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Antibacterial activity and effects of aromatic derivatives on denture base polymerization.

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Abstract: This study assessed the effect of copolymerization on the microbiota of the oral cavity. The plant extraction was converted into aromatic derivatives, which were added to methyl methacrylate monomer. Fourier transform infrared spectroscopy spectra showed no negative effects of these additives on the polymerization process. All the assayed derivatives displayed some degree of antibacterial activity.

Keywords: Polymethyl methacrylate; denture bases; Fourier transform infrared spectroscopy; eugenol; anti-bacterial agents.

INTRODUCTION.

Acrylic resin is widely used as a denture base material beside its many other dental applications. The wearing of complete dentures may have adverse effects on the health of both oral and denture supporting tissues, and may result in denture-induced stomatitis, which is a common recurring disease for edentulous patients.

Despite its multifactorial etiology, great importance can be attributed to bacterial and *Candida sp*. infections, especially when associated to a poor oral hygiene. Therefore, it is important to ensure that elderly denture wearers are provided with adequate oral hygiene, and daily hygienic management of the prosthetic surface is recommended, such as mechanical cleaning or immersion of the denture in specific cleansers containing synthetic or natural products, or a combination of both methods for denture hygiene. However, it has been reported that mechanical cleaning methods are insufficient for a complete reduction of microorganisms on denture bases.

On the other hand, most denture cleansers are not effective in reducing plaque accumulation and residual microorganism retention has been observed after immersion in some denture cleaning solutions. Thus, modifying denture base materials to resist or decrease microbial accumulation would be beneficial for daily oral hygienic management.[1-3]

Dimethylaminododecyl methacrylate (DMADDM) has been reported to reduce the number of viable microbes in multi-species biofilms.[4] Denture base resins containing immobilized quaternized ammonium monomers provided high antibacterial activity, but the flexural strength and flexural modulus of the denture base resins decreased.[5] The Kirby-Bauer antibiotic testing method is widely used to test antibiotics; It is a simple way of determine the activity of antibiotics against microorganisms. The effectiveness of antimicrobic agents

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is based on the diameter of the zone of inhibition that surrounds a disk that has been impregnated with a specific concentration of the tested agent. Only by considering all these variables can a reliable method be worked out.[6] This paper investigated the incorporation of eugenol, eugenol benzoate, eugenol acetate and eugenol methyl ether into acrylic resin to improve its antimicrobial activity.

MATERIALS AND METHODS.

Preparation of Eugenol

One hundred and twenty milliliters was of steam distillation from 15g of cloves a in 150 ml of distilled water was collected for about 100 minutes. We extract three 15ml-portions of diethyl ether. Three portions of 25ml of 5% NaOH were added to the ether layer and shaked well. Three portions of 25ml of 5% NaOH were added to the aqueous layer and shaked well. Ether solvent was evaporated after anhydrous magnesium sulfate was added.

Preparation of Eugenol Benzoate

We swirled 3.05mmol eugenol with 10ml of 10% aqueous sodium hydroxide; 8.59mmol benzoyl chloride was added with constant shaking for 30 minutes. The solid fraction was collected by suction and washed with cold water.

Preparation of Eugenol Acetate

We shaked 1.5:4:3 eugenol, pyridine and acetic anhydride in a dry flask for 8 hrs. The mixture was extracted with ether after pouring into ice-cold water and washed with 1N HCl, 5% $NaHCO_3$ respectively. The solvent was evaporated under vacuum after drying with anhydrous sodium sulphate.

Preparations of Eugenol Methylate

Eugenol and sodium hydroxide (0.06:0.0625 moles) in 20ml of water were cooled in a ice-salt bath. We added 0.06 moles dimethyl sulfate with stirring during three hours. The mixture boiled vigorously over a hotplate for fifteen hours. The organic portion was extracted twice with 10ml of ether. It was dried over anhydrous magnesium sulphate for 24hrs after washing with water.

