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"EFFICACY OF OZONATED OIL AND SODIUM PERBORATE AGAINST CANDIDA ALBICANS AS DENTURE CLEANSERS AND ITS EFFECT ON COLOR STABILITY IN PMMA RESINS"- A PILOT STUDY

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Abstract: There are many commercially available denture cleansers in the market, but yet there is a need to find natural denture cleanser. One such example is stated in this article.

AIM OF THE STUDY:

Evaluation of efficacy of ozonated oil and sodium perborate against candida Albicans as denture cleansers and its effect on color stability in PMMA resins.

OBJECTIVES OF THE STUDY:

1. To evaluate the efficacy of ozonated olive oil, as a denture cleanser against Candida Albicans.

2. To evaluate the efficacy of sodium perborate as a denture cleanser against Candida Albicans.

3. To evaluate the color stability of denture base after rinsing with ozonated olive oil.

4. To evaluate the color stability of denture base after rinsing with sodium perborate.

5. To compare the efficacy of ozonated oil (immersion) and sodium perborate against Candida Albicans.

6. To compare the color stability of denture base after rinsing in ozonated olive oil and sodium perborate.

Results: ozonated olive oil showed superior properties in both anticandidal efficacy and color stability as a denture cleanser on PMMA resins

Keywords: Ozonated olive oil, Sodium perborate, Distilled water, Denture cleanser

Introduction:

Polymethylmethacrylate (PMMA) was first introduced by Walter Wright in 1937 as a denture base resin and became superior overall materials by 1940. It is been used successfully for various applications in dentistry for many years.[1] It is the material of choice because of its ease of processing, low cost, lightweight, favorable physical and mechanical properties, water sorption, solubility, and ability to repair easily.[2]

The resin consists of polymer chains, which in turn affect the properties of the polymer material based on its length, branching, and cross-linking. With increasing polymer chain length, the links between polymer chains increase hence making it more challenging to undergo deformation. Cross-linking within the polymer affects the physical and mechanical properties of the polymer. Cross-linking can decrease solubility and increase the strength and rigidity of the polymer.

Basic requirements for denture base polymers include adequate mechanical properties, sufficient esthetics, hygiene, and easy handling. Post-insertion instructions given to patients during insertion appointment help in maintaining healthy oral mucosa. Denture hygiene is important for general health, especially in elderly patients who cannot adequately clean their dentures because of various reasons like disease, dementia, and poor dexterity.[3] The optimal environment is provided for adhesion and multiplication of both pathogenic and non-pathogenic organisms by dentures in the mouth.[4] The denture base material can get colonized and infected by microorganisms on long-term use.

Around 60-65% of denture wearers are affected by Candida-associated denture stomatitis, which is usually seen on the palatal mucosa beneath the fitting surface of the upper denture. One of the most prominent contributing factors for this is deficient denture hygiene habits. High prevalence of Mutans streptococci, Lactobacilli, Staphylococci, and yeasts are seen in the oral cavity of continuous denture wearers.

A spongy denture tissue surface, full of nutritive substances, is an ideal incubator for species such as Candida albicans. Candida albicans is a commensal in the oral cavity of 45-65% of healthy individuals with a higher prevalence found in children and young adults. In denture wearers, the prevalence of Candida increases to 60-100% and the organism can be opportunistic, which can be explained by the fact that dentures decrease the flow of oxygen and saliva to the underlying tissue producing a local acidic and anaerobic microenvironment that favors yeast overgrowth.[5] Additionally, Candida has an affinity for the acrylic surface of dentures and nonrenewing surfaces such as teeth, dental fillings. Surface characteristics of denture base acrylic resins, such as hydrophobicity, have generally been acknowledged to be one of the factors contributing to the adhesion, which is a crucial step in biofilm formation. Candida albicans biofilms are frequently associated with the occurrence of denture stomatitis, but non-albicans species, such as C. glabrata, C. tropicalis, C. krusei, C. parapsilosis, and C. dubliniensis, can also contribute to the development of this infection. Its primary location is the posterior tongue and other oral sites such as the mucosa, while the film that covers the dental surfaces is colonized secondarily. Cells in this unique environment are equipped to withstand host defenses and survive antifungal therapy. Effective removal of biofilm is a significant challenge by both chemical and mechanical methods. Various methods advocated for denture cleaning include mechanical, chemical, and a combination of both. [6]

