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The influence of tee tree oil and sage oil (immersing and incorporation) on candida albicans adhesion on acrylic denture base material

Graduation Project Submitted to the Council of the College of Health and Medical Technologies, Al-Mustaqbal University as a partial Fulfilment of Requirements for the degree of Bachelor of Dental Technician Department

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿دَعْوَاهُمْ فِيهَا سُبْحَانَكَ اللَّهُمَّ وَتَحِيَّتُهُمْ
فِيهَا سَلَامٌ ۚ وَآخِرُ دَعْوَاهُمْ
أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ﴾

{ يونس: 10}

الاهداء

بسم الله الرحمن الرحيم

"وآخر دعواهم أن الحمد لله رب العالمين"

الحمد لله الذي بنعمته تتم الصالحات، الحمد لله الذي وفقني وأعانني حتى بلغت هذا اليوم الذي طالما انتظرته وسعيت لأجله.

إلى أساتذتي الأفاضل

لكم كل التقدير والاحترام، فأنتم من زرعتم فيّ حب التعلم وكنتم منارات أضاءت طريقي بالعلم والمعرفة، لن أنسى فضلكم ما حييت، وأسأل الله أن يجزيكم خير الجزاء.

إلى الذي زيّن اسمي بأجمل الألقاب، من دعمني بلا حدود ومن علّمني أن الدنيا كفاح وسلاحه العلم والمعرفة، داعمي الأول في مسيرتي، وسندي وقوتي وملاذي بعد الله...

إلى فخري واعتزازي... أبي الغالي...

إلى من جعل الله الجنة تحت أقدامها واحتضنتني قلبًا قبل يدها وسهّلت لي الشدائد بدعائها، إلى القلب الحنون والشمعة التي كانت لي في الليالي المظلمات، سر قوتي ونجاحي...

أمي الغالية...

إلى ضلعي الثابت وأمان أيامي، إلى من تشدّدت عضدي بهم فكانوا لي ينابيع ارتوي منها إلى قرة عيني

إخوتي وأخواتي...

لكل من كان عونًا وسندًا في هذا الطريق... للأصدقاء الأوفياء ورفقاء السنين، لأصحاب الشدائد والأزمات أهديكم هذا الإنجاز وثمرة نجاحي الذي طالما تمنيته، ها أنا اليوم أكملت وتذوقت أولى ثمراته بفضل الله سبحانه وتعالى، فالحمد لله على ما وهبني.

شكر وتقدير

الحمدُ لله أولًا وآخرًا، الذي وفقني لإتمام هذا العمل، ومنّ عليّ بالصبر والقوة لإكماله

أتقدم بخالص الشكر والتقدير إلى أستاذتي المشرفة (الدكتورة رقيه جعفر باقر)

لما قدّمته من توجيهٍ قيّم ودعمٍ مستمر، كان له الأثر الكبير في إنجاز هذا البحث

كما أتقدم بجزيل الشكر إلى أساتذتي الكرام في قسم لما بذلوه من جهودٍ في تعليمنا وإرشادنا طوال فترة دراستنا

ولا يفوتني أن أعبر عن امتناني العميق لعائلتي الكريمة، التي كانت السند والداعم الأول لي

فبفضل دعائهم وتشجيعهم وصلتُ إلى هذه المرحلة

وأتوجه بالشكر إلى أصدقائي وزملائي، لكل من قدّم لي يد العون والمساندة، وساهم

ولو بكلمة طيبة في دعمي خلال هذه الرحلة

وفي الختام، أسأل الله أن يوفق الجميع لما فيه الخير، وأن يجعل هذا العمل خالصًا لوجهه الكريم

Commitment

We, the undersigned, pledge that the entitled research "**The influence of tee tree oil and sage oil (immersing and incorporation) on candida albicans adhesion on acrylic denture base material**" by "**Taiba Assad - Mays Ali - Shahd Firas - Mariam Kamel - Baneen Mazen - Hawraa Abdul kareem - Tabarak hameed - Safa Haider - Zahraa Ibrahim**" is not completely stolen or copied from any source, that the sources have been referred to faithfully, and that it has been completed under the supervision of the supervising professor.

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Student name:

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Committee Certification

We certify that we have read the entitled research " **The influence of tee tree oil and sage oil (immersing and incorporation) on candida albicans adhesion on acrylic denture base material**" and as the Examining Committee, we have examined the student "**Taiba Assad - Mays Ali - Shahd Firas - Mariam Kamel - Baneen Mazen - Hawraa Abdul kareem - Tabarak hameed - Safa Haider - Zahraa Ibrahim**" in its contents in 2026, and that, in our opinion, it is adequate as research for the degree of Bachelor of Dental Technician Department.

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Abstract

Denture stomatitis, primarily caused by the adhesion and colonization of *Candida albicans* on the porous surfaces of Poly (methyl methacrylate) (PMMA), remains a prevalent clinical challenge for prosthetic patients. Conventional chemical cleansers often induce adverse effects on the material's properties, prompting the search for biocompatible natural alternatives.

This study aimed to evaluate and compare the antifungal efficacy of Tea Tree Oil (TTO) and Sage Oil (SO) against *Candida albicans* using two application methods: immersion and incorporation into the heat-cured PMMA denture base material.

Thirty PMMA samples (10mm x 10mm x 5mm) were prepared and divided into groups. Fungal growth was monitored using spectrophotometry at a wavelength of 600 nm, where the optical density (OD) of the inoculated broth increased from 0.562 to 1.381 within 24 hours. In the immersion method, samples were soaked in 100% oil for 10 minutes. In the incorporation method, 15% TTO or SO was mixed with the PMMA monomer prior to polymerization. Fungal colony diameters were measured after 48 hours and one week.

The results demonstrated that the immersion method provided superior antifungal protection compared to incorporation. Tea Tree Oil (TTO) exhibited higher and more consistent inhibitory effects than Sage Oil (SO) across both methods. In the incorporation trial, the mean colony diameter for TTO was 3.29 mm (SD ±1.16), while SO recorded a higher mean of 3.71 mm (SD ±1.91). Control groups showed "Very High" fungal density with no inhibition zones, confirming the lack of inherent antimicrobial activity in conventional PMMA.

Tea Tree Oil is a potent natural antifungal agent suitable for denture hygiene. While immersion is the most effective topical protocol for immediate disinfection, incorporation offers a potential self-disinfecting mechanism, provided that concentrations are optimized to maintain the mechanical integrity of the denture base.

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Chapter One
Introduction
And
Basic Concepts

Chapter One: Introduction and Basic Concepts

1.1 Background of Denture Base Materials

Since its introduction to the field of dentistry in the 1930s, Poly (methyl methacrylate) (PMMA) has remained the undisputed gold standard and the most predominantly used material for the fabrication of complete and partial denture bases. PMMA offers significant advantages in terms of cost-effectiveness, ease of manipulation, simple laboratory processing equipment, and the ability to be easily repaired or relined when clinical adjustments are required **(Fatin A. Asim, Studying the Physical and Biological Characteristics of Denture Base Resin PMMA Reinforced with ZrO2 and TiO2 Nanoparticles, 2022).**

However, despite these excellent functional characteristics, PMMA is far from being an ideal dental material. It possesses inherent physical and mechanical shortcomings that directly affect its long-term clinical performance. One of the most critical structural flaws of PMMA is the occurrence of inherent surface and internal porosity. Porosity can develop due to various factors during the laboratory processing phase, such as the rapid volatilization of the unreacted monomer if the curing temperature exceeds the boiling pointو along with the natural surface roughness of the acrylic resin, create a highly favorable microenvironment that facilitates the mechanical entrapment of food debris and the subsequent adhesion and colonization of various oral microorganisms **(M. S. Zafar, Polymeric Denture Base Materials: A Review, 2020)**, the physical properties of the denture base are directly correlated with the biological complications observed in completely edentulous patients.

1.2 Denture Stomatitis and Fungal Adhesion

The continuous wearing of a removable acrylic prosthesis fundamentally alters the micro-ecological balance of the oral cavity. The denture base acts as a physical barrier that isolates the underlying palatal mucosa from the mechanical cleansing action of the tongue and the protective, antimicrobial buffering capacity of saliva. This isolation results in a localized microenvironment characterized by low oxygen tension, decreased pH, Clinical studies suggest that denture stomatitis affects approximately 20% to 80% of complete denture wearers globally, **(Gordon Ramage, Early Adhesion of Candida albicans onto Dental Acrylic Surfaces, 2017)**.

the primary microbiological agent universally implicated in its pathogenesis is the opportunistic fungal pathogen, Candida albicans. C. albicans possesses a remarkable affinity for the polymethyl methacrylate surfaces. The pathogenesis begins with the initial adherence of the yeast cells to the porous acrylic surface, a process mediated by complex physicochemical interactions including hydrophobic forces, electrostatic interactions, and the presence of a salivary pellicle on the denture surface, this transition allows the fungus to deeply penetrate the microscopic irregularities of the PMMA resin, ultimately leading to the maturation of a resilient, three-dimensional structured biofilm. This firmly attached biofilm acts as a continuous infectious reservoir, making it exceedingly difficult to eradicate the infection **(J. Chandra, Sensitivity of Candida Albicans Biofilm Cells Grown on Denture Acrylic to Antifungal Proteins and Chlorhexidine, 2001).**

1.3 The Limitations of Conventional Disinfection Therapies

The standard clinical management of denture-associated candidiasis typically involves a dual approach: the prescription of topical or systemic antifungal medications, The resilient architectural matrix of the Candida biofilm provides a protective shield for the embedded fungal cells, significantly upregulating their resistance to standard antifungal agents compared to free-floating, planktonic cells. This often leads to recurrent infections once the antifungal therapy is discontinued. Furthermore, the rising global concern regarding the development of multi-drug resistant strains of Candida albicans necessitates the exploration of alternative antimicrobial therapies **(J. Chandra, Sensitivity of Candida Albicans Biofilm Cells Grown on Denture Acrylic to Antifungal Proteins and Chlorhexidine, 2001)**.

