REVOLUTIONISING DENTURE CARE: THE INNOVATIVE EUGENOL-BASED DENTURE CLEANSER TABLET-AN IN-VITRO PILOT STUDY

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RESEARCH REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ORAL SCIENCE [PROSTHODONTICS]

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ORIGINAL LITERARY WORK DECLARATION

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Field of Study: Denture cleanser

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ABSTRACT

Denture biofilm, which is resistant to conventional cleaning methods, can lead to infections such as denture stomatitis, often caused by *Candida albicans*. Eugenol, a natural extract derived from plants, has demonstrated remarkable antimicrobial activity against wide range of fungi, gram-negative and gram-positive bacteria. This study presents a novel approach to denture care by developing an innovative effervescent denture cleanser tablet infused with eugenol. The aim of the present study is to produce a novel eugenol-based denture cleansing tablet and to evaluate its antifungal and antibacterial efficiency. The study was conducted in five phases. In the first phase, effervescent denture cleanser tablets were prepared using two formulations: one containing eugenol and the other as a placebo. The second phase involved analysing the physicochemical properties including hardness, thickness, diameter, pH, effervescence time of novel eugenol-based denture cleansing tablet, placebo, Polident® tablet and content analysis of the novel eugenol-based denture cleansing tablet. In the third phase, microbial strains were cultivated, focusing on three selected microbes: *Candida albicans* (fungus), *Streptococcus mutans* (gram-positive bacterium), and *Escherichia coli* (gram-negative bacterium). The fourth phase involved preparing denture cleansing solutions of novel eugenol-based denture cleansing tablets, placebo tablet, distilled water (negative control), 0.12% Chlorhexidine (positive control) and Polident® (commercially available denture cleanser). Finally, the fifth phase utilised the agar well diffusion test, where triplicate petri dishes for each microbial group were used by measuring the inhibition zones around the five wells inoculated with prepared denture cleansing solutions. All zones of inhibition were recorded meticulously to evaluate the antimicrobial effectiveness of the eugenol-based tablets compared to the placebo and controls. The formulation of the novel eugenol-based denture cleanser tablet met most physicochemical parameters

within the limits, although the hardness of the tablet was lower than ideal. The eugenol concentration of 5.294% in the tablet was deemed acceptable for topical use after rinsing, aligning with safety guidelines. The eugenol-based tablet recorded a mean inhibition zone of 21.26 mm against *Candida albicans*, indicating superior antifungal activity, compared to 0.12% Chlorhexidine, which had a mean inhibition zone of 12.53 mm. The novel eugenol-based denture cleanser solution exhibited a mean inhibition zone of 8.20mm with moderate antibacterial properties to 0.12% Chlorhexidine with a mean inhibition of 19.79 mm against *Streptococcus mutans*. For *Escherichia coli* test group, the novel eugenol-based denture cleanser solution exhibited a mean inhibition zone of 9.21 mm, indicating moderate antibacterial activity, while the 0.12% Chlorhexidine showed a mean inhibition zone of 14.21 mm, demonstrating superior antibacterial potential. Commercially available Polident® had a mean inhibition zone of 6.66 mm against *Escherichia coli* and no sensitivity in other groups. Both the placebo tablet solution and distilled water showed no antimicrobial activity in all the groups. The novel eugenol-based denture cleanser tablet demonstrated promising antifungal activity against *Candida albicans* and moderate antibacterial activity against *Streptococcus mutans* and *Escherichia coli* exhibiting its potential as an effective denture cleanser.

Keywords: Novel eugenol-based denture cleansing tablet, agar well diffusion test, mean inhibition zone, antifungal, antibacterial

ABSTRAK

Biofilem gigi palsu, yang tahan terhadap kaedah pembersihan konvensional, boleh membawa kepada jangkitan seperti stomatitis gigi palsu, selalunya disebabkan oleh *Candida albicans*. Eugenol, ekstrak semula jadi yang diperoleh daripada tumbuh-tumbuhan, telah menunjukkan aktiviti antimikrobial yang luar biasa terhadap kulat dan pelbagai jenis bakteria gram-negatif dan gram-positif. Kajian ini membentangkan inovasi dalam penjagaan gigi palsu dengan menghasilkan tablet pencuci gigi palsu yang diformulasikan dengan eugenol. Matlamat kajian ini adalah untuk menghasilkan tablet pencuci gigi palsu berasaskan eugenol dan menilai keberkesanan antikulat dan antibakteria tablet tersebut. Kajian ini dijalankan dalam lima fasa. Pada fasa pertama, tablet pencuci gigi palsu disediakan menggunakan dua formulasi: mengandungi eugenol dan plasebo. Fasa kedua melibatkan menganalisis sifat fizikokimia bagi tablet yang disediakan, plasebo dan Polident® dari segi kekerasan, ketebalan, diameter, pH, masa efervescence dan menganalisa kandungan tablet pembersih gigi palsu berasaskan eugenol. Dalam fasa ketiga, strain mikrob yang terpilih dikultur, memfokuskan kepada: *Candida albicans* (kulat), *Streptococcus mutans* (bakteria gram-positif), dan *Escherichia coli* (bakteria gram-negatif). Fasa keempat melibatkan penyediaan larutan pembersihan gigi palsu bagi tablet pembersihan gigi palsu berasaskan eugenol, tablet plasebo, air suling (kawalan negatif), 0.12% Chlorhexidine (kawalan positif) dan Polident® (pencuci gigi palsu komersial). Akhir sekali, fasa kelima menjalankan ujian difusi telaga agar, di mana tiga petri agar disediakan untuk setiap kumpulan mikrob bagi mengukur zon rencatan di sekitar lima telaga yang disi dengan larutan pembersih gigi palsu. Semua zon rencatan direkodkan dengan teliti untuk menilai keberkesanan antimikrobial tablet berasaskan eugenol berbanding plasebo dan kawalan. Tablet pencuci gigi palsu berasaskan eugenol memenuhi kebanyakan kelayakkan fizikokimia, walaupun kekerasannya lebih rendah. Kepekatan eugenol sebanyak 5.294% dalam tablet sesuai

untuk kegunaan topikal selepas dibilas, selaras dengan garis panduan keselamatan. Bagi ujian difusi telaga agar, larutan tablet berasaskan eugenol merekodkan purata zon rencatan sebanyak 21.26 mm terhadap *Candida albicans* menunjukkan aktiviti antikulat yang ketara berbanding 0.12% Chlorhexidine, yang mempunyai purata zon rencatan sebanyak 12.53 mm. Larutan tablet berasaskan eugenol juga menunjukkan purata zon rencatan 8.20mm yang rendah berbanding dengan 0.12% Chlorhexidine dengan purata zon rencatan sebanyak 19.79 mm terhadap *Streptococcus mutans*. Untuk *Escherichia coli*, larutan tablet berasaskan eugenol menunjukkan purata zon rencatan sebanyak 9.21 mm menunjukkan aktiviti antibakteria yang sederhana manakala 0.12% Chlorhexidine menunjukkan purata zon rencatan sebanyak 14.21 mm dan potensi antibakteria yang ketara. Polident®, pencuci gigi palsu komersial mempunyai purata zon rencatan 6.66 mm terhadap *Escherichia coli* dan tiada zon rencatan dalam kumpulan lain. Larutan tablet plasebo dan air suling tidak menunjukkan sebarang aktiviti antimikrobial untuk semua kumpulan. Tablet pencuci gigi palsu berasaskan eugenol menunjukkan aktiviti antikulat yang berpotensi tinggi terhadap *Candida albicans* dan aktiviti antibakteria yang sederhana terhadap *Streptococcus mutans* dan *Escherichia coli* serta menunjukkan potensinya sebagai pencuci gigi palsu yang berkesan.

Katakunci: Tablet pencuci gigi palsu berasaskan eugenol, ujian difusi telaga agar, zon rencatan, antikulat, antibakteria

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LIST OF SYMBOLS AND ABBREVIATIONS

AR	:	Analytical Reagent
ATCC	:	American Type Culture Collection
BHI	:	Brain Heart Infusion
CFU	:	Colony Forming Unit
CHX	:	Chlorhexidine
CO2	:	Carbon Dioxide
ESBL	:	Extended-Spectrum Beta-Lactamase
FDA	:	Food and Drug Administration
GRAS	:	Generally Recognized As Safe
ISO	:	International Organization for Standardization
MBC	:	Minimum Bactericidal Concentration
MIC	:	Minimum Inhibitory Concentration
MTCM	:	Manual Tablet Compaction Machine
NTCC	:	National Type Culture Collection
PBS	:	Phosphate Buffered Saline
SDA	:	Sabouraud Dextrose Agar
SDB	:	Sabouraud Dextrose Broth
SD	:	Standard Deviation
USP	:	United States Pharmacopeia
WHO	:	World Health Organization

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CHAPTER 1: INTRODUCTION

1.1 Background

Denture biofilm is a complex amalgamation of oral bacteria, fungi, and various microorganisms, enveloped by a dense, hydrated, viscous exopolysaccharide matrix (Nikawa et al., 1998). This biofilm exhibits remarkable tenacity, proving resistant to host-defence mechanisms, disinfectants, and other antimicrobial agents (Katsikogianni & Missirlis, 2004). While controlled and immature biofilms are generally non-problematic, poor denture hygiene allows the biofilm to develop into a more advanced, mature, and pathogenic form (Nikawa et al., 1998).

Candida albicans, a common commensal species in the oral microflora of healthy humans, stands out as a prominent contributor to the development of denture stomatitis (Scully & Felix, 2005). Denture stomatitis is characterised by widespread or localised erythematous inflammation in the area where dentures are placed (Gendreau & Loewy, 2011). This prevalent condition affects 20% - 80% of denture wearers globally (Aguayo et al., 2017; Bilhan et al., 2009; Gendreau & Loewy, 2011). In Malaysia, denture stomatitis is one of the most frequently diagnosed oral mucosal lesions with a prevalence of approximately 33.5% (Zain et al., 1997).

Additionally, other Candida-associated lesions, such as median rhomboid glossitis and angular cheilitis may coexist with denture stomatitis, which in partially dentate patients is linked to a higher risk of dental caries and periodontitis (McReynolds et al., 2023). Among older, frail, medically compromised, and nursing home populations, the presence of denture stomatitis, coupled with poor denture hygiene and nighttime denture use, has been linked to potentially life-threatening aspiration pneumonia (Baumgartner et al., 2015; Nishi et al., 2014; Sjögren et al., 2016).

The usual way to clean dentures is by brushing them with soap and water, which alone does not remove all the biofilm (Mylonas et al., 2022). Combining mechanical and chemical approaches has demonstrated promise in reducing adherent microorganisms on denture surfaces (Kumar et al., 2012). However, certain prosthesis wearers, such as elderly patients and those with limited motor capacity, may struggle to maintain cleanliness through mechanical biofilm control alone (Kulak-Ozkan et al., 2002).

In Malaysia, Polident®, Steradent, and Pearlie White, which are alkaline peroxide-type denture cleansers, are widely available (Tarib et al., 2018). Previous studies have indicated that while denture cleansers are effective against polymicrobial organisms, they may not provide complete efficacy against *Candida albicans* species (de Andrade et al., 2011; Lucena-Ferreira et al., 2013; Yadav et al., 2013). Besides that, immersing dentures in a 0.12% Chlorhexidine (CHX) solution for 20 minutes has demonstrated the ability to eradicate Candida biofilms (de Andrade et al., 2011). However, 0.12% CHX solution resulted in the most noticeable discolouration of artificial teeth, leading to clinically unacceptable outcomes (Kaypetch et al., 2023).

On the other hand, there is a growing interest in natural extracts with exceptional antifungal properties as a viable alternative for denture cleansing solutions. Eugenol, primarily derived from clove oil, has been a staple in dental applications due to its notable analgesic, anti-inflammatory, anaesthetic, and antimicrobial properties including both antifungal and antibacterial efficacy for the purpose of relieving pain arising from oral clinical conditions including pulpitis and dentinal hypersensitivity (Marchese et al., 2017). Eugenol has demonstrated exceptional antimicrobial activity in studies, proving effective against fungi as well as a broad spectrum of gram-negative and gram-positive bacteria (Marchese et al., 2017).

1.2 The statement of problem

Denture biofilm, resistant to conventional cleaning, leads to infections such as denture stomatitis, often caused by *Candida albicans*. Existing denture cleansers either inadequately combat *Candida albicans* or cause damage to dentures in the process. Eugenol, with proven antimicrobial properties, has not well established in denture cleansing tablets. Hence, this study aims to produce eugenol as an effervescent denture cleansing tablet serving as an alternative denture cleanser for the prevention and treatment of denture related infections, especially denture stomatitis.

1.3 The aim of the research

This study aims to produce a pioneering denture cleansing tablet infused with eugenol and assess its antifungal and antibacterial effectiveness compared to a commercially available denture cleanser and 0.12% chlorhexidine.

1.4 Objectives of the research

1) To produce a novel eugenol-based denture cleansing tablet.
2) To evaluate the antifungal and antibacterial efficiency of the novel eugenol-based denture cleansing solution.

1.5 The null hypothesis of the research

Novel eugenol-based denture cleansing tablets' solution has no observational effect on antifungal and antibacterial properties compared to commercially available effervescent denture cleansing tablets' solution and 0.12% chlorhexidine (CHX).

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Recognising the importance of maintaining denture hygiene to prevent fungal and bacterial infections, this literature review aims to establish a comprehensive understanding of various denture cleansing methods, including both mechanical and chemical approaches. It will highlight the role of antifungal therapy in maintaining oral health. Furthermore, the review will explore the properties and historical use of eugenol in dental applications, focusing on its antifungal and antibacterial efficacy. Additionally, it will examine common denture pathogens and provide insights into their potential impact on causing denture-induced diseases.

2.2 Denture wearer and denture stomatitis

Many elderly individuals with dentures struggle to maintain cleanliness due to reduced dexterity associated with ageing, resulting in poor oral hygiene (Kulak-Ozkan et al., 2002). A study found that only 11.9% of patients had clean dentures and most denture wearers did not clean their dentures satisfactorily (Dikbas et al., 2006). Contaminated dentures directly contribute to mucosal diseases like denture stomatitis, which is highly prevalent among denture users (Kossioni, 2011). In Malaysia, denture stomatitis is one of the most frequently diagnosed oral mucosal lesions, with a prevalence of approximately 33.5% (Zain et al., 1997). Maintaining proper cleanliness of dentures and the mucosal tissue of the edentulous mouth is crucial for good health, especially in the elderly.

2.3 Denture cleansing methods

Patients are generally advised to use both mechanical and chemical methods to remove plaque and debris from their dentures (Lee et al., 2011). Denture cleaning can be broadly categorised into mechanical and chemical methods (de Souza et al., 2009; Kaypetch et al., 2023; Mylonas et al., 2022). Mechanical methods involve manual

cleaning with a brush or vibrational cleaning using ultrasonic or sonic baths (Kaypetch et al., 2023). Adjuncts to assist manual cleaning can be divided into pastes, gels, foams, or powders, each with similar ingredients and modes of action, are designed to enhance the effectiveness of normal manual cleaning methods (Kumar et al., 2017). However, using a toothbrush and toothpaste is ineffective against microbial activity on denture biofilms and is inappropriate as it can alter the denture material's texture, leading to plaque formation or hindering its removal (Harrison et al., 2004).

Chemical methods, designated to disinfect oral prostheses, are categorised based on their chemistry and mode of action (Kaypetch et al., 2023). These include bleach-based cleaners (such as sodium hypochlorite or sodium hydroxide), effervescent types (like peroxide, bicarbonate, percarbonate, or persulfate), and other categories including mineral acid-based, enzyme-based, oral rinses, and flexible denture cleansers (Mylonas et al., 2022). Soaking dentures in disinfectant solutions with chemical agents has been shown to effectively reduce the number of contaminating organisms (Harrison et al., 2004), although some of these agents are known to damage acrylic resin and metal alloy materials (Lee et al., 2011).

Additionally, a review highlights that using a standard domestic microwave oven to heat a denture in tap water has been shown to eradicate microorganisms such as *Candida albicans* and *Pseudomonas aeruginosa* on the denture surface but, this method lacks standardised methodology, and microwave irradiation for denture disinfection remains contentious and is not recommended (Brondani & Siqueira, 2018). A new denture cleaning method features a wipe infused with an antibacterial solution, intended for discreet cleaning when conventional mechanical and chemical methods are impractical (Axe et al., 2019).

2.4 Chemical denture cleansers

2.4.1 Bleach-based denture cleansers

Bleach-based cleansers, containing 1.5% or 2% sodium hypochlorite and/or 1.7% sodium hydroxide, offer superior antimicrobial capabilities due to the action of hydroxyl (OH^-) and chloride ions (Cl^-), which dissolve microbial cell walls and degrade lipids (Estrela et al., 2002). These cleansers can be used for short durations of about 10 to 20 minutes or overnight, as per manufacturer instructions (Mylonas et al., 2022). A minimum of 0.5% hypochlorite solution, used for at least three minutes daily, effectively combats *Streptococcus mutans* and *Candida albicans* without affecting the acrylic colour, surface roughness, or mechanical properties (de Sousa Porta et al., 2015). However, these cleansers can cause acrylic discolouration and degrade metal components, depending on the concentration and duration of immersion (Kulak-Ozkan et al., 2002; Salles et al., 2015).

2.4.2 Effervescent tablet-based denture cleansers

Effervescent tablets contain oxidants such as sodium bicarbonate, sodium percarbonate, and sodium persulphate, which generate carbon dioxide bubbles when dissolved in water while hydrogen peroxide cleansers, on the other hand, release oxygen (Yildirim-Bicer et al., 2014). To boost biofilm removal and cleaning efficacy, sodium lauryl sulphate is commonly included as a detergent (Papadiochou & Polyzois, 2018). While effervescent cleansers are gentler than bleach-based alternatives, they are generally safe for metallic dentures and do not cause corrosion issues (Senna et al., 2011). However, they are not suitable for dentures with acrylic reline materials due to potential degradation and increased porosity over time (Chittaranjan, 2011).

2.4.3 Enzyme-based denture cleansers

Enzyme-based cleaners incorporate enzymes such as lipases, amylases, and proteases, which effectively degrade fats, glycoproteins, and proteins, thereby bolstering their antimicrobial efficacy (Mylonas et al., 2022). These cleaners are predominantly employed for dentures featuring soft reline materials, and research indicates they have negligible adverse impacts on these typical reline materials (Chittaranjan et al., 2011).

2.4.4 Mineral acid-based cleansers

Mineral acid-based cleansers, which typically contain hydrochloric or phosphoric acids, effectively dissolve calcified biofilm deposits and the cell membranes of microorganisms (Mylonas et al., 2022). Nonetheless, their application with metal alloy dentures is cautioned against due to the potential for significant tarnishing and corrosion (Chittaranjan, 2011).

2.4.5 Oral rinses

Oral rinses, including mouthwash products like 0.2% chlorhexidine gluconate, 0.05% salicylate solution (a derivative of salicylic acid), and phenolic-based mouthwashes such as Listerine, exhibit notable antimicrobial properties (Mylonas et al., 2022). A five-minute soak with 4% chlorhexidine solution has been particularly effective against *Candida albicans* and *Streptococcus mutans* on acrylic dentures and those with soft silicone linings, surpassing the efficacy of mechanical brushing and effervescent cleaning tablets (Mantri et al., 2013).

However, prolonged daily use of chlorhexidine solutions (0.2 to 4%) can lead to staining of dentures, similar to natural teeth (Chittaranjan, 2011). For instance, dentures soaked in a 2% chlorhexidine solution showed noticeable brown discolouration after seven days, compared to those soaked in a 0.5% sodium hypochlorite solution daily over

90 days, indicating that chlorhexidine should be used for short periods only (de Sousa Porta et al., 2015).

Chlorhexidine-based mouthwashes at a concentration of 0.2% are commonly recommended for oncology patients with oral prostheses, although their antimicrobial efficacy can vary significantly (de Castellucci Barbosa et al., 2008). Despite its widespread use as an antimicrobial agent in dentistry, chlorhexidine has been reported to have adverse effects such as inducing inflammatory reactions and tissue necrosis associated with the inflammatory response (Faria et al., 2009).

2.4.6 Flexible denture cleansers

Flexible dentures are constructed from thermoplastic polyamide resins such as nylon, offering a limited degree of flexibility, with each manufacturer issuing specific care guidelines (Hundal & Madan, 2015). Typically, these dentures are maintained using a silicone-bristled denture brush or a standard toothbrush along with a designated cleanser designed for flexible dentures (Hundal & Madan, 2015). The formulation of these flexible denture cleansers closely resembles effervescent-type denture cleansers, containing oxidants like potassium peroxymonopersulphate or potassium peroxydisulphate, as well as acids such as sodium benzoate and citric acid (Mylonas et al., 2022).

Table 2.1 serves as a comprehensive reference outlining the compatibility between various denture cleansers and the diverse array of denture materials available. It offers a summary that aids in understanding which cleansers are suitable for specific types of dentures, considering factors such as material composition and cleaning agent formulation (Mylonas et al., 2022).

Table 2.1: Compatibility between various denture cleansers and denture materials (Mylonas et al., 2022)

Denture cleaning method	Acrylic dentures	Metal dentures	Denture modified with soft or resilient linings	Flexible dentures	Polymer - based dentures
Denture brush	✓	✓	✕	✕	✓
Toothbrush	✓	✓	✕	✕	✓
Silicone brush	✓	✓	✕	✓	✓
Bleach-based	✓	✕	✓	✕	✓
Effervescent type	✓	✓	✕	✕	✓
Mineral acid-based	✓	✕	✕	✕	✓
Enzyme-based	✓	✓	✓	✕	✓
Oral rinses	✓	✓	✓	✕	✓
Flexible denture cleanser	✓	✓	✓	✓	✓
Symbols: ✓ = Compatible ✕ = Not compatible					

However, it is also reported that chemical and mechanical denture hygiene methods may damage the denture surface, causing a rougher surface with an increased propensity for microbial colonization (Gad & Fouda, 2020).

2.5 Antifungal therapy

Antifungal agents are frequently prescribed in conjunction with other therapies for established cases of denture stomatitis (Abuhajar et al., 2023). The most commonly utilised antifungal medications, typically administered over a 14-day period, fall into two primary classes: polyenes (such as nystatin and amphotericin B) and azoles, which encompass triazoles (itraconazole and fluconazole) and imidazoles (clotrimazole, ketoconazole, isoconazole, miconazole, and tioconazole) (Swamy et al., 2018).

Topical antifungal treatments, like nystatin, demonstrate effectiveness against most Candida species and are available in various forms including dry powders for

mouthwash, slow-dissolving lozenges (pastilles), and oral suspensions for swishing, gargling, and swallowing (Abuhajar et al., 2023). Patients with full or partial dentures are advised to nightly soak their dentures in nystatin oral suspension to eradicate the fungus, with consideration given to replacing dentures if this approach proves ineffective (Abuhajar et al., 2023). Miconazole, available as an oral gel, can be applied sparingly over affected areas or to the fitting surface of upper dentures prior to placement in the mouth, potentially offering greater efficacy and lower relapse rates compared to other formulations (Zhang et al., 2016).

Systemic therapy is recommended when topical treatments fail, particularly for patients requiring special care or those with systemic conditions like diabetes or immunosuppression (Swamy et al., 2018). Fluconazole and itraconazole are the most extensively studied and effective systemic antifungal drugs (Swamy et al., 2018). Azole antifungals can interact with the oral anticoagulant warfarin, necessitating close monitoring and potential dosage adjustments under physician guidance (Abuhajar et al., 2023).

Despite the availability of numerous antifungal medications, recurrence of denture stomatitis is common post-treatment due to persistent Candida biofilms on mucosal and prosthetic surfaces (Gendreau & Loewy, 2011). Complete recovery is often hindered by factors such as poor patient compliance, immunodeficiency, and development of drug resistance from prolonged and inappropriate use of antifungals (de Souza et al., 2017).

2.6 Common denture pathogens

Denture biofilms serve as reservoirs for multiple pathogenic microorganisms, including Candida spp., *Staphylococcus aureus, Streptococcus mutans,* and *Escherichia coli* (Kaypetch et al., 2023). Candida species, particularly *Candida albicans*, are known for their strong electrostatic affinity to salivary pellicle-coated Polymethylmethacrylate

(PMMA) denture base material, especially rough surfaces like the unpolished intaglio of a denture (McReynolds et al., 2023). In the oral environment, *Candida albicans* forms mixed microbial communities on both soft tissues and prosthetic surfaces, contributing to mucosal infections. Coexistence within these biofilms with bacterial strains such as Staphylococcus and Streptococcus has been observed during denture infections (Baena-Monroy et al., 2005; Cavalheiro & Teixeira, 2018; Thein et al., 2009). Research indicates that the presence of oral streptococci alongside *Candida albicans* can enhance invasiveness, exacerbating infection and tissue damage in mucosal areas (Cavalcanti et al., 2015; Diaz et al., 2012; Koo et al., 2018).

Notably, *Streptococcus mutans* is frequently found on denture surfaces in individuals with denture stomatitis (Baena-Monroy et al., 2005). Streptococcus species are recognised as primary colonizers during plaque formation (Rosan & Lamont, 2000). *Candida albicans* can co-aggregate with Streptococcus spp., facilitating biofilm development on saliva-coated surfaces (Bamford et al., 2009; Redfern et al., 2022). The acidic environment generated by *Streptococcus mutans* likely supports yeast proliferation (Redfern et al., 2022). Additionally, less common microorganisms in oral flora, such as respiratory pathogens like *Escherichia coli*, Pseudomonas spp., and Klebsiella spp., have been isolated from dentures (Senpuku et al., 2003; Sumi et al., 2002).

2.7 Eugenol and antimicrobial properties

In recent decades, there has been a growing interest in plant extracts possessing antimicrobial properties as viable alternatives (Siqueira et al., 2000). Eugenol, originally isolated in 1929, exhibits good solubility in organic solvents and reasonable solubility in water (Marchese et al., 2017). Derived primarily from clove oil, eugenol has been a cornerstone in dental applications due to its significant analgesic, anti-inflammatory,

anaesthetic, and antimicrobial properties, effectively addressing oral conditions such as pulpitis and dentinal hypersensitivity (Marchese et al., 2017).

Eugenol (4-allyl-2-methoxyphenol) features a straightforward structure with three active sites: hydroxyl, allylic, and aromatic groups (Abdou et al., 2021). The presence of a free hydroxyl group in the eugenol molecule contributes significantly to its potent antimicrobial activity (Marchese et al., 2017). Found in plants like *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae), eugenol plays a crucial role in traditional medicine, offering a wide range of pharmacological effects including acne treatment, allergy relief, rheumatoid arthritis management, wart removal, scar reduction, asthma alleviation, and serving as an analgesic, antispasmodic, and general antiseptic in dental practices (Marchese et al., 2017).

Eugenol exhibits bactericidal (killing bacteria) or bacteriostatic (inhibiting bacterial growth) properties depending on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), while also demonstrating antiviral and antifungal effects (Devi et al., 2010). The MIC refers to the lowest concentration of an antimicrobial agent that completely inhibits growth, disregarding any slight haze or isolated colony resulting from the initial inoculum (Marchese et al., 2017).

Studies have highlighted eugenol's efficacy against various pathogens: it produces an inhibition zone of 15.9 ± 0.4 mm against *Escherichia coli* O157:H7 ATCC 35150 (Mith et al., 2014); exhibits MIC values ranging from 249 to 999 µg/mL against Extended-Spectrum Beta-Lactamase (ESBL) isolates of *Escherichia coli* (Dhara & Tripathi, 2013); demonstrates a MIC of 100 µg/mL against *Streptococcus mutans* ATCC 25175 (Moon et al., 2011); and has a MIC exceeding 250 µg/mL against *Candida albicans* (Carrasco et al., 2012), with an inhibition zone of 13.5 mm against *Candida albicans* ATCC 10231 (Siqueira et al., 2000).

Eugenol has shown effectiveness against pathogens like *Salmonella typhi*, *Proteus mirabilis* (Devi et al., 2010; Devi et al., 2013), *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* (Walsh et al., 2003), and *Listeria monocytogenes* (Filgueiras & Vanetti, 2006). Notably, eugenol exhibits concentration-dependent antifungal properties against *Candida albicans* (Latifah-Munirah et al., 2015), consistent with findings reported by a previous study (WH et al., 2011).

Recent research has explored eugenol's potential in denture cleansers, highlighting its efficacy against *Candida albicans* compared to commercial alternatives. A 2% eugenol solution as a denture cleanser demonstrated comparable effectiveness in removing initial adherence of *Candida albicans* on denture base materials as commercially available cleansers (Zanul Abidin et al., 2023). Furthermore, clove-mediated effervescent denture cleansing granules have proven effective against *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*, suggesting their potential for routine denture hygiene to prevent infections like denture stomatitis (Ezhilarasan & Subha, 2019). Eugenol has also been proposed as an alternative treatment for Candida spp. infections in denture wearers, with promising in vitro activity (Marcos-Arias et al., 2011).

The degree of oral soft tissue response linked to eugenol varies with dosage (Deshpande et al., 2014). Higher concentrations of eugenol adversely affect fibroblast and osteoblast-like cells, leading to localised necrosis and impaired healing (Alpar et al., 1999). Conversely, lower concentrations provoked localised hypersensitivity reactions in the oral mucosa, commonly referred to as "contact stomatitis" (Sarrami et al., 2002). With eugenol being safe for topical application at concentrations below 5% (Opdyke, 1975), its chemical properties underscore its potential for developing novel drugs that could address human health challenges (Abdou et al., 2021). The World Health Organization

(WHO) has classified eugenol as a non-mutant and generally recognised it as a safe (GRAS) molecule (Nisar et al., 2021). Eugenol's antiseptic properties have led to its use in mouthwashes as a disinfectant, and it is also combined with tooth fillers for its pain-relieving, antiseptic, and analgesic effects (Jadhav et al., 2004). While eugenol has been investigated as a denture cleanser solution (Zanul Abidin et al., 2023) and in the form of effervescent denture cleansing granules (Ezhilarasan & Subha, 2019), this study will be the first to formulate it into an effervescent tablet.

2.8 Effervescent tablet

"Effervescent tablet is a tablet intended to be dissolved or dispersed in water before administration" as per the revised definition proposed to the US FDA (Palanisamy et al., 2011). Effervescent tablets are more convenient to transport compared to liquid medication since water is only added when they are ready to be used (Palanisamy et al., 2011). Effervescent tablets have storage advantages for keeping the drug dry, stable and safe compared with suspension forms or syrup (Ipci et al., 2016).

Effervescent tablets are designed to dissolve in water, releasing carbon dioxide in the process produced through an acid-base reaction (Patel & Siddaiah, 2018). These tablets contain organic acids and alkali metal carbonate salts, with the primary components being sodium bicarbonate and citric acid (Ipci et al., 2016). Acids such as tartaric, malic, fumaric, citric, and adipic are used, with citric acid being preferred for its citrus-like taste (Ipci et al., 2016). After granulation, a lubricant, typically magnesium stearate, is added to the tablets to improve flow and prevent sticking (Ipci et al., 2016). Materials like calcium silica, talc, fumed silica, or cornstarch are added to enhance flowability (Ipci et al., 2016; Stahl, 2003).

The production process involves precise ratios of these different materials to ensure the desired properties of the effervescent tablets are achieved (Stahