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Conference paper

Evaluation of the effect of coating Vertex denture lining ma terial with plant oils on microbial growth and hardness.

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Abstract: Aims: To evaluate the effect of surface coating with natural plant oils (*Salvia officinalis*, *ginger* and *eucalyptus*) on *Candida* growth and the hardness of Vertex denture lining material. Materials and method: Forty five specimens were prepared from soft acrylic lining material, twenty five of which were 10x10x2mm in size for testing antifungal activity, and twenty samples were 20mm in diameter and 12mm in thickness, for testing shore A hardness after coating samples with three types of natural oils (*Salvia officinalis*, ginger and eucalyptus oils). Significant differences among the groups at ($p \leq 0.05$) level of significance were determined statistically with one way analysis of variance and Duncan's multiple range test Result: Antifungal assay showed a significant difference between five groups regarding *Candida albicans* growth ($p \leq 0.05$). For the hardness test, comparing different times of storage in water (1 day, 7 day, 14 day, 30 days) revealed a significant difference within all groups ($p \leq 0.05$). While comparing the groups coated with natural oils with the control group, significant differences were found between different times of storage in water (1 day, 7 day, 30 day) ($p \leq 0.05$), except at 14 days of water storage there was no significant difference between groups ($p > 0.05$). Conclusion: All tested natural oils were effective as fungicidal agents and increased the softness and duration of soft acrylic lining material.

Keywords: Hardness tests; denture liners; Candida albicans; Salvia officinalis; ginger; eucalyptus oil.

INTRODUCTION.

Soft denture lining materials are used in complete and partial removable dentures to distribute masticatory loads homogeneously on denture-bearing tissues. These materials are suggested in cases of thin atrophic mucosa, irregular bone resorption, immediate dentures, for patients with xerostomia and bruxism, and during healing after implant placement.[1] Surface roughness and hardness changes are predisposing factors for microbial accumulation that compromise the material's durability.[2] The most widespread and important opportunistic fungal infections in the oral cavity is candidiasis, caused by yeasts belonging to the *Candida* genus, such as *Candida albicans*. The best way to prevent oral infections is microbial biofilm inhibition. Herbal extracts use denotes a new era for antimicrobial treatment after development of microbial resistance to antibiotics.[3] Ginger,

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eucalyptus and *Salvia officinalis* oils are examples of plants that exhibit great antifungal effects.[3-5]

The aim of this research is to evaluate the effect of surface coating of the natural oils from S*alvia officinalis* ginger and eucalyptus on *Candida albicans* growth and hardness of Vertex denture lining material.

MATERIALS AND METHODS.

Antifungal Study

Preparation of Specimens: Twenty-five specimens were prepared from soft acrylic lining material (Vertex, Holland). The wax pattern (10x10x2mm) was made to fabricate stone mold in a metal flask.[6] The material was mixed according to the manufacturer's instructions and packed in the mold, the flask was then re-closed and pressed using a hydraulic press. Specimens were cured at a water bath (90 min at 73ºC and 30 min at 100°C) according to the manufacturer's instructions. Then the flash of access materials was trimmed with a scalpel.

Sterilization and Contamination of Specimens: All specimens were sterilized at an autoclave at 121°C for 15 minutes; the *Candida albicans* strain used in this study was isolated in a routine smear from patients with denture stomatitis then sub-cultured and the suitable identification biochemical tests were perfomed (germ tube test and sugar fermentation test).

On the first day, *Candida albicans* was inoculated to a turbidity of 0.5 McFarland which correspond to 10^8 organism/ml in 10ml of Brain Heart Infusion Broth (BHIB) and incubated at 37°C for 24 hrs. On the following day, 50ml of inoculated BHI broth were added to a test tube containing 10ml of sterile BHI broth, each sterile specimen tested was aseptically placed into a test tube, then it was sealed with foil and incubated for 24 hrs at 37°C.[7]

Antifungal Study

The method used for antifungal study was described by Al-Irhayim *et al.*,[6] a total of 25 specimens were divided after contamination into five groups:

Group A (Negative control): Five specimens individually placed in sterile BHI broth.

Group B (Positive control): Five specimens were coated in chlorohexidine-gluconate (4% w\v) (Amman P.O. Jordan), diluted to 0.5% and left for eight hours.

Group C: Five specimens were coated in ginger oil and left for eight hours.

Group D: Five specimens were coated in *Salvia officinalis* oil and left for eight hours.

Group E: Five specimens were coated in eucalyptus oil and left for eight hours.