Additionally, the routine use of commercial chemical denture cleansers poses a threat to the integrity of the prosthesis itself. Prolonged and continuous overnight immersion in strong chemical agents, particularly sodium hypochlorite solutions, has been extensively documented to induce detrimental alterations in the physical and mechanical properties of the PMMA denture base. These structural degradations include surface bleaching, increased surface roughness, and a reduction in flexural and transverse strength, which ultimately compromises the clinical longevity of the denture **(M. S. Zafar, Polymeric Denture Base Materials: A Review, 2020)**.

1.4 Phytotherapy and Essential Oils in Dentistry

Due to the side effects associated with manufactured chemical treatments, dental research has undergone a radical shift towards natural, plant-derived alternatives. These alternatives, which exploit the medicinal properties of plants and their extracts, represent a promising approach in prosthetic dentistry. Essential oils, which are concentrated, volatile mixtures of chemical compounds extracted from plant parts, have demonstrated biological activity. This activity includes potent antimicrobial, antifungal, as well as antioxidant properties. They offer a highly biocompatible strategy for disinfecting dental materials, theoretically reducing microbial load without causing fungal resistance or degrading the polymer structure of the denture base **(L. M. Al-Nema, Evaluation of Addition of Plant Fixed Oil Extracts on Some Properties of Heat Cured Denture Base Material, 2014).**

1.5 Mechanisms of Tea Tree Oil and Sage Oil

Tea Tree Oil (TTO) and Sage Oil have shown exceptional promise in the eradication of oral fungal pathogens. Tea Tree Oil, extracted via steam distillation from the leaves of the native Australian plant Melaleuca alternifolia, is globally recognized for its profound antimicrobial efficacy. Its potent antifungal action against Candida albicans is primarily attributed to its high concentration of Terpinen-4-ol, a monoterpene that vigorously interacts with the fungal cell envelope, Research indicates that specific concentrations of TTO can significantly diminish the adherence of candidal cells to heat-cured acrylic resins **(Hussein Mohammed, Tea Tree Oil: Anew Antifungal Agents Against Candida Albicans Cells on Heat – Cured Acrylic Resin Denture Base Material, 2019).**

Sage Oil, derived from the leaves of Salvia officinalis, is deeply rooted in traditional medicine and possesses powerful bioactive constituents, including camphor, thujone, and 1,8-cineole. Sage oil exhibits a robust inhibitory effect on the morphological transition of Candida albicans from yeast to the virulent hyphal form, effectively stunting the progression of biofilm formation. Furthermore, its inherent anti-inflammatory properties can aid in soothing the inflamed oral mucosa associated with denture stomatitis, making it a highly desirable dual-action therapeutic agent in prosthetic dentistry **(L. M. Al-Nema, Evaluation of Addition of Plant Fixed Oil Extracts on Some Properties of Heat Cured Denture Base Material, 2014).**

1.6 Methods of Application: Immersion vs. Incorporation

To effectively translate the antifungal capabilities of Tea Tree Oil and Sage Oil into clinical prosthodontic practice, two distinct methodological approaches are evaluated: surface immersion and direct material incorporation. The **immersion method** functions as an external, topical disinfection protocol. It requires the patient to soak the fabricated PMMA complete or partial denture in an aqueous solution containing formulated concentrations of the essential oils for a specified duration (e.g., overnight). This method aims to physically detach the adhering biofilm and chemically eradicate the surface-bound fungal cells without altering the internal chemical composition of the acrylic resin **(Hussein Mohammed, Tea Tree Oil: Anew Antifungal Agents Against Candida Albicans Cells on Heat –Cured Acrylic Resin Denture Base Material, 2019).**

Conversely, the **incorporation method** represents a fundamental modification of the material's structural chemistry. This technique involves the precise addition and thorough blending of liquid essential oils directly into the unpolymerized PMMA matrix—either mixed with the liquid monomer or the polymer powder—prior to the heat-curing polymerization process. The primary objective of incorporation is to engineer a "smart," functionally modified acrylic resin that acts as a self-disinfecting material. By trapping the bioactive volatile compounds within the polymerized matrix, the denture base may theoretically provide a sustained, long-term release of antifungal agents directly at the tissue-prosthesis interface, preventing initial fungal adhesion. However, introducing foreign organic liquids into the intricate cross-linked network of PMMA may act as a plasticizer, potentially compromising the crucial mechanical properties of the denture, such as its transverse and flexural strength, and increasing the amount of toxic residual monomer **(Entessar H.A. Al-Mosaweb, Properties of the Modified Polymethyl Methacrylate as Denture Base Materials: A Comprehensive Review, 2023)**. Therefore, a critical comparative analysis is strictly required.

1.7 Statement of the Problem

Candida albicans adhesion to the porous surface of PMMA denture base materials is the primary etiological factor for denture stomatitis. While traditional chemical cleansers and synthetic antifungal drugs are used to control these biofilms, they suffer from severe drawbacks including the induction of microbial drug resistance,

potential toxicity to the patient, and progressive degradation of the physical and mechanical properties of the acrylic resin prosthesis. There is a pressing clinical need to explore natural, biocompatible, and effective plant-derived antifungal agents that can prevent fungal adhesion without compromising the durability of the denture base.

1.8 Aims and Objectives of the Study

1. To evaluate and quantify the isolated antifungal influence of Tea Tree Oil and Sage Oil on the adhesion of *Candida albicans* to heat-cured PMMA denture base materials.
2. To scientifically compare the efficacy of two different delivery methods: external surface Immersion versus internal material Incorporation of the essential oils.
3. To determine the optimal concentration of these oils that yields maximum antifungal properties while maintaining the structural and physical integrity of the acrylic denture base.

1.9 Research Hypothesis

- **Null Hypothesis (H0):** There is no statistically significant difference in the adhesion of *Candida albicans* on PMMA denture base materials treated with Tea Tree Oil and Sage Oil (via immersion or incorporation) compared to the untreated control group.
- **Alternative Hypothesis (H1):** The treatment of PMMA denture base materials with Tea Tree Oil and Sage Oil, whether through external immersion or internal incorporation, significantly decreases the adhesion and colonization of *Candida albicans* compared to the conventional control group.

Chapter Two

Review of Literature

Chapter Two: Review of Literature

2.1 Denture Base Materials

2.1.1 Evolution and Chemistry of Polymethyl Methacrylate (PMMA)

The pursuit of an ideal denture base material has been a primary focus in the field of prosthodontics for over a century. Before the 1930s, materials such as vulcanite rubber, celluloid, and even porcelain were utilized, but they all presented severe limitations in terms of aesthetics, weight, and dimensional stability. The introduction of Poly (methyl methacrylate) (PMMA) revolutionized the fabrication of removable prostheses. PMMA is an acrylic resin formed through the free-radical addition polymerization of methyl methacrylate monomer. The heat-cured variant, which is the most widely adopted in clinical practice, utilizes a powder-liquid system. The powder consists of prepolymerized PMMA spheres and an initiator (benzoyl peroxide), while the liquid contains unreacted methyl methacrylate monomer and a cross-linking agent (such as ethylene glycol dimethacrylate). The resulting polymer provides an exceptional combination of tissue compatibility, color stability, and the ability to mimic the natural appearance of the gingiva, which explains its continued dominance as the material of choice **(Muhammad Sajid Zafar, Prosthodontic Applications of Polymethyl Methacrylate (PMMA): An Update, 2020)**.

2.1.2 Physical and Mechanical Limitations

Despite its universal acceptance, PMMA exhibits inherent physical and mechanical deficiencies that compromise its long-term clinical success. Two of the most significant structural flaws are surface roughness and porosity. Surface roughness is heavily influenced by the laboratory polishing protocols and the inherent properties of the resin itself. Porosity, on the other hand, is a volumetric defect that occurs during the processing stage. It is primarily caused by either the vaporization of the unreacted monomer when the curing temperature exceeds 100.8°C, or by an inadequate mixing ratio of powder to liquid, leading to trapped air. These structural irregularities are not merely aesthetic concerns; they dramatically increase the surface area of the denture base. This increased surface area, combined with the hydrophobic nature of PMMA, creates micro-retentive niches that are highly protected from the mechanical cleansing action of the tongue and saliva. Consequently, these pores serve as ideal initial attachment sites for organic debris

and pathogenic oral microorganisms **(Fatin Ahmed Asim, Studying The Physical and Biological Characteristics of Denture Base Resin PMMA Reinforced With ZrO2 and TiO2 Nanoparticles, 2022)**.

