by Dr. Yasmin M. Hassan, Botany Department, Faculty of Science, Suez Canal University, Egypt. A reference specimen was deposited at the herbarium of the department.

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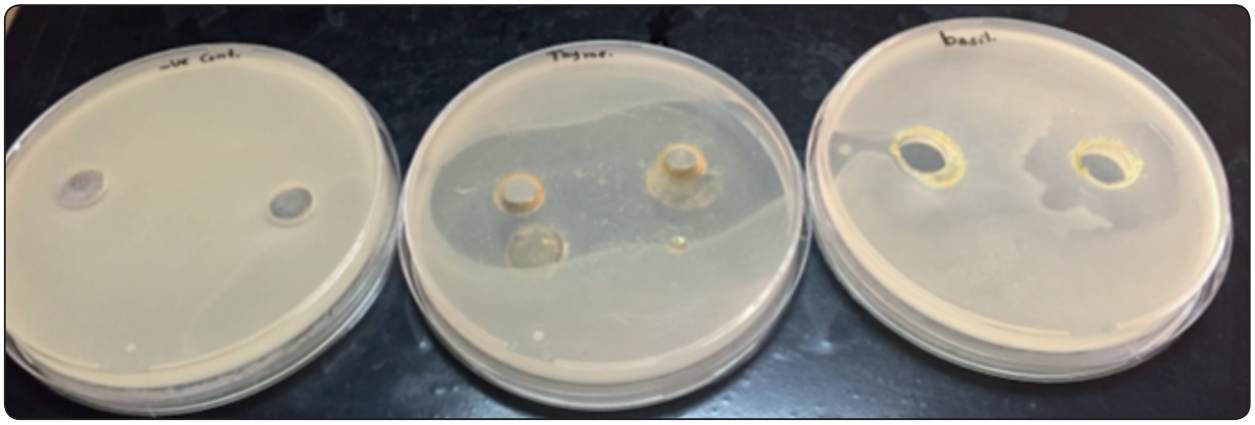

Fig. (1) : (-ve) Control group, thyme group and basil group samples in Dextrose agar

RESULTS

The results in Table (1) and Figure (2) showed that there were significant differences in each of groups long cycle, short cycle and Karadent before and after treatment in both thyme and basil extracts, except group short cycle PMMA in basil that did not differ from before and after treatment at P<0.05.

Analysis of variance table showed significant difference among groups (long cycle PMMA, short cycle PMMA and Karadent) after treated with thyme and basil extract for antifungal activity using one way ANOVAs at P<0.05, Table 2.

The results in table (3) showed the comparison among denture base materials in each extract and between extracts in each group. The denture base materials were differed with treated by thyme extract (P **<0.001**), and the high value was recorded in Karadent (62.5) while long and short cycle PMMA groups were very closed each other with 50.0 and 49.5 respectively. For basil extract, the denture base materials were differed significantly with treated by Basil extract (P <0.001), the high value was recorded in Karadent group (82.5) followed by long cycle PMMA (38.5) while short cycle did not give any response after being treated with basil extract.

TABLE (1) Comparison between before and after treatment with plant extracts in each denture base materials.

		Before	After	Paired-t	p value
Thyme	Long cycle	0.0±0.0	49.5±1.37	99.00	0.006**
	Short cycle	0.0±0.0	50.0±2.09	99.50	0.006**
	Karadent	0.0±0.0	62.5±0.54	100.2	0.001**
Basil	Long cycle	0.0±0.0	38.5±0.1.05	77.00	0.008**
	Short cycle	0.0±0.0	0.0±0.0	0.00	1.00
	Karadent	26±3.65	82.5±5.00	8.69	0.04*