The most common and effective procedure for biofilm removal on the prosthesis is by mechanical methods. In patients with a lack of motor coordination, such methods may be ineffective, so alternative methods like chemical cleaning are advisable. The rate at which deposits accumulate on dentures varies from individual to individual and is affected by certain factors like saliva composition, surface texture, dietary intake, duration of denture wearing.[7]

Many systemic diseases have been associated with oral candidiasis. The primary cause is attributed to the decreased salivary secretion, leading to the reduced concentration of

immunoglobulin in the saliva and less efficient humoral-mediated host defense against C. Albicans.

For patients with diabetes mellitus, besides the reduced salivary flow, the high level of blood glucose also plays a significant role and is associated with reduced salivary pH and facilitates oral candidal overgrowth and colonization.[8]

As an opportunistic infection, oral candidiasis is also associated with a wide spectrum of systemic diseases that suppress the host autoimmunity. Several drugs may cause the development of oral candidiasis by many mechanisms. The pharmacological action of the broad-spectrum group of antibiotics may break the balance within the normal oral flora, resulting in the overgrowth of C. Albicans. Drugs such as corticosteroids may suppress either the nonspecific inflammatory response or the Tcell-mediated immunity, which can, in turn, predispose individuals to oral candidiasis. Drugs that have xerogenic effects can cause oral candidiasis by directly reducing the salivary flow. It appears that the association between oral candidiasis and systemic diseases, intake of medications has been well established.

Several disinfectants have been suggested for the disinfection of dentures. The disinfectant should possess certain features of the ideal agent while not altering the structure of the dentures. An ideal denture cleanser should exhibit bactericidal and fungicidal effects, should have antibiofilm activity, should be biocompatible and nontoxic to the structure of denture, should effectively remove deposits, should have an acceptable taste, and should be easy to use.

Commonly used denture cleansers include sodium hypochlorite and alkaline peroxides. The peroxide solution formed on dissolving sodium perborate in water releases oxygen, which enables mechanical cleaning by oxygen bubbles in addition to the chemical cleaning.[9] Sodium hypochlorite has been suggested as an effective hygiene agent, acting on the organic biofilm matrix, it is bactericidal and fungicidal as well as a stain remover.

Though they are proven efficient, they have some disadvantages. One of the disadvantages of sodium hypochlorite is that it may cause the whitening of the acrylic resin. Regarding peroxides, there have been reports on the occurrence of damage to the acrylic resin and metallic components of the prosthetic device in the form of surface oxidation. Frequent use of chemical cleansers causes the whitening of the denture, and this is related to the use of the high temperature of water used in the solution. They are also harmful to the environment as they are not biodegradable.

Natural products can be used as an alternative to chemical cleansers. Natural products and essential oils are promising therapeutic tools for oral infection. Essential oils obtained from plants are complex mixtures of volatile compounds, and they also possess antioxidant and antimicrobial properties against a wide range of pathogens, including Candida albicans and dermatophytes. When compared with chemical cleansers, these natural products are biodegradable and cause less harm to the environment. Many specialized products are available in the market for denture cleansing, but there is decreased access for a continuous supply of such materials for the elderly population having dentures. Hence there is a need to introduce a few natural products to clean dentures, which are easily and economically available.

Ozone is a natural gaseous molecule made up of three oxygen atoms. The word originates from the Greek word ozein, which means odor, and was first used in 1840 by german chemist Christian Friedrich Schonbein “the father of ozone therapy”. The first dentist to use ozone

therapy in his practice was E.A.Fisch in the 1930's, to aid in disinfection and wound healing during dental surgeries.