All tubes containing negative control specimens (group A) were vortexed vigorously (Tucker instruments LTD/ England) for one minute and rested for nine minutes followed by a short vortex to re-suspend any present organisms. To determine the number of microorganism in 10^{-5} and 10^{-6} dilution replicate specimens, 100µL of the resulting suspension were transferred onto a plate of Sabouraud agar containing 5 mg/ml gentamicin. The plates were incubated for 48 hrs at 37°C.

Yeast colonies in each plate specimen were counted. The colony forming units per milliliter (CFU/ml) was then calculated. Other specimens (B,C,D,E) were individually placed in sterile glass test tubes containing 10 ml sterile BHIB and treated identically to the negative control specimens.[7]

Hardness study

Preparation of Specimens: Twenty specimens were prepared from soft acrylic lining material (Vertex, Holland), 20mm in diameter and 12mm in thickness.[8]

A total of 20 specimens were divided into four groups[6]:

a) Five specimens were immersed in distilled water as a control group.

b) Five specimens were coated in ginger oil for eight hours then immersed in distilled water.

c) Five specimens were coated in *Salvia officinalis* oil for eight hours then immersed in distilled water.

d) Five specimens were coated in eucalyptus oil for eight hours then immersed in distilled water.

Hardness test

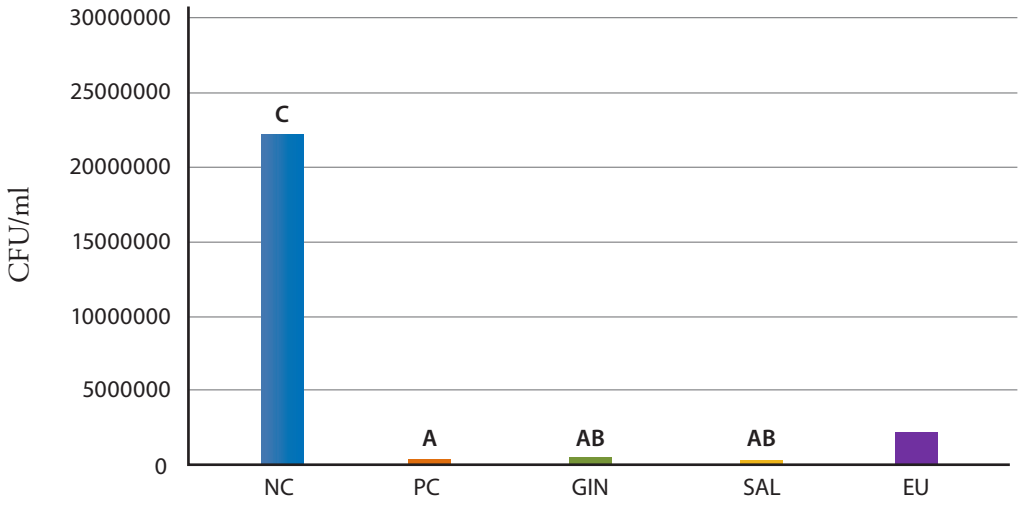
The measurement of hardness of soft lining materials was carried out by using a Shore A hardness durometer, the measurement method is based on penetration of the specimens by an indentor under specified conditions.

The readings were taken 1 second after a firm contact was achieved three readings were taken for each sample and the mean of those readings was calculated.[9]

The specimens were tested for hardness after different time intervals (1, 7, 14, and 30 days). For each specimen the measurements were recorded three times and mean values

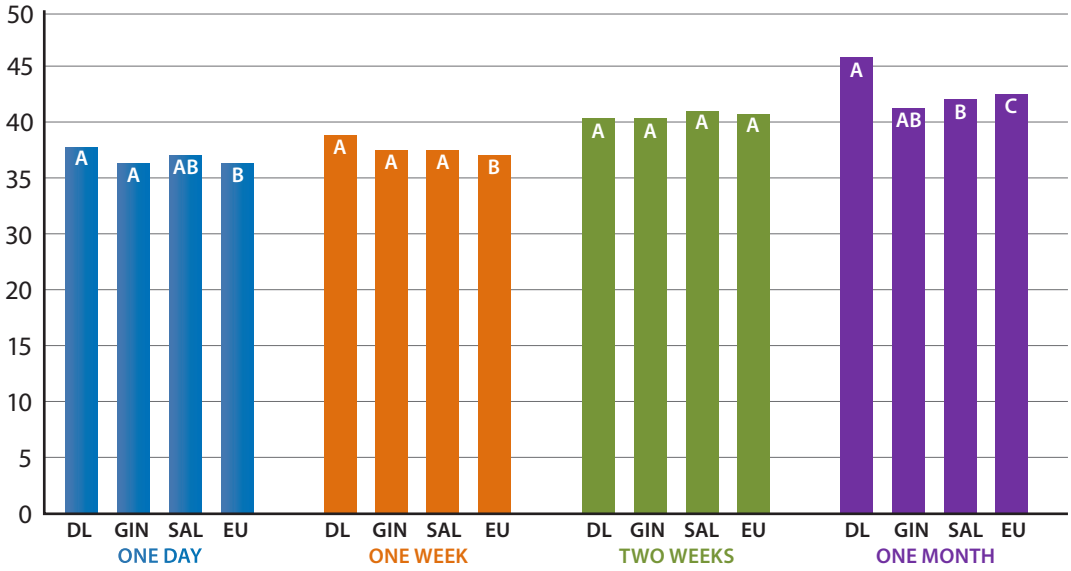
were calculated and compared statistically with one way Analysis of Variance and Duncan's multiple range test to determine the significant difference among the groups at $p \leq 0.05$ level of significance by using SPSS version 11.5.