***** Means signific ant difference between before and after in each groups under different extract***

TABLE (2) ANOVA table

Source of variance		Sum of Squares	df	Mean Square	F	Sig.
Thyme	Between Groups	651.00	2	325.50	119.08	<0.001
	Within Groups	41.00	15	2.733		
	Total	692.0	17			
Basil	Between Groups	20449.0	2	10224.5	1170.74	<0.001
	Within Groups	131.0	15	8.733		
	Total	20580	17			

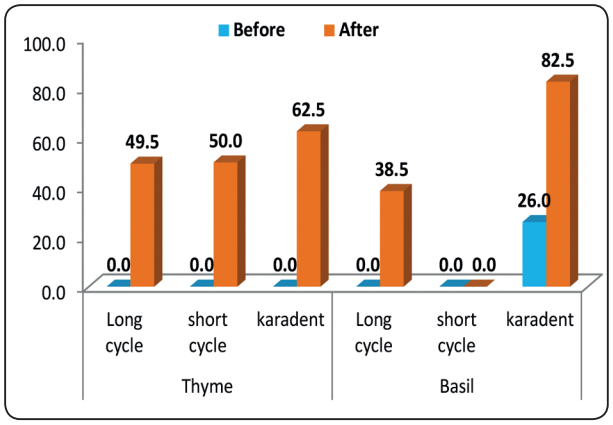

Fig. (2): Comparison between before and after treatment with plant extracts in each denture base materials.

To compare between thyme and basil in each group, table (3) and figure (3) the results showed significant differences between basil and thyme in all groups. In general, all groups gave high values when treated with thyme extract in both groups long cycle and short cycle PMMA, but in Karadent the values of basil were higher than those of thyme.

To compare between thyme and basil extracts, table (3) and figure (4) the results showed significant differences between basil and thyme extracts.

TABLE (3) Comparison between groups after coating with thyme and basil

	Long cycle	Short cycle	Karadent	F	p<0.05
thyme	49.5±1.37^{bA}	50±2.09^{bA}	62.5±0.54^{aA}	**119.08**	**<0.001*****
basil	38.5±1.05^{bB}	0±000^{cB}	82.5±5.00^{aA}	1170.74	**<0.001*****
Independent.T-test p<0.05	**<0.001****	**<0.001****	**<0.001****		
Extract					
	Thyme	**Basil**	**-ve control**		
Extract	44.0±2.82^{a}	36.5±2.12^{b}	0.0±0.0^{c}	**256.96**	**<0.001*****

*****, a,b,c: means significant difference between groups under different extract***

*****,A,B: means significant difference between extracts for each group***

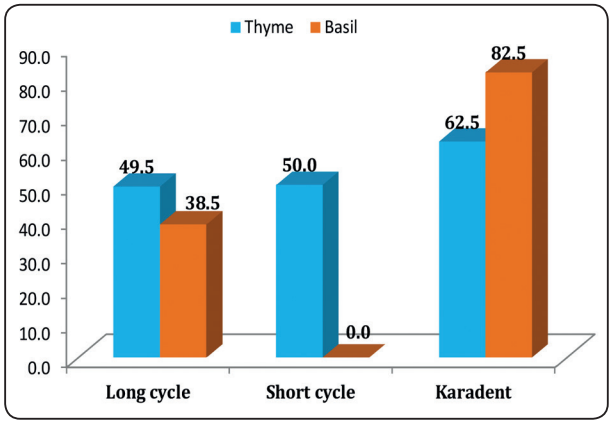

Fig. (3): comparison between different denture base materials after treatment with thyme and basil extracts