Preparation and Polymerazion of Resin Samples

Methyl methacrylate monomer was mixed with each of the synthesized compounds far from light at the following percentages weight by weight: 0.0, 2.5, 5.0, 10.0, 15.0, 20.0.

Biostar Discs measuring 5mm were used to perform stone molds. Mixing and processing of acrylic resin were done at a 3:1 powder:liquid ratio. The mix was packed into the stone molds to polymerize in a boiling water bath for 30 minutes after the mixture reached the dough phase, and the top and the bottom surfaces were left without any treatment to perform antimicrobial assays.

Kirby-Bauer Disc Diffusion

An agar plate was inoculated with bacterial and fungal cultures as described below, and the resins to be tested were applied to the surface of an agar plate using sterile forceps. Following incubation for 18 to 24 hours, the diameter of the inhibition zones was measured. Three types of microorganisms isolated from the oral cavity at the Dental Basic Sciences Department at the Dentistry College were used: *Staphylococcus* sp. as a Gram-positive bacterial representative, *Escherichia Coli* as a Gram negative bacterial representative and *Candida albicans* as a fungal representative. Nutrient agar was used for *Staphylococcus* growth, MacConkey was used for *E. coli* and Sabouraud was used for *Candida albicans.* A loop full of each culture was taken and inoculated in 4ml of brain heart infusion broth, mixed well and then a swab from each suspension was spread evenly on the surface culture and left to dry for 5-10 min. Discs containing resin supplemented with five concentrations of eugenol, eugenol benzoate, eugenol acetate and eugenol methyl were added to the surface of each agar plate using forceps. Plates were inverted and incubated at 37°C for 18-24 hrs. The inhibition zone was measured in mm.

RESULTS.

Eugenol preparation: the pale yellow oily liquid was analyzed by FTIR spectroscopy. The resulting spectra showed these peaks: 3518.36 (br, s, OH str.), 3076.60 and 3003.84 (=CH aromatic and olefinic, mw), 2975.56, 2938.54, 2842.38(m, C-H str., aliphatic), 1638.06 (m, sharp, C=C olefinic), 1612.67, 1514.04, 1452.19 (m-s, sharp, C=C aromatic), 1432.38 (m, sharp, CH_2, aliphatic), 1367.11 (m, sharp, CH_3), 1268.08, 1234.16 (s, C-O str.). This fraction was identified as 4-allyl-2-methoxyphenol.

Eugenol benzoate preparation: eugenol benzoate was obtained as white needles in a (73%) yield, (mp 66-

Figure 1. FTIR spectra of dental base material supplemented with aromatic derivatives.