2.2 Denture-Associated Microorganisms and Pathogenesis

2.2.1 The Biology of *Candida albicans*

The oral cavity hosts a complex and dynamic microbiome. While a healthy oral environment maintains a symbiotic balance, the introduction of an acrylic prosthesis disrupts this equilibrium. The most critical opportunistic pathogen associated with denture wearing is *Candida albicans*. *C. albicans* is a polymorphic fungus, meaning it has the unique biological capability to transition between different morphological states: a unicellular budding yeast form, pseudohyphae, and true multicellular hyphae. In a healthy individual, it exists primarily in the harmless yeast form. However, under the specific localized environmental conditions created by a denture—such as low pH, decreased oxygen availability, and compromised salivary flow—the fungus transitions into its pathogenic hyphal form. This morphological switching is a critical virulence factor, as the elongated hyphae can exert mechanical force and actively invade the microscopic porosities of the PMMA resin, making them exceptionally difficult to eradicate **(Gordon Ramage, Early Adhesion of Candida albicans onto Dental Acrylic Surfaces, 2017).**

2.2.2 Adhesion and Biofilm Formation

The pathogenesis of denture-related fungal infections is entirely dependent on the initial adhesion of the yeast cells to the acrylic surface. This is a complex, multi-step process governed by nonspecific physicochemical forces (such as van der Waals forces and hydrophobicity) and specific biological interactions mediated by fungal cell wall proteins known as adhesins. Once initial attachment occurs, the cells proliferate and secrete a thick, protective extracellular polymeric substance (EPS). This EPS matrix encases the fungal community, forming a mature, three-dimensional biofilm. Biofilms represent a sophisticated survival strategy; the EPS acts as an impenetrable physical barrier that prevents host immune cells and topically applied antimicrobial agents from reaching the embedded fungal cells. The structural resilience of the *Candida* biofilm on PMMA is the primary reason why clinical

infections are notoriously recalcitrant to standard therapies **(Jyotsna Chandra, In vitro growth and analysis of Candida albicans biofilms, 2001).**

2.2.3 Denture Stomatitis

The direct clinical manifestation of chronic *Candida* biofilm formation on the fitting surface of a prosthesis is Denture Stomatitis (DS). This inflammatory condition affects a vast majority of complete denture wearers, particularly the elderly population. It is clinically graded into three types (Newton's classification), ranging from localized pinpoint hyperemia to generalized mucosal erythema and, in severe chronic cases, inflammatory papillary hyperplasia of the hard palate. The constant release of hydrolytic enzymes (such as secreted aspartyl proteinases and phospholipases) and metabolic toxins by the fungal biofilm directly damages the underlying epithelial tissues, inciting a persistent host inflammatory response. Therefore, effectively managing the biofilm on the denture surface is paramount to resolving the mucosal inflammation **(Gordon Ramage, Early Adhesion of Candida albicans onto Dental Acrylic Surfaces, 2017).**

2.3 Current Denture Cleansing Protocols and Limitations

2.3.1 Mechanical and Chemical Disinfection

Routine denture hygiene is critical for preventing DS. The current clinical consensus recommends a combination of mechanical and chemical methods. Mechanical cleaning involves brushing the prosthesis with water and mild soap or dentifrice. While necessary for removing gross debris, vigorous brushing with abrasive pastes causes microscopic scratches on the PMMA surface, paradoxically increasing surface roughness and facilitating faster subsequent microbial recolonization. Chemical disinfection typically involves immersing the denture in solutions such as sodium hypochlorite, chlorhexidine, or effervescent alkaline peroxides. These agents are designed to chemically dissolve the biofilm matrix and lyse the fungal cells **(Muhammad Sajid Zafar, Prosthodontic Applications of Polymethyl Methacrylate (PMMA): An Update, 2020)**.

2.3.2 Detrimental Effects on PMMA Integrity

While chemical cleansers can exhibit potent antimicrobial activity in the short term, their prolonged and repetitive use is highly destructive to the structural integrity of the acrylic resin. Studies have definitively shown that overnight immersion in

sodium hypochlorite solutions causes a significant degradation of the polymer network. This chemical attack results in severe color alteration (bleaching), increased surface roughness, and a drastic reduction in the mechanical properties of the denture base, specifically its flexural strength and surface microhardness. Furthermore, the rising global crisis of antimicrobial resistance is affecting fungal pathogens as well. Prolonged exposure to sub-lethal doses of standard antifungal medications embedded in denture creams has led to the emergence of resistant *Candida* strains. This necessitates a desperate shift toward alternative, non-synthetic antimicrobial agents that do not degrade the prosthetic material **(Hussein Ali Mohammed, Tea Tree Oil: Anew Antifungal Agents Against Candida Albicans Cells on Heat –Cured Acrylic Resin Denture Base Material, 2019)**.

2.4 Phytotherapy and Essential Oils in Dentistry

2.4.1 The Shift Toward Natural Antimicrobials

To overcome the toxicity and material degradation associated with synthetic chemicals, modern dental research has heavily pivoted toward phytotherapy. This approach harnesses the bioactive compounds found in medicinal plants. Essential oils (EOs) are highly concentrated, volatile aromatic liquids extracted from various plant parts. Because they are complex mixtures of hundreds of different biochemical compounds (terpenes, alcohols, aldehydes), they attack microbial cells through multiple simultaneous mechanisms. This multi-target approach makes it exceedingly difficult for fungi like *Candida albicans* to mutate and develop genetic resistance. Furthermore, essential oils are generally recognized as safe, highly biocompatible with human tissues, and possess profound anti-inflammatory properties that aid in mucosal healing **(Luma Mahmoud Al-Nema, Evaluation of Addition of Plant Fixed Oil Extracts on Some Properties of Cured Denture Base Material, 2014).**

2.5 The Investigated Essential Oils

2.5.1 Tea Tree Oil (TTO)

Extracted from the leaves of the Australian native plant *Melaleuca alternifolia*, Tea Tree Oil is one of the most extensively researched essential oils in modern microbiology. Its composition is strictly regulated by international standards, requiring a minimum of 30% Terpinen-4-ol and a maximum of 15% 1,8-cineole. Terpinen-4-ol is the primary antimicrobial agent. It is highly lipophilic, allowing it

to easily partition into the lipid bilayer of the fungal cell membrane. Once integrated, it severely disrupts the structural organization of the membrane, increasing its permeability. This causes a lethal leakage of essential intracellular potassium ions and disrupts cellular respiration. Extensive in vitro studies have demonstrated that TTO can not only inhibit the initial adhesion of *C. albicans* to acrylic surfaces but can also penetrate established biofilms and inhibit the critical transition from yeast to the invasive hyphal form **(K. A. Hammer, Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil, 2003)**.

2.5.2 Sage Oil (*Salvia officinalis*)

Sage is a historically significant medicinal plant utilized for its broad-spectrum therapeutic benefits. The essential oil extracted from *Salvia officinalis* is rich in oxygenated monoterpenes, specifically thujone, camphor, and 1,8-cineole. While its direct antifungal mechanism is similar to TTO in terms of membrane disruption, Sage oil distinguishes itself through its exceptional anti-inflammatory and antioxidant capacities. In the context of denture stomatitis, the application of Sage oil provides a dual therapeutic effect: it suppresses the proliferation and adhesion of the candidal biofilm while simultaneously modulating the local immune response of the palatal mucosa, reducing the painful erythema and edema associated with the infection **(Thanyaporn Sookto, In vitro effects of Salvia officinalis L. essential oil on Candida albicans, 2013)**.

2.6 Methods of Application: Immersion vs. Incorporation

2.6.1 The Immersion Protocol

The clinical application of these essential oils can be executed via two fundamentally different methodologies. The immersion method relies on utilizing the essential oils as an external disinfectant bath. The completed PMMA prosthesis is soaked in an aqueous or emulsified solution of the targeted oils. This method is highly effective for chemical debridement of the surface and preventing initial daily colonization. Its primary advantage is that it does not alter the core chemical composition or the physical strength of the acrylic resin. However, the efficacy of the immersion method is entirely dependent on strict patient compliance, and its antimicrobial effect is temporary, lasting only until the denture is reintroduced into the oral cavity

(A. Catalán, In vitro and in vivo activity of Melaleuca alternifolia mixed with tissue conditioner on Candida albicans, 2008).

2.6.2 The Incorporation Protocol and Material Modification

The incorporation method attempts to engineer a smart, self-disinfecting biomaterial. This involves the direct intermixing of varying concentrations of liquid essential oils into the PMMA monomer liquid or polymer powder prior to the heat-curing process. The objective is to trap the volatile bioactive compounds within the polymerized acrylic matrix, allowing for a slow, sustained release of the antifungal agents at the interface between the tissue and the prosthesis. While this method successfully bypasses the need for patient compliance, it introduces a critical biomechanical challenge. The addition of liquid essential oils into the intricate cross-linked network of PMMA can act as an unintended plasticizer. Plasticizers lodge themselves between the long polymer chains, increasing the intermolecular distance and reducing the attractive forces between them. While this may theoretically increase the impact strength (making the denture less brittle), it simultaneously severely decreases the transverse and flexural strength of the material, making the denture highly susceptible to catastrophic fracture under the heavy forces of mastication. Therefore, identifying the exact optimum concentration of oil that provides adequate antimicrobial resistance without inducing unacceptable mechanical degradation is the core challenge of current research **(Entessar Hussein Al-Mosaweb, Properties of the Modified Polymethyl Methacrylate as Denture Base Materials: A Comprehensive Review, 2023)**.