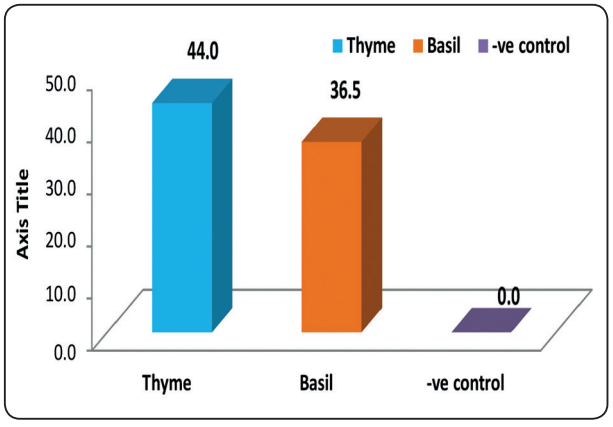

Fig. (4): Comparison in antifungal effect of thyme and basil extracts

DISCUSSION

Most denture wearers' saliva contains these yeasts, which have an affinity for adhesion to methacrylate resin.[22] Denture cleanliness is essential in the prevention and treatment of denture stomatitis.[23] In this investigation, plant extracts were used as mouthwash and disinfectant solutions to eliminate Candida albicans from the denture surface.

Many plant species, including Peppermint, Cinnamon, Lemon, Clove, and Tee tree, generate extracts that could be used to prevent or treat common oral pathogens (Candida albicans). Each of these extracts is effective against the microbes responsible for complete denture wear-related oral mucosal disorders.[12]

All of the extracts tested were successful in disinfecting of long cycle, short cycle heat cured acrylic resin acrylic resin and thermoplastic monomer free microcrystalline polymer from C. albicans. In our study thyme extract show antifungal efficacy than basil extract. Thyme extract has more antifungal effect than basil extract with long and short curing cycle acrylic resin, but in Karadent the values of basil were higher than those of thyme extract. Thyme contains carvacrol, thymol and thymoquinone in enough proportions to be used for the treatment of DS. In fact, they might reduce the time and concentration of antibiotics needed in the therapeutic approaches. This was agreed with different researchers for the antifungal action of these extracts.

Shamseddine and Chidiac [24] found that *Origanum Syriacum* have different antimicrobial efficiency against most strains related to DS with a higher efficiency associated to carvacrol content. Carvacrol is possibly one of the major components linked to Origanum Syriacum's anti-fungal effectiveness. The major component of oregano EO is carvacrol, according to the most recent studies.[25,26] The presence of thymol in the EO could improve its function by disrupting cell membranes, decreasing the functioning of ATPase, and releasing intracellular ATP and other components.[27] A link was discovered between thymoquinone and antifungal efficacy of thyme by prevention of C. albicans adhesion and proliferation on prosthesis.[28]

BEO had an excellent efficiency against fungi.[29] The six major chemotypes of linalool, eugenol, estragole, methyl eugenol, 1, 8-cineole and geraniol, which can be subdivided into smaller groups.[30] The metabolic response of Candida albicans to BEO at a subinhibitory concentration, which revealed significant changes in central carbon metabolism, amino acid metabolism, polyamine metabolism, and lipid metabolism. The development of a phytochemical-target-metabolite interactive network revealed a panel of important components in BEO, primarily terpenoids and phenyl-propanoids, to be potentially responsible for the inhibition of C. albicans.[31]

The antifungal efficacy of acrylic resin (long curing cycle or short curing cycle) and thermoplastic resin disc samples was compared in this study, and thermoplastic resin was found to have a higher antifungal efficacy than acrylic resin for the thyme and basil groups. This could be explained by the fact that C. albicans adherence is regulated by a variety of parameters, including the chemical composition of the denture base material, which is critical in influencing the capacity of pathogenic yeast cells to attach and form biofilms.[32] The findings of this study matched with recent researches that found that thermoplastic denture base material has a lower biofilm formation and candidal count than heat cure acrylic resin.[33,34] They believe this is due to the porosity of heat-cured acrylic resin, which allows food particles and microorganisms to easily accumulate inside.[34]

CONCLUSION

Within the constraints of this research, Origanum syriacum and Ocimum basilicum extracts have antifungal activity against candida flora identified in denture stomatitis. The results of this study suggest that thyme and basil extracts could be used to treat denture stomatitis.

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