Figure 1. *Candida albicans* colony forming units per milliliter (CFU/ml) of tested samples.



PC: Positive control. **NC:** Negative control. **DL:** Distiled water. **GIN:** Ginger oil. **SAL:** *Salvia officinalis* oil. **EU:** Eucalyptus oil.

Figure 2. Duncan multiple range test of hardness of Vertex denture lining material after different times after coating with plant oils.



DL: Distiled water. **GIN:** Ginger oil. **SAL:** *Salvia officinalis* oil. **EU:** Eucalyptus oil.

RESULTS.

The results obtained from the study are shown in Figure 1 and Figure 2, which show the analysis of variance (one way ANOVA) and Duncan multiple range test, and where it can be observed that the differences were highly significant at $p \leq 0.05$ between the five groups for *Candida albicans* growth as measured by CFU/ml in BHI broth. *Salvia officinalis* oil showed the highest antifungal action (379,500) followed by ginger oil (462,500), while eucalyptus oil showed the lowest antifungal action (2,215,500). The antifungal activity of *Salvia officinalis* oil is due to the presence of active ingredient (flavonoid glycosides, phenolic acids, salvins and monomethyl) and this result is in agreement with Gorbani *et al.*,[5] whose studies revealed a wide range of pharmacological activity of S*alvia officinalis*.

The antifungal result for ginger oil is supported by Aghazadeh *et al.*,[3] who found that ginger extract

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has good antifungal and antibiofilm activity against *Candida albicans* and *Candida krusei*. The results we obtained for eucalyptus oil are in agreement with Agarwal *et al.,*[4] who revealed that peppermint, eucalyptus, ginger grass and clove oils act as effective antifungal agents against *Candida albicans*, and performed better than fluconazole.

DISCUSSION.

The analysis of variance (one way ANOVA) and Duncan multiple range test revealed a significant difference ($p \leq 0.05$) in hardness of the denture lining material between different times of storage in water (1, 7, 14, and 30 days) within four groups coated with plant oils and the control group. Acrylic resin-based product hardness increased when stored in water and the initial softness is due to the quantity of plasticizer in the liquid, and since plasticizers are responsible for maintaining the softness in acrylic -based liner materials and plasticizers leach, causing hardening as duration of immersion increases, in line with Mese *et al.,*[8] and Pahuja *et al.,*[9] who found that the hardness of soft lining materials are higher with increased duration of storage in water.

Figure 2 shows results of analysis of variance (one way ANOVA) and Duncan multiple range test that revealed a significant difference ($p \leq 0.05$) in hardness for denture lining materials between groups coated with plant oils and control group within different times of water storage (1, 7, 30 day), except within 14 days of water storage when there was no significant difference ($p > 0.05$) between groups. Coating soft lining materials with natural plant oils increased softness for materials due to the oil diffusing into the material and acting as a plasticizer. The explanation of decreasing the hardness for soft lining materials means an increase of softness with oils addition together with maintaining softness for materials for longer also probably due to the oil reducing the amount of plasticizer leaching out during storage in water. This result is in agreement with Francis *et al.,*[10] who concluded that the sealing of soft lining materials increases the softness and plays an important role in the preservation of the hardness of some reline materials.

CONCLUSION.

The soft acrylic lining material when coated with selected natural plant oils has antifungal activity as compared with uncoated material but there was no significant difference among the oils (*Salvia officinalis*, ginger and eucalyptus oils). In addition these oils increased the softness and duration of this material.

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