Chapter Three

Materials and methods

3. chapter three: Materials and methods

3.1 Materials and Equipment (Oil Treatment):

3.1.1 Materials Used in this Study:

1. Nutrient Broth (India)
2. Potato Dextrose Agar (India)
3. Tae tree oil (T.T.O) (Germany)
4. Sega oil (S.O) (India)
5. Distilled Water
6. Acrylic Samples

3.1.2 Equipment used in the study:

1. Microbalance
2. Graduated Cylinder
3. Erlenmeyer Flask
4. Gloves
5. Petri Dish
6. Laboratory Spatula
7. Test Tube
8. Laboratory Spatula
9. cuvette
10. ruler
11. Oral swab
12. Normal saline
13. Nylon

3.1.3 devices used in the study:

1. Autoclave
2. Incubator
3. Spectrophotometer
4. Fume Hood

Figure (1) shows the Tae tree Oil and Sage Oil .

Figure (2) shows the acrylic samples

Figure (3) shows the tools and materials .

3.2 Methods:

3.2.1 Specimens grouping:

Thirty samples were divided into main groups consisting of:

1. 7 samples combined with tea tree oil
2. 7 samples combined with sage oil
3. 7 samples treated with tea tree oil as a wash
4. 7 samples treated with sage oil as a wash
5. 2 control samples

3.2.2 Preparation of test specimens

In this study, 30 samples were made out of modeling wax of the high-impact PMMA with dimension of 10mm X 10mm X 5mm Are used

Figure (4) molding wax

The obtained specimens were categorized into two groups depending on the type of test utilized to detect Candida albicans adherence on denture base
16 specimens are made by mixing the polymer, monomer (using a powder-to-liquid ratio of (2:1) by weight according to manufacturer's instructions of high impact.
7 sample, the polymer, monomer, and T.T.O amounts have been
determined using a digital balance to weigh powder and monomer and a micropipette to measure liquids. Afterwards, the polymer and monomer were blended using a powder-to-liquid ratio of (2:1) by weight according to manufacturer's instructions of high impact acrylic with concentrations of 100% pure, Tea Tree oil 15%.

the required amount of TTO was measured in a dry, clean glass beaker, with a micropipette, reduced from the volume of the monomer, and then combined with the

monomer for one minute وAfter adding this mixture to the acrylic powder, which was thoroughly mixed, the curing process suggested by the manufacture

the last 7 sample incorporate polymer and monomer with sage oil as the same techniques of TTO, Swap take from patient in prosthodontics clinic - college of dentistry Al-Mustaqbal university, and the SDA prepare in the microbiology lab. By adding

3.3 part one, immersing

3.3.1 Methods for preparing agar and culturing fungi

Preparing the Culture Media

Using a balance, measure the agar content. Using a graduated cylinder, measure the distilled water content. Mix the agar with the distilled water in an Erlenmeyer flask. Add the agar, then gradually add the water, stirring the mixture with a laboratory spatula. Seal the flask tightly with cotton and then place it in an autoclave (a device similar to a pressure cooker that operates at high temperatures). The autoclave will incubate for one to one and a half hours until the mixture is sterile and clear, free of impurities. After the autoclaving period was completed, cool the mixture slightly until it is lukewarm. Then pour it into Petri dishes. This step must be done in a laminar flow hood, which should be heated to prevent any contamination of the agar mixture. Pour the mixture into the dishes, each dish needing approximately 20 ml, and allow them to cool for a few minutes (cooling is essential). After pouring the agar into Petri dishes, the plates were left uncovered inside the laminar flow hood near the flame until the medium solidified. This step was necessary to prevent condensation from forming on the lids and to avoid unwanted microbial growth. Once the agar had completely solidified and cooled, the plates were covered with their lids and stored in the refrigerator until use.

Figure (5) shows the calculation of agar weight.

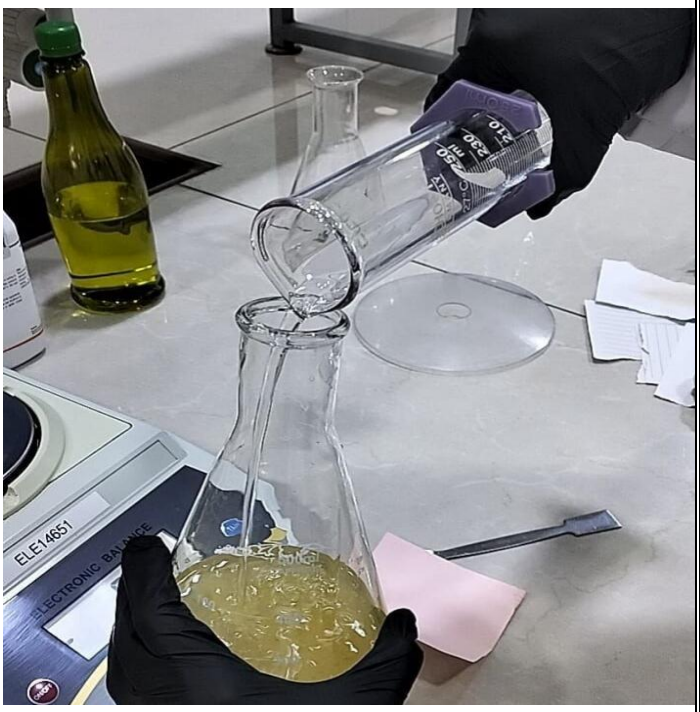


Figure (6) shows the mixing of agar and distilled water (their weights were calculated).

Figure (7) shows the placement of agar mixture in the autoclave.

Fungal Culture

We take cotton swabs from the mouths of several patients (we collected swabs from all ages, but the primary target group was the elderly; we also collected swabs from areas with black and curare accumulation). After collecting the required number of swabs, we take the agar (the culture medium stored in the refrigerator) and apply the swabs to it. This step should be done in a hood or near a flame to avoid any contamination. The swab should be applied to the agar in a wavy, cross-shaped motion. After completing the culture and recording the information on each culture plate, we place them in the incubator for approximately two days to allow the fungi to grow.

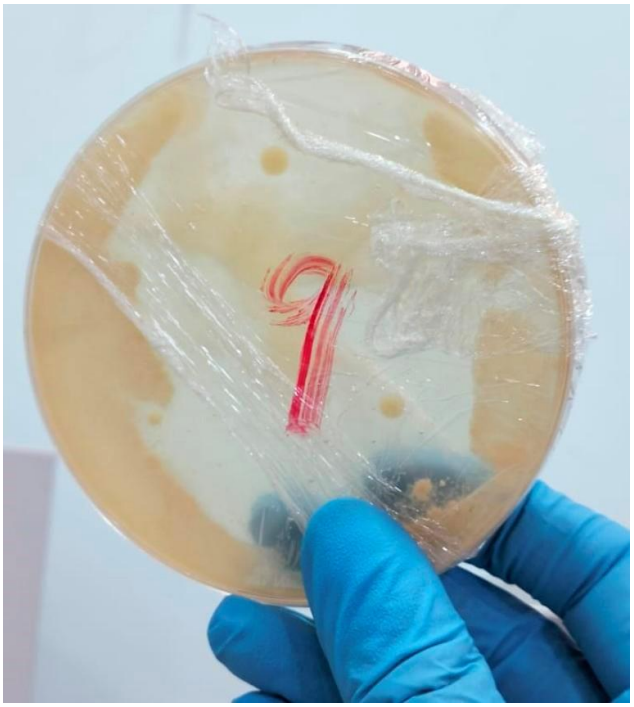


Figure (8) shows the take of saliva swabs.

Figure (9) shows the fungal growth after 48 hours of incubation. From patient.

3.3.2 Methods for preparing the Broth and Oil Soaking

Preparing the Broth:

Measure the required amount of Broth and then calculate the amount of distilled water. Mix the measured proportions of Broth with the water. Autoclave the solution. After removing it from the autoclave, allow it to cool slightly and then distribute it into the tubes (the distribution step must be done near the flame in the hood to avoid any contamination). Place the tubes in the incubator for two days, then inoculate them with the cultured fungi.

Inoculation Step: After two days, remove the Broth from the incubator and the cultured fungi. Begin inoculating them near the flame in the hood. Using a loop, take some fungi and place them in the Broth. Place one piece of acrylic in each prepared tube. After completing all the acrylic samples, place them in the incubator for another two days.

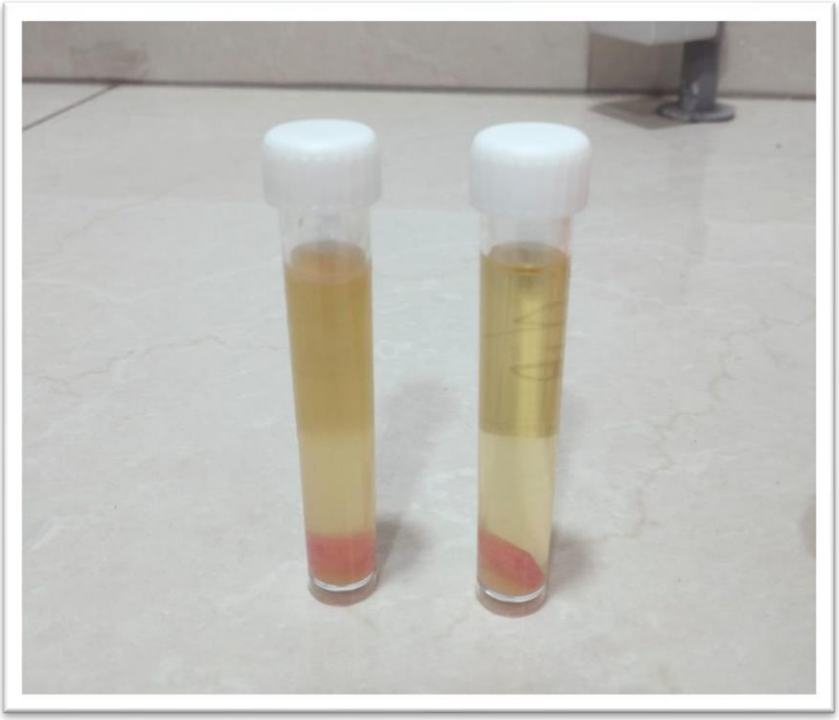


Figure (10) shows broth media in test tubes containing acrylic samples.