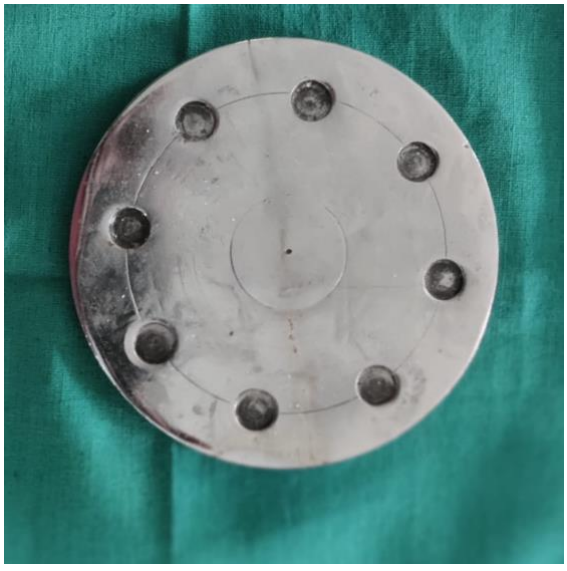
Ozonated oil is a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses. Oxygen is one of the most classic elements on the earth, from which ozone is obtained. Ozone can be used mainly in three forms in dentistry: ozone gas, ozone oil, ozonated water. As ozone is in gaseous form it needs to be incorporated in certain media like oil or water.

In this study ozonated oil is used because when ozone gas is incorporated into oil, it has more shelf life than that incorporated into the water. The properties of ozone remain for a long duration when incorporated into oil rather than when incorporated into the water. The most commonly used oil is olive oil because of its various benefits, ozonated olive oil addresses the problem of athlete's foot and toenail fungus. It acts as an antifungal and cleans the skin. Studies show hydroxytyrosol, an antioxidant found in olive oil, kills the molds, yeasts, and dermatophytes associated with both toenail fungus and athlete's foot. Hence, in this study ozonated oil incorporated into olive oil is used.

Methodology:

This study was conducted in the post-graduate laboratories of the Department of Prosthodontics and Crown & Bridge and Department of Oral Pathology and Microbiology in Lenora Institute of Dental Sciences.

For this study, a total of 30 specimens of heat-cured acrylic denture base resin were fabricated from metal dies, out of which 30 are of 10mmx2mm for checking candida efficacy and the other 30 are 15 mm x 4 mm (ADA specification no.12) for color stability test. First, a metal die of circular measurements 10 mm x 2 mm(Figure- 1) was fabricated for checking candida efficacy.



(Figure-1)

The lubricating gel was applied in the die space. The molten wax was poured into the mold space and allowed to set (Figure-2).

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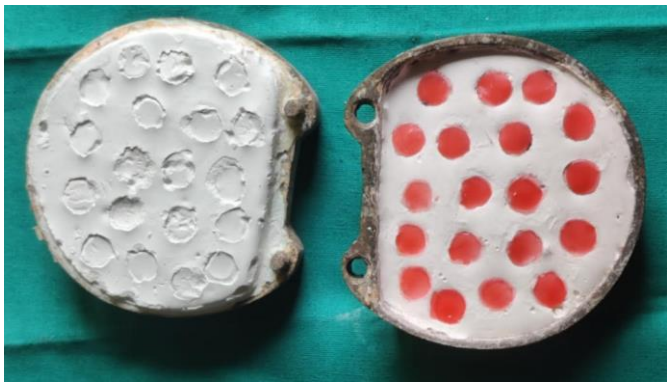
(Figure- 2)

These wax samples were invested in the lower part of the flask with model plaster (Figure-3) and dewaxed to form molds.



(Figure-3)

The molds formed were packed with heat cure acrylic resin and acrylization was done according to the manufacturer instructions under conventional technique. Finally, deflasking, finishing, and polishing were carried out.

The specimens were stored in distilled water for 37^0c for 48 hrs before immersing into denture cleansers for the removal of residual monomer.

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A similar procedure was carried out for checking color stability but with circular specimens of measurement 15 mm x 4 mm (Figure- 4). 

(Figure- 4)

The lubricating gel was applied in the die space. The molten wax was poured into the mold space and allowed to set (Figure- 5).

(Figure-5)

These wax samples were invested in the lower part of the flask with model plaster (Figure-6) and dewaxed to form molds.

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(Figure – 6)

The molds formed were packed with heat cure acrylic resin and acrylization was done according to the manufacturer instructions under conventional technique(Figure-7).

(Figure-7)

Finally, deflasking, finishing, and polishing was carried out (Figure-8)

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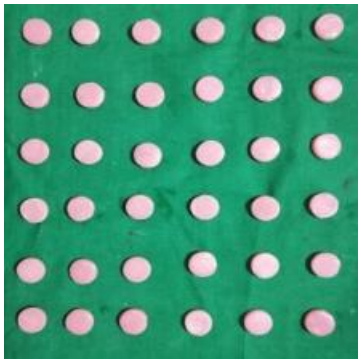


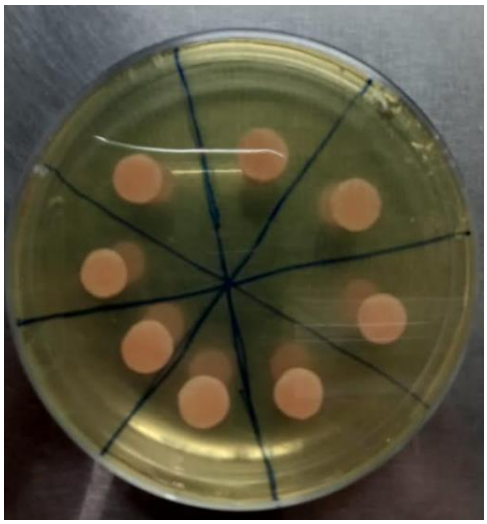
(Figure-8)

A total of 60 acrylic specimens were grouped into 2 groups each consisting of 75 specimens. Group, I contained circular discs of dimension 10mm x 2mm and group contained circular discs of dimension 15 mm x 4 mm. Each group was then divided into 3 subgroups. Group Ia and IIa consisted of the control group, Group Ib and IIb consisted of sodium perborate. Group Ic and IIc consisted of ozonated olive oil.

Immersion of specimens:

For testing antifungal activity:

- Group Ia (control group): 10 samples were placed in candida solution for 2 days at 37^0c. These samples were removed and rinsed with saline to remove the free microbes and were placed in distilled water. After that these samples were removed and were placed into the tubes containing saline solution and then the cells were dispersed by vortexing. From this initial solution, aliquots of 0.1 ml were plated on sabroud's dextrose agar (Figure-9) and were counted immediately, after 24 hrs and after 48 hrs, and then compared and calculated in CFU/ml.



(Figure- 9)

- Group Ib (sodium perborate): 10 samples were placed in candida solution for 2 days at 37^0c. These samples were removed and rinsed with saline to remove the free microbes and were placed in sodium perborate. After that these samples were removed and was placed into the tubes containing saline solution and then the cells were dispersed by vortexing.

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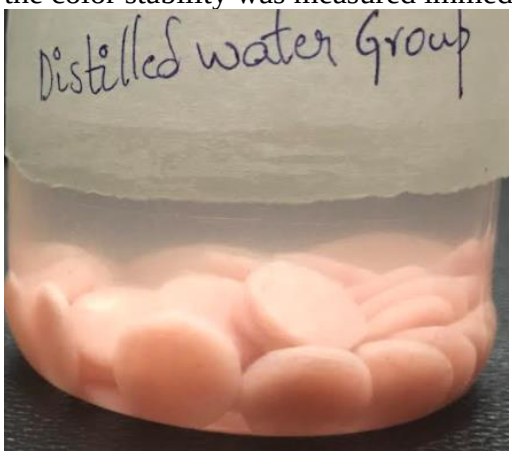
From this initial solution, aliquots of 0.1 ml were plated on sabroud's dextrose agar and were counted immediately, after 24 hrs and after 48 hrs, and then compared and calculated in CFU/ml.

- Group Ic (Ozonated olive oil): 10 samples were placed in candida solution for 2 days at 37^0c. These samples were removed and rinsed with saline to remove the free microbes and were placed in ozonated olive oil. After that these samples were removed and placed into the tubes containing saline solution and then the cells were dispersed by vortexing. From this initial solution, aliquots of 0.1 ml were plated on sabroud's dextrose agar and were counted immediately, after 24 hrs and after 48 hrs, and then compared and calculated in CFU/ml.

For checking color stability:

A metal die of circular measurements 15 mm x 4 mm will be fabricated for checking color stability. A lubricating gel will be applied in the die space. Molten wax will be poured into the mold space and allowed to set. These wax samples will be invested in the lower part of the flask with model plaster and dewaxed to form molds. The molds formed will be packed with heat cure acrylic resin and acrylization will be done according to the manufacturer's instructions under conventional technique. Finally, deflasking, finishing, and polishing will be carried out. The specimens will be stored in distilled water for 37^0c for 48 hrs before immersing into denture cleansers for the removal of residual monomer.

- Group IIa (control group): 10 samples will be placed in distilled water (Figure-10) and the color stability was measured immediately, after 24 hrs and after 48hrs.



(Figure-10)

- Group IIb(sodium perborate): 10 samples will be placed in sodium perborate (Figure-11) and the color stability was measured immediately, after 24 hrs and after 48hrs.

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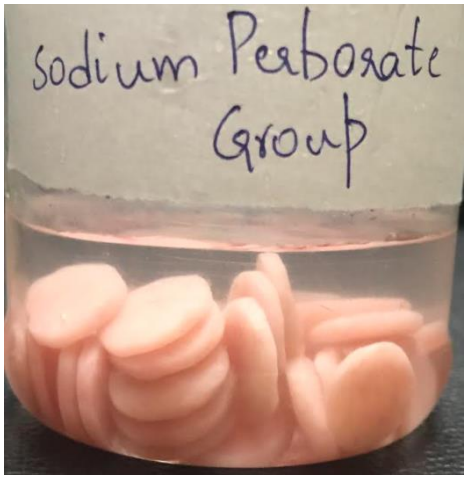

(Figure- 11)

- Group IIc (Ozonated olive oil): 10 samples will be placed in Ozonated olive oil (Figure – 12) and the color stability was measured immediately, after 24 hrs, and after 48hrs.

(Figure-12)

Color stability was measured using a spectrophotometer. The spectrophotometer was calibrated according to the manufacturer's instructions before each measurement period using the white calibration plate supplied by the manufacturer. The color differences were evaluated using the Commission Internationale de l'Eclairage L*a*b* colorimetric system. This system is based on three parameters for defining color: L*, a*, and b *. L* represents lightness, a* represents red-green, and b* represents yellow-blue. The color change (ΔE) of each specimen was calculated as follows: $\Delta E = [(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*) 2]½$ where a* and b* are on the chromatic scale and represent red as +a* and green as - a*, while yellow corresponds to + b* and blue to -b*. Delta L*, Δa*, and Δb* represent the differences measured in L*, a*, and b* values before and after immersion. The levels of color change (ΔE) have been quantified by the National Bureau of Standards (NBS) with the NBS units of color difference. NBS units are expressed by the following formula: NBS unit = $\Delta E \times 0.92$ S = $3PL/2bd^2$.

Discussion:

For the present study ozonated oil was used as a denture cleanser because of its antimicrobial property, and Fittydent (sodium perborate) was selected as it has better properties among commercially available denture cleansers. Studies conducted by Peracini A et al[3]., Raj N and D'Souza M[10], Paranhos HFO[5] et al., and Sharma P[11] et al., where they compared fittydent with hypochlorites and peroxides, proved that fittydent did not show any significant difference in the properties of denture base resins. Therefore, it was used for comparing with ozonated oil.

According to Arita M[12] et al., a combination of ozonated water and ultrasonication had a strong effect on the viability of C. Albicans adhering to the acrylic resin plates. There were no significant differences in antimicrobial activity against C. Albicans between plates immersed in ozonated water with ultrasonication and those treated with commercially available denture cleaners. But whereas in my study there is a marked difference in the candidal count when ozonated oil was used against candida species.

The results obtained for anticandidal efficacy in the present study stated that there is a marked decrease in the candida count when ozonated oil was used as denture cleanser when compared with distilled water and sodium perborate.

Agarwal V[13] et al. stated that natural oils serve as a source of compounds with therapeutic potential against Candida-related infections. Similarly, in my study ozonated olive oil, a natural oil was used for testing candida efficacy.

Results obtained in the current study for color stability noticed the significant color change in the denture base acrylic resin immersed in fittydent solution and this was in agreement with the study conducted by Peracini A[3] et al., as they also observed significant color change of the acrylic resins tested with Correga tabs, but they claim that the result obtained in their study may be due to the short simulation period (30 days) and visual comparisons made by photographs. My study result was also in accordance with the study by Piskin B[14] et al., where a significant change in color of the acrylic resins was observed after immersion in various chemical disinfectants with various concentrations.