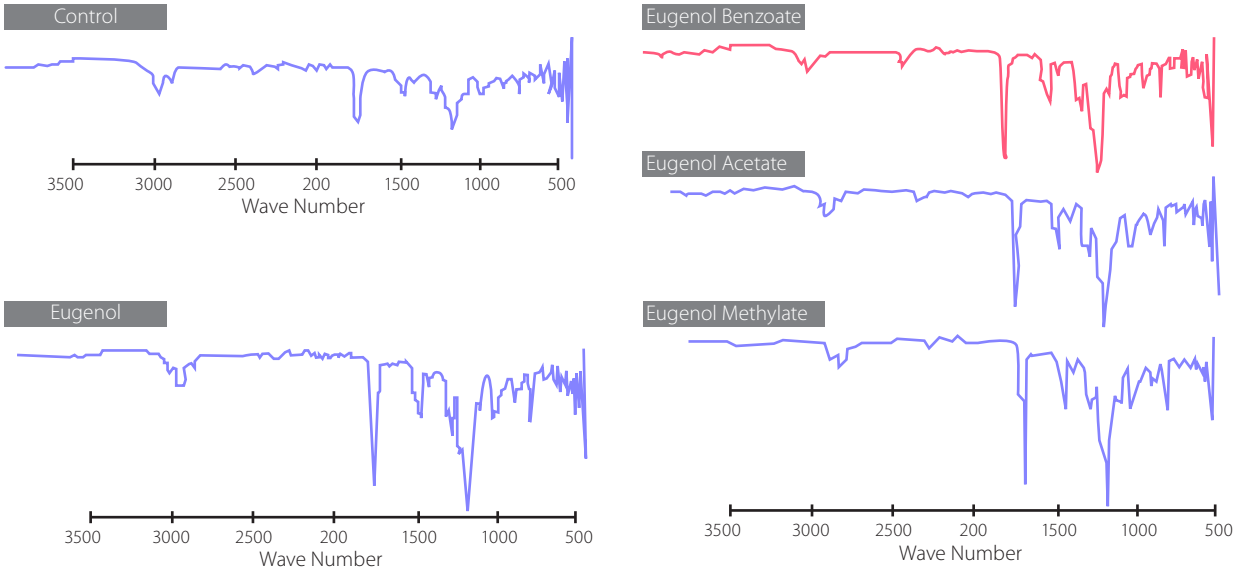

Table 1. Antimicrobial activity of denture base material supplemented with eugenol and its derivatives.

		Growth inhibition zone of *Staphylococcus* sp.					Growth inhibition zone of *E. coli*				
No	Additive (%Wt/Wt)	2.5%	5%	10%	15%	20%	2.5%	5%	10%	15%	20%
1	Control	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm
2	Eugenol	0mm	0mm	0mm	5mm	5mm	0mm	0mm	0mm	0mm	0mm
3	Eugenol Benzonate	2mm	5mm	9mm	10mm	12mm	10mm	22mm	22mm	25mm	30mm
4	Eugenol Acetate	0mm	0mm	0mm	0mm	0mm	8mm	10mm	12mm	20mm	20mm
5	Eugenol Methylate	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	0mm	2mm

None of the assayed compounds showed antifungal activity.

67°C). The structure of the product confirmed this. It displayed an absorption at 1738.35 s, sharp, C=O str. Eugenol acetate preparation: this compound was a pale yellow liquid with a freezing point at 30°C. The product structure was confirmed through absorption at 1770.03s, sharp, C=O ester str. The absence of the phenol signal (3518.36 br, s, OH str.) in the FTIR spectrum of both eugenol benzoate and eugenol acetate was noted as an indication of successful conversion of the eugenol to its derivatives. Eugenol methylate preparation: this compound was a dark yellow oil with a boiling point of 146–147°C. This material evaporate readily at room temperature and slowly thickens when exposed to air to melt at ⁻2°C. The successful conversion was confirmed by the iron chloride test and the absence of the phenol signal on the FTIR spectrum. The additives provided some degree antibacterial activity to the denture base material although no anti-fungal activity was noted (Table 1).

Base material supplemented with eugenol benzoate showed activity against both Gram negative and Gram negative bacteria at a wide range of concentrations.

DISCUSSION.

The additives were added to methyl methacrylate monomer at different percentages. The FTIR spectrum demonstrated no evidence of any chemical effect on the polymerization process, as expected. [8-10]

The antimicrobial activity of these additives was assessed on the following microorganisms commonly found in the oral cavity: *Staphylococcus* sp., *E.coli* and *C. albicans*. The

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release of antimicrobial properties was demonstrated *in vitro*.[7] The results indicated that both Gram negative and Gram negative bacteria were sensitive to some derivatives of eugenol. Eugenol benzoate showed the best and widest activity, whereas Eugenol acetate had effect on the growth of *E. coli* only and no effect on Gram positive bacteria nor fungi.

CONCLUSION.

Eugenol derivatives showed no effects on the polymerization of the dental base material. The assayed compounds showed activity against either Gram negative or Gram positive bacteria, with eugenol benzoate showing activity against both of the bacterial species tested. None of the compounds showed activity against *Candida albicans*.

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