3.3.3 Preparing for Oil Soaking

At these steps, we prepare agar plates to inoculate the acrylic samples. Preparation is standard.

Steps for Soaking:

Prepare 16 tubes:

- 7 tubes containing 2 ml of tea tree oil
- 7 tubes containing 2 ml of sage oil
- 2 tubes containing 2 ml of normal saline for the control samples

Prepare 14 tubes for washing the oil-soaked samples, each tube containing 2 ml of normal saline.

Figure (11) Oil distribution, normal saline, and tube numbering

Soaking Steps: (in the hood and near the fire)

- We Take 7 samples (acrylic pieces) from the broth and place them in tubes of tea tree oil (T.T.O.). Soak them for 10 minutes. After 10 minutes, remove them from the oil and place them in tubes of normal saline (N.S.) for 2 minutes (washing).
- We Take 7 samples (acrylic pieces) from the broth and place them in tubes of sage oil (S.O.). Soak them for 10 minutes. Then remove them from the oil and place them in tubes of normal saline (N.S.) for 2 minutes (washing).
- We have 2 samples (acrylic pieces) left in the broth. Remove them from the broth and soak them in normal saline (N.S.) for 10 minutes (control).

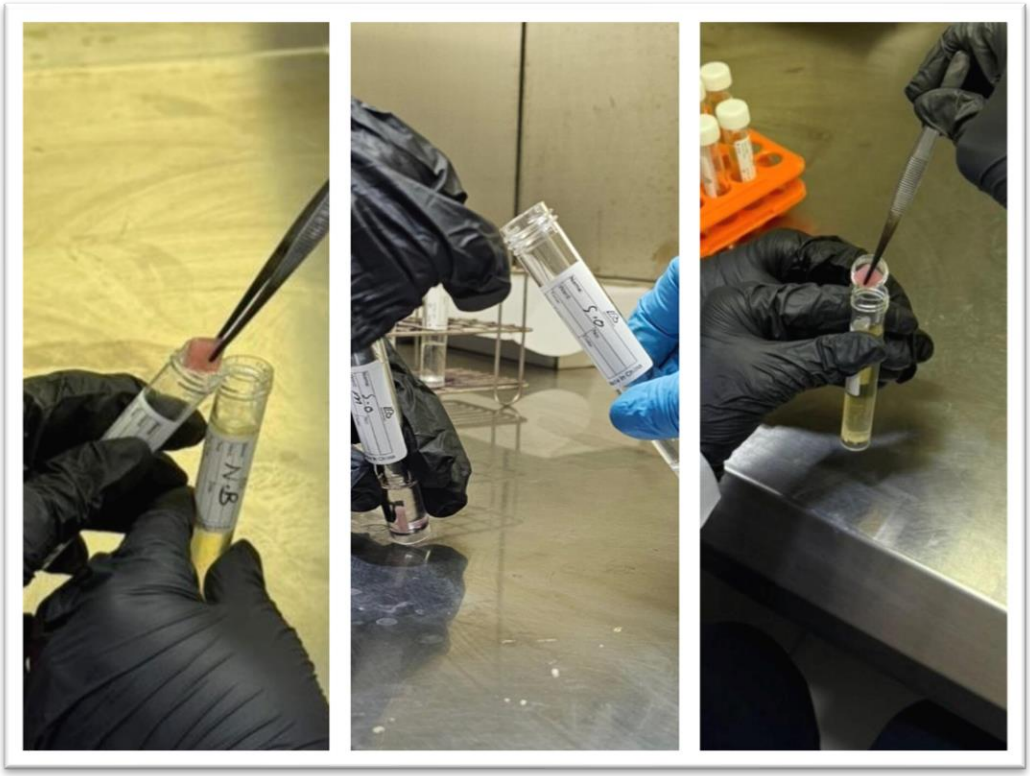


Figure (12) Soaking Steps

3.3.3.1 Steps of Sample Inoculation

All procedures were carried out near the laminar flow hood and close to a flame to maintain aseptic conditions.

Preparation of Culture Plates:

Eight agar plates were prepared. The plates were divided into three groups.

Seven plates were labeled and each plate was divided into two sections:

The first section was labeled T.T.O (Tea Tree Oil)

The second section was labeled S.O (Sage Oil)

One plate was labeled as Control (C), with each section representing a control sample.

Washing of Samples:

After immersing 14 acrylic samples in the oils for 10 minutes, they were washed using normal saline (N.S) for 2 minutes before starting the inoculation process.

Inoculation of Oil-Treated Samples:

Samples immersed in tea tree oil were placed on the agar in the section labeled T.T.O.

Samples immersed in sage oil were placed on the agar in the section labeled S.O.

The plates were covered immediately after placing the samples.

Control Samples:

The control samples were immersed in normal saline (N.S) for 10 minutes, then directly placed onto the agar, each sample in a separate section of the plate.

Incubation:

After completing the inoculation process, all plates were placed in the incubator for 48–72 hours (2–3 days).

Figure (13) Distribution of acrylic samples.

3.4 Part two, incorporating

3.4.1.1 Steps for measuring the wavelength of the broth:

1. Wash the glass tubes with distilled water and clean the transparent side.
2. Set the spectrophotometer to 600 nm (the wavelength for Candida).
3. Zero the spectrophotometer.

3.4.1.2 Experimental Steps:

1. measure the broth (free of any contaminants) in the glass tubes, zero the spectrophotometer, and place the glass tubes in the spectrophotometer.
2. measure the broth contaminated with fungi on the same day.
3. measure the broth contaminated with fungi after a full day.

3.4.1.3 Results of measuring the broth absorbance:

a) obtained in units of 0.022 .
b) Sputum contaminated with fungi at the moment of contamination is 0.562 .
c) Sputum contaminated with fungi after a day is 1.381 .

Figure (14) shows the spectrophotometer used for. measuring sample absorbance.

Figure (15) shows the Contamination of broth with fungi.

3.4.2 Placing the oil-embedded samples in the broth

After the broth is ready and we have completed measuring its wavelength, we place the oil-embedded samples into tubes.

T.T.O. samples are placed in tubes labeled T.T.O., and S.O. samples in tubes labeled S.O. We leave them in the incubator for two days, then remove them.

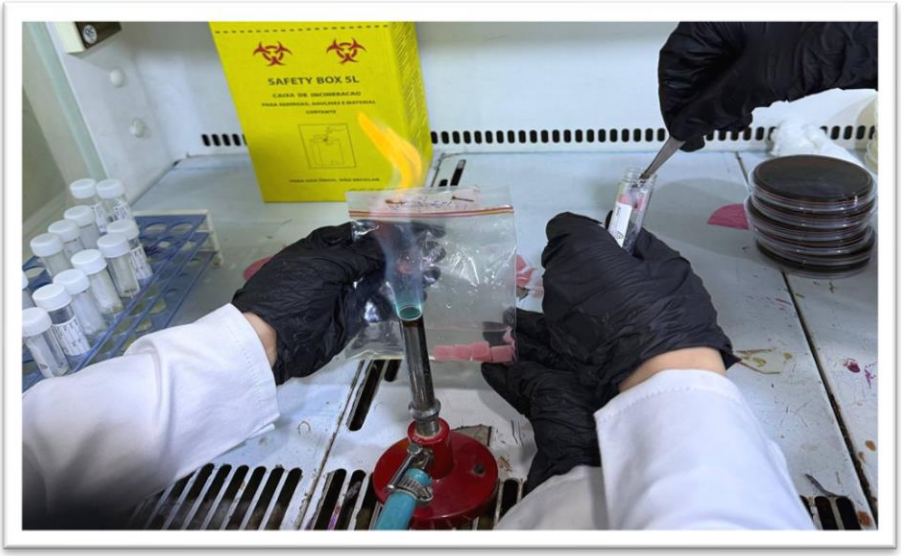
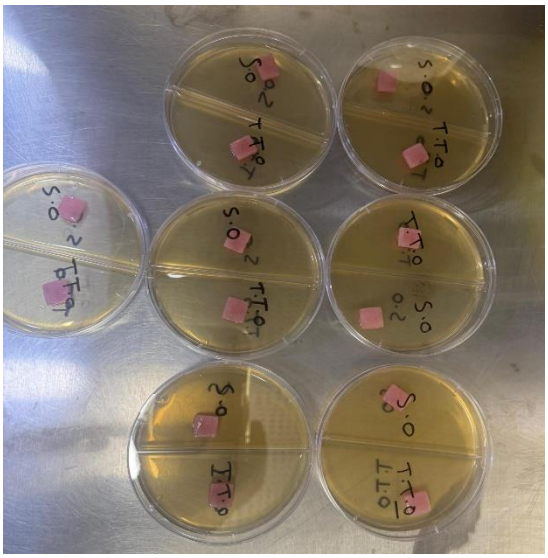


Figure (16) Placing the oil-embedded samples in the broth

3.4.3 Transferring the oil-treated samples from the broth to agar plates

Agar was prepared as described in Section 3.3.1 .After completing all these steps, we begin the inoculation process .We take T.T.O samples from the broth and place them on the agar in the section labeled T.T.O . We take S.O .samples from the broth and place them on the agar in the section labeled S.O .



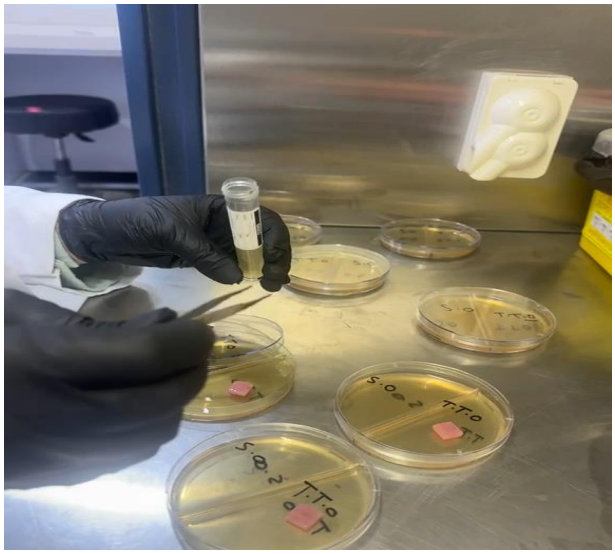


Figure (17) Transferring the oil-treated samples from the broth to agar plates

- After completing the cultivation process, we collect all the dishes in food-grade plastic wrap, leaving a slightly open space without covering, and record all the information on them before placing them in the incubator for 48 hours.

Figure (18) shows the placement of the samples inside the incubator.

- After 48 to 72 hours, we measured the existing colonies, and these are the measurement results.

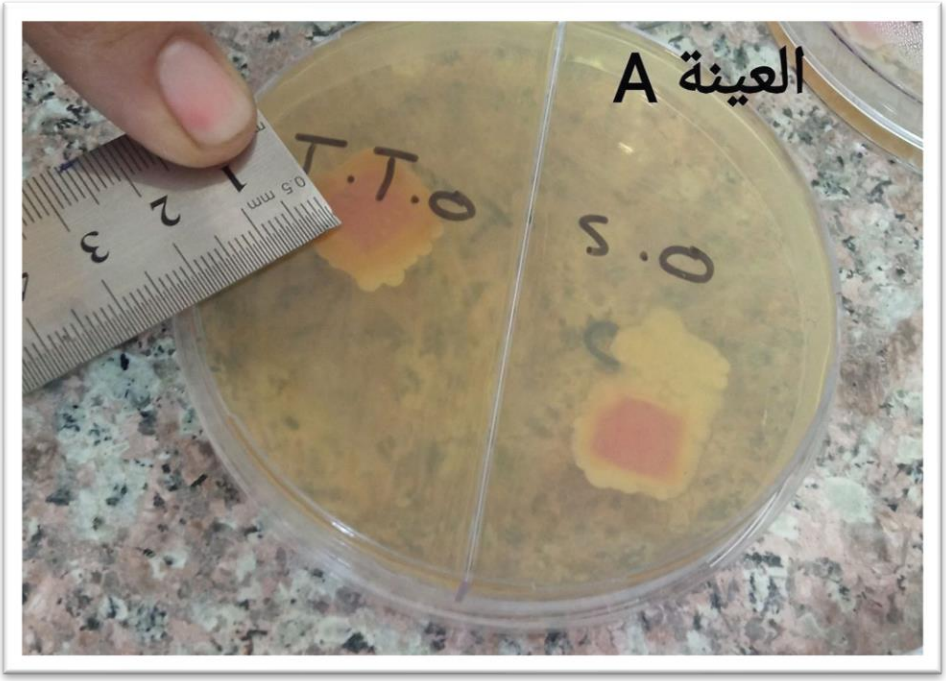


Figure (19) Results after 48 to 72 hours (A)

Figure (20) Results after 48 hours to 72 (B)

Chapter Four

Results

Chpter four : Results

4.1 Immersion method experiment (Oil Treatment)

The results obtained using the oil immersion method demonstrated a clear difference in fungal growth among the three groups (Tea Tree Oil, Sage Oil, and Control).

After 48 hours of incubation, the control group exhibited the highest level of fungal growth, characterized by dense and widely distributed colonies with no observable inhibition zone. In contrast, the samples treated with Tea Tree Oil (TTO) showed a noticeable reduction in fungal growth, as the colonies were fewer in number and smaller in size, along with the presence of a slight inhibition zone around the samples. The Sage Oil (SO) group demonstrated moderate to high fungal growth, greater than that observed in the TTO group but lower than the control group, with minimal or no detectable inhibition zone.

After one week, fungal growth increased in all groups. However, the control group continued to exhibit the most extensive and dense fungal growth. The TTO group maintained a relatively strong inhibitory effect, with lower colony density and more limited spread compared to both the control and SO groups. In contrast, the Sage Oil group showed heavy fungal growth, approaching that of the control group, with no observable inhibition effect.

Overall, Tea Tree Oil demonstrated the strongest and most sustained antifungal activity, followed by Sage Oil with limited effectiveness, while the control group exhibited the highest fungal growth due to the absence of any antifungal agent.

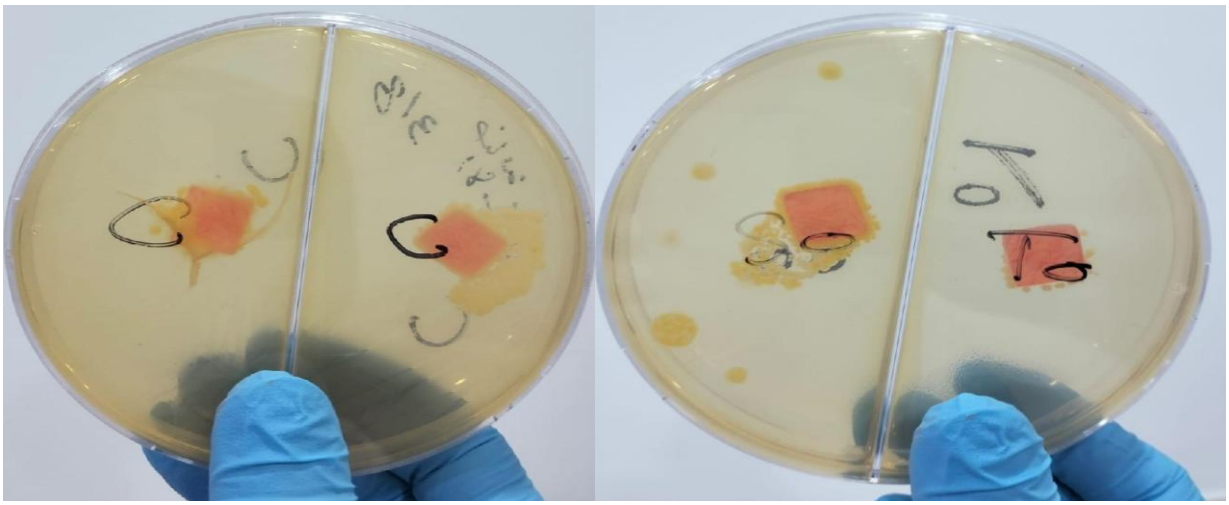


Figure (21) results after 48 hours

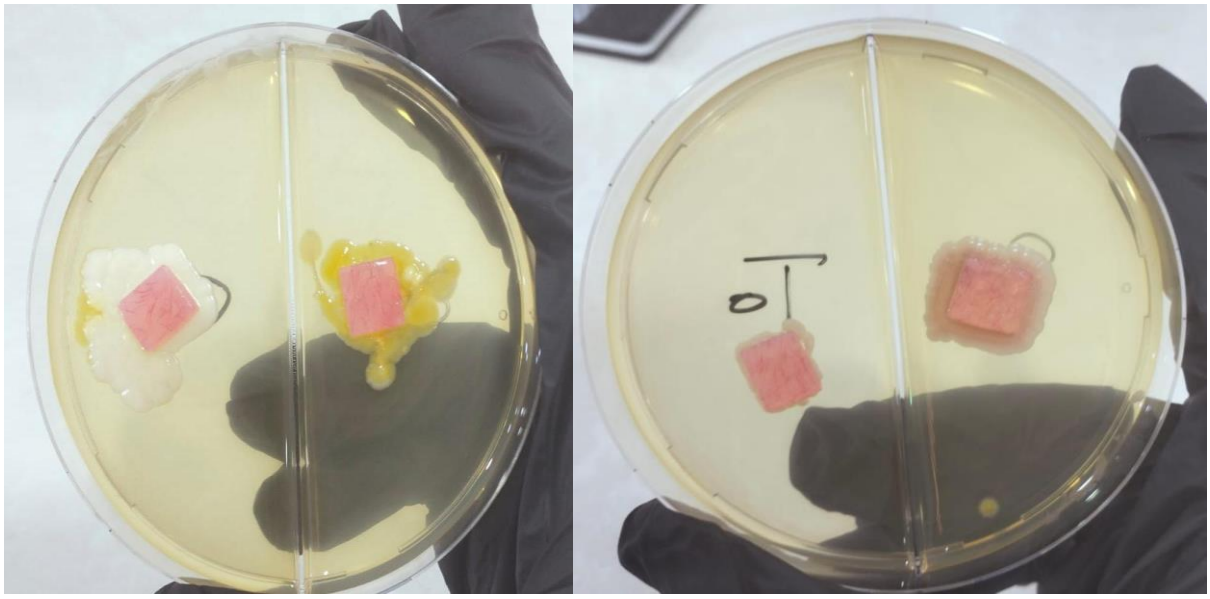


Figure (22) Results after 1 Week

Parameter	Control _48 h	T.T.O _48 h	S.O _48 h	Control _1week	T.T.O _1week	S.O _1week
Fungal growth density	Very high	Low	Moderate to high	Very high	Moderate	Very high
Colony size	Large	Small	Medium	Very large	Medium	Large
Colony distribution	Widely spread	Limited	Spread	Extensive	Moderate	Extensive
Inhibition zone	Absent	Slight	Absent/weak	Absent	Partial	Absent
Antifungal effectiveness	None	High	Low	None	Moderate to high	Very low

Table NO.(1)

The control group exhibited the highest fungal growth, confirming the absence of antifungal activity, while Tea Tree Oil demonstrated superior inhibitory effects compared to Sage Oil.

4.2 incorporation method experiment

The results of the incorporation method showed observable fungal growth in both groups after incubation. However, a difference in antifungal activity was noted between Tea Tree Oil (TTO) and Sage Oil (SO).

The TTO group demonstrated relatively lower fungal growth, with smaller colony diameters and more consistent results among samples. In contrast, the SO group showed slightly higher fungal growth with larger colony diameters and greater variability between samples.

These findings indicate that Tea Tree Oil has a stronger and more consistent antifungal effect compared to Sage Oil when incorporated into the material.

Statistical Analysis

Tea Tree Oil (TTO)

Values (mm): 5, 2, 3, 5, 3, 2, 3

Mean = 3.29 mm

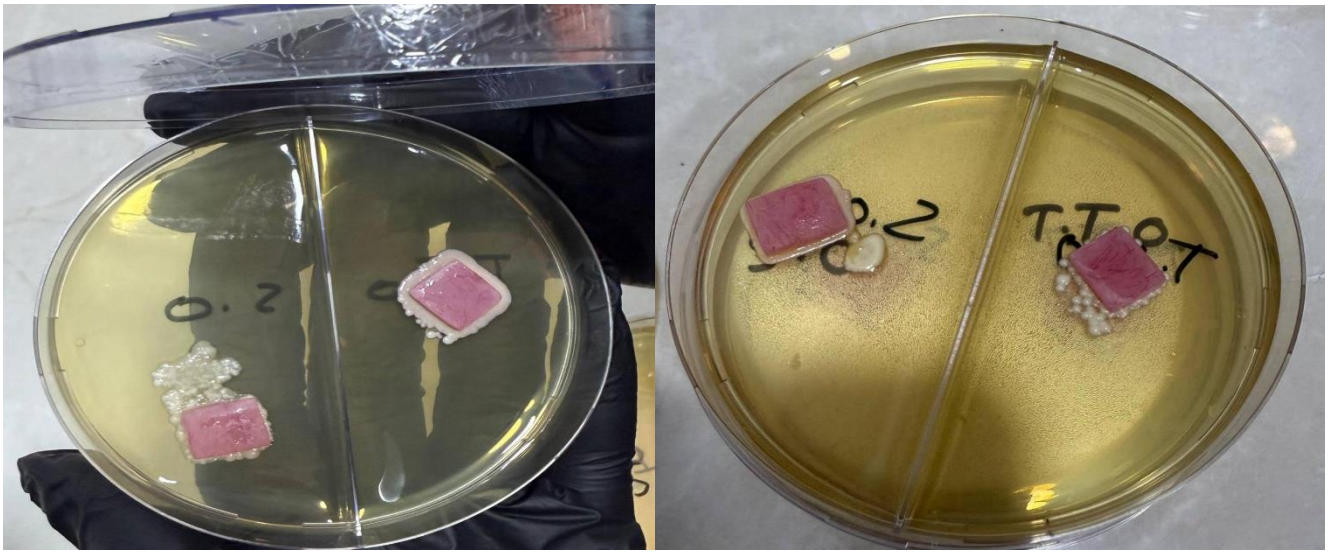
Standard Deviation = ±1.16

Sage Oil (SO)

Values (mm): 3, 7, 3, 2, 5, 1, 5

Mean = 3.71 mm

Standard Deviation = ±1.91



Figur (23) Result after 48 hour

Parameter	Tea Tree Oil (TTO)	Sage Oil (SO)
Mean (mm)	3.29	3.71
Standard Deviation (±SD)	±1.16	±1.91
Fungal growth level	Lower	Higher
Consistency of results	More consistent	More variable
Antifungal effectiveness	Higher	Lower

Table NO. (2)

Tea Tree Oil demonstrated lower mean fungal growth and less variability compared to Sage Oil, indicating a stronger and more consistent antifungal activity in the incorporation method.

4.3 The comparison between the immersion and incorporation methods revealed a clear difference in antifungal effectiveness.

The immersion method demonstrated a stronger antifungal effect, particularly in the Tea Tree Oil (TTO) group, where a clear inhibition zone was observed along with a noticeable reduction in fungal growth density. This indicates that direct exposure of the samples to the oil enhances its antifungal activity.

In contrast, the incorporation method showed a weaker antifungal effect, as fungal growth was still present in both groups with no observable inhibition zone. Although Tea Tree Oil performed better than Sage Oil, the overall effect was less pronounced compared to the immersion method.

These findings suggest that the method of application plays a significant role in antifungal efficiency, with immersion providing more direct and effective contact between the oil and the fungal cells.

Parameter	Immersion Method	Incorporation Method
Antifungal effectiveness	High	Moderate
Fungal growth density Moderate	Low (especially with T.T.O	Moderate
Inhibition zone	Present	Absent
Colony spread	Limited	More extensive
Consistency of results	Higher	Lower
Overall performance	Better	Less effective

Table NO. (3)

The immersion method showed superior antifungal activity compared to the incorporation method, indicating that direct exposure to the oil is more effective in inhibiting fungal growth.

Chapter Five

Discussions

Chapter Five: Discussion

5.1 Quantitative and Pathological Analysis of Fungal Colonization

The findings of this study, as evidenced by the experimental data, underscore the critical biological challenge posed by *Candida albicans* to the integrity of prosthetic dental materials. To understand the baseline virulence of the fungi utilized, the spectrophotometric analysis of the inoculated broth provides a vital quantitative foundation. The initial optical density (OD) of the pure nutrient broth was recorded at **0.022**, representing a sterile baseline. Upon inoculation with patient-derived oral swabs, the absorbance increased to **0.562** at time zero, reflecting the initial microbial load. Remarkably, after only 24 hours of incubation, the absorbance values surged to **1.381**, representing a significant exponential growth phase. This rapid proliferation confirms that the oral environment and the moisture-rich conditions under a denture base serve as an ideal incubator for fungal pathogens **(Gordon Ramage, Early Adhesion of Candida albicans onto Dental Acrylic Surfaces, 2017).**

The clinical consequence of this high growth rate is observed in the Control Group samples. Without any antifungal intervention, the heat-cured PMMA samples exhibited "Very High" fungal growth density, characterized by large, widely spread colonies that became even more extensive after one week. The absence of an inhibition zone in the control group further validates that conventional PMMA lacks inherent antimicrobial properties. This high baseline of colonization is the primary etiological factor for denture stomatitis, as the porous surface of the acrylic resin allows the fungi to penetrate the material and form resilient biofilms **(M. S. Zafar, Polymeric Denture Base Materials: A Review, 2020).**

5.2 Biochemical and Therapeutic Significance of the Investigated Oils

The strategic selection of Tea Tree Oil (TTO) and Sage Oil (SO) as alternatives to harsh chemical cleansers was based on their documented biocompatibility and broad-spectrum activity.

- **Tea Tree Oil (TTO):** The superior performance of TTO throughout this research is attributed to its high concentration of Terpinen-4-ol. This monoterpene is highly lipophilic, enabling it to partition into the fungal cell membrane and disrupt its structural integrity. This leads to a lethal leakage of

intracellular components and inhibits cellular respiration. In our immersion trials, TTO maintained a "High" antifungal effectiveness even after one week, demonstrating its potential as a sustainable long-term disinfectant **(Hussein Mohammed, Tea Tree Oil: Anew Antifungal Agents Against Candida Albicans Cells on Heat –Cured Acrylic Resin Denture Base Material, 2019).**

- **Sage Oil (SO):** While SO demonstrated a "Low" to "Moderate" antifungal effect compared to TTO, its clinical value remains significant. The presence of camphor and 1,8-cineole in sage oil contributes to inhibiting the transition of *Candida* from a harmless yeast form to a virulent hyphal form. Furthermore, its anti-inflammatory properties offer a dual therapeutic benefit by soothing the palatal mucosa, which is often inflamed in patients suffering from denture-induced candidiasis **(L. M. Al-Nema, Evaluation of Addition of Plant Fixed Oil Extracts on Some Properties of Heat Cured Denture Base Material, 2014).**

5.3 Comparative Evaluation of Application Methods (Immersion vs. Incorporation)

5.3.1 Clinical Efficacy of the Immersion Method

The immersion method demonstrated the highest immediate antifungal potency. By exposing the acrylic surface directly to 100% concentrations of the oils for 10 minutes, the fungal cells were eradicated before they could establish a mature biofilm. The results after 48 hours and one week showed that TTO immersion maintained a "Slight" inhibition zone and "Limited" colony spread. This suggests that surface treatment is highly effective for daily disinfection, though its success is dependent on patient compliance with the immersion protocol **(Hussein Mohammed, Tea Tree Oil: Anew Antifungal Agents Against Candida Albicans Cells on Heat –Cured Acrylic Resin Denture Base Material, 2019).**

5.3.2 Statistical Analysis of the Incorporation Method

The incorporation of oils directly into the PMMA matrix aimed to create a self-disinfecting material. The analysis of the individual colony diameters (measured in mm) for the seven samples in each group yielded critical statistical evidence:

1. **Tea Tree Oil (TTO) Incorporation:**

The recorded values were (**5, 2, 3, 5, 3, 2, 3**).