Present study results for color stability were also on par with a study by Da Silva LCH[5] et al., where they evaluated the color stability of disc-shaped specimens fabricated with denture tooth acrylic resin after being subjected to various chemical disinfectants and observed alteration in the color of the acrylic resin depending on the duration of immersion period. However, Sato S[15] et al., in their research study did not identify any color changes in the acrylic resins with the use of chemical agents.

According to Amin F[16] et al., the color change was noticed in acrylic resins after immersion in different denture cleansers, and it was more affected after treatment with Fittydent denture cleansing tablets when compared to Dentipur denture cleanser tablets. They noticed a color change not only in the experimental group but also in the control group, i.e., Distilled water group, and justified their result by mentioning that color change may be due to irreversible damage caused to acrylic resins by water, because of the formation of microcracks due to consecutive sorption and desorption cycles. This results in hydrolytic degradation of the polymer by causing damage to the ester linkages and slow weakening of the infrastructure of the polymer, which leads to the development of acrylic zones with different optical properties, which can be unesthetic and detected visually. In the current study also color change was observed in the acrylic resin after immersion in denture cleansers, which may be due to the reason mentioned by Amin F et al.

Porwal A[17] and his colleagues in their study reported an increase in color change for sodium perborate denture cleansers when compared with sodium hypochlorite. In my study also color change was noticed in acrylic resins immersed in sodium perborate solution.

In the current study color stability values for the specimens immersed in ozonated oil denture cleanser was found to be more than that of the sodium perborate group and control group., i.e., a change in color of the acrylic resin was observed less in an ozonated group than sodium perborate group and control group which is a positive sign.

Results:

Anti Candidal efficacy was measured using an Electron microscope. Specimens were inoculated into SDA and anticandidal efficacy was measured Immediately, after 24 hours and 48hours. It was done with the help of Grams staining and finally after which they were examined for anticandidal species under an electron microscope. Anti candidal efficacy results were analyzed by the Kruskal-Wallis H test and mean comparison test. The p-value < 0.05 is considered statistically significant.

The color stability was measured using a spectrophotometer. The color differences were evaluated using the Commission Internationale de I'Eclairage L*, a*, b* colorimetric system. Color stability test results were analyzed statistically by one–way ANOVA analysis and mean comparison test. The p-value < 0.05 is considered statistically significant.

Conclusion:

Ozonated oil is a powerful antimicrobial agent against bacteria, fungi, protozoa, and viruses. Oxygen is one of the most classic elements on the earth, from which ozone is obtained. It is nothing but, a gaseous molecule made up of three oxygen atoms. When compared with chemical cleansers, these products are biodegradable and cause less harm to the environment. Hence there is a need to introduce a few newer products as denture cleansers, which are easily and economically available.

Considering the methodological limitations of this study, the following conclusions were drawn:-

Ozonated olive oil may possess absolutely no or minimal side effects and can be used as a denture cleanser. The properties of ozone remain for a long duration when incorporated into oil rather than when incorporated into the water.

Color stability of heat-cured acrylic denture base resin after immersion in ozonated olive oil reported that there was no alteration in the color of the resin samples.

Anticandidal efficacy was more after immersing with ozonated olive oil ie., the candida count was reduced after immersion in ozonated olive oil.

On comparing the anticandidal efficacy of ozonated olive oil and sodium perborate against candida Albicans, there was found to be marked reduction of candida count when immersed in ozonated olive oil than sodium perborate.

On comparing the color stability of denture base after rinsing in ozonated olive oil and sodium perborate there was no significant color change with ozonated olive oil when compared to sodium perborate.

From this study, we can conclude that ozonated oil can be used as a denture cleanser because of its antimicrobial and anticandidal properties, and also it has no adverse affects on the physical properties of denture base material.

A single variant of ozonated oil was used in this study, there are many variants available. Further studies using different variants can be undertaken in the future to know the effect of Ozonated oil as a denture cleanser on the properties of polymethylmethacrylate.

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