- The **Mean** diameter was **3.29 mm**, with a **Standard Deviation (±SD)** of **±1.16**. The relatively low SD indicates that TTO provided a consistent and stable inhibitory effect across the samples, effectively limiting the expansion of the colonies from within the material.

2. **Sage Oil (SO) Incorporation:**

The recorded values were (**3, 7, 3, 2, 5, 1, 5**).

- The **Mean** diameter was higher at **3.71 mm**, with a much higher **Standard Deviation (±SD)** of **±1.91**. This higher variability suggests that the antifungal activity of Sage Oil is less consistent when trapped within the acrylic matrix, possibly due to the uneven distribution or degradation of its volatile compounds during the heat-curing process.

5.3.3 Comparative Conclusion and Clinical Recommendations

A final comparison of the methodologies indicates that the **Immersion method** provides superior antifungal protection, particularly when using Tea Tree Oil. The presence of a measurable inhibition zone and a consistently lower fungal density makes it the most effective topical protocol.

In contrast, while the **Incorporation method** eliminates the need for patient compliance, its overall effectiveness is "Moderate" and lacks a clear inhibition zone. The statistical data confirms that TTO is the more reliable choice for incorporation due to its lower mean growth (**3.29 mm**) and higher consistency compared to SO. However, researchers must be cautious with the incorporation method, as adding liquid oils to the PMMA monomer can act as a plasticizer. These organic molecules can increase the distance between polymer chains, potentially leading to a decrease in the flexural and transverse strength of the denture base, which may increase the risk of fracture under masticatory loads **(Entessar H.A. Al-Mosaweb, Properties of the Modified Polymethyl Methacrylate as Denture Base Materials, 2023)**. Therefore, the immersion method remains the gold standard for maintaining the biological and mechanical integrity of the prosthesis.

Chapter Six

Conclusions and Recommendations

Chapter Six: Conclusions and Recommendations

6.1 Conclusions

Based on the laboratory results and statistical analysis conducted in this study regarding the influence of Tea Tree Oil and Sage Oil on the adhesion of *Candida albicans* to acrylic denture base materials, the following conclusions can be drawn:

1. **High Fungal Adherence Susceptibility:** The study confirmed that conventional Polymethyl Methacrylate (PMMA) denture base material (Control Group) completely lacks inherent antifungal activity. It exhibited very high fungal growth density and extensive colony spread, confirming that it serves as a highly favorable environment for the onset of denture stomatitis if no preventive treatments are applied.
2. **Superiority of Tea Tree Oil (TTO):** Tea Tree Oil demonstrated a decisive numerical and biological superiority over Sage Oil in both application methods. In the incorporation experiment, TTO achieved a lower mean colony growth of **3.29 mm** compared to Sage Oil, indicating a higher capacity to penetrate and disrupt the fungal cell envelope.
3. **Efficiency of the Immersion Method:** The immersion technique proved to be the most effective method for the immediate eradication of surface fungal cells. This method resulted in the appearance of a clear "Inhibition Zone" around the samples, a feature that was entirely absent in the incorporation method. This suggests that direct and concentrated contact between the oil and the fungi provides a stronger surface protection.
4. **Limitations of the Incorporation Method:** Although incorporating the oils at a 15% concentration provided the material with sustained internal protection, its efficacy was lower than that of immersion. Furthermore, Sage Oil recorded significant variability in results with a high standard deviation of **±1.91**, indicating unstable effectiveness during the chemical interaction with the acrylic resin during polymerization.
5. **Accelerated Biological Activity:** Spectrophotometric measurements confirmed that *Candida albicans* possesses a massive replication capacity in contaminated environments, with absorbance values rising from **0.562** to

1.381 within 24 hours. This emphasizes the vital importance of early intervention using essential oils to prevent the formation of mature biofilms.

6.2 Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. **Assessment of Mechanical Properties:** It is recommended to conduct future studies to evaluate the effect of incorporating essential oils at a 15% concentration on the "Flexural Strength" and "Surface Hardness" of the acrylic to ensure that the oils do not act as plasticizers that weaken the durability of the prosthesis **(Entessar H.A. Al-Mosaweb, Properties of the Modified Polymethyl Methacrylate as Denture Base Materials, 2023).**
2. **Testing Variable Concentrations:** Future research should investigate lower concentrations of essential oils (e.g., 1%, 2%, or 5%) in the incorporation method to determine if they provide a better balance between antifungal efficacy and the preservation of the material's mechanical integrity.
3. **Clinical In Vivo Studies:** Transitioning from *in vitro* laboratory studies to clinical trials on patients is necessary to evaluate the oral tissue compatibility of essential oils during long-term use and to assess patient acceptance of the taste and odor of the oils.
4. **Surface Roughness Analysis:** Detailed measurements of "Surface Roughness" should be performed after treating the acrylic with essential oils, as surface texture is a critical factor in subsequent fungal recolonization.
5. **Development of Enhanced Cleansers:** It is recommended to utilize Tea Tree Oil as a primary active ingredient in commercial denture cleansing solutions due to its high antifungal efficacy demonstrated numerically in this research.

Chapter seven

Reference List

Chapter Seven: Reference List

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الملخص

يُعدّ التهاب الفم الناتج عن أطقم الأسنان، والذي ينجم أساسًا عن التصاق واستعمار فطر المبيضات البيضاء (Candida albicans)على الأسطح المسامية لمادة بولي (ميثيل ميثاكريلات) (PMMA) ، تحديًا سريريًا شائعًا لمرضى أطقم الأسنان، غالبًا ما تُسبب المنظفات الكيميائية التقليدية آثارًا ضارة على خصائص المادة، مما يدفع إلى البحث عن بدائل طبيعية متوافقة حيويًا.

هدفت هذه الدراسة إلى تقييم ومقارنة فعالية زيت شجرة الشاي (TTO) وزيت المريمية (SO) كمضادات للفطريات ضد فطر المبيضات البيضاء (Candida albicans) باستخدام طريقتين للتطبيق (الغمر والدمج) في مادة قاعدة طقم الأسنان المصنوعة من مادة PMMA المعالجة حراريًا.

تم تحضير ثلاثين عينة من مادة PMMA (10 مم × 10 مم × 5 مم) وقُسّمت إلى مجموعات. رُصد نمو الفطريات باستخدام قياس الطيف الضوئي عند طول موجي 600 نانومتر، حيث زادت الكثافة الضوئية (OD) للمرق الملقح من 0.562 إلى 1.381 خلال 24 ساعة. في طريقة الغمر، نُقعت العينات في زيت نقي لمدة 10 دقائق، أما في طريقة الدمج، فقد مُزج 15% من زيت شجرة الشاي أو زيت المريمية مع مونومر بولي ميثيل ميثاكريلات قبل عملية البلمرة، وقُيس قطر مستعمرات الفطريات بعد 48 ساعة وأسبوع.

أظهرت النتائج أن طريقة الغمر توفر حماية فائقة ضد الفطريات مقارنةً بطريقة الدمج. وقد أظهر زيت شجرة الشاي تأثيرات مثبطة أعلى وأكثر اتساقًا من زيت المريمية في كلتا الطريقتين، في تجربة الدمج، بلغ متوسط قطر المستعمرة لزيت شجرة الشاي 3.29 ملم (بانحراف معياري ±1.16)، بينما سجل زيت المريمية متوسطًا أعلى بلغ 3.71 ملم (بانحراف معياري ±1.91)، أظهرت مجموعات التحكم كثافة فطرية "عالية جدًا" دون وجود مناطق تثبيط، مما يؤكد عدم وجود نشاط مضاد للميكروبات في بولي ميثيل ميثاكريلات التقليدي.

يُعد زيت شجرة الشاي عاملًا طبيعيًا قويًا مضادًا للفطريات، وهو مناسب لنظافة أطقم الأسنان. على الرغم من أن الغمر هو البروتوكول الموضعي الأكثر فعالية للتطهير الفوري، إلا أن الدمج يوفر آلية تطهير ذاتي محتملة، شريطة أن يتم تحسين التركيزات للحفاظ على السلامة الميكانيكية لقاعدة طقم الأسنان.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة المستقبل

تأثير زيت شجرة الشاي وزيت المريمية (بالنقع والدمج) على التصاق المبيضات البيضاء بمادة قاعدة طقم الأسنان الأكريليكية

مشروع تخرج مقدم إلى مجلس كلية التقانات الصحية والطبية بجامعة المستقبل كجزء من متطلبات الحصول على درجة البكالوريوس في قسم تقنيات صناعة الأسنان

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بإشراف

الدكتورة. رقية جعفر باقر

1447 هـ **2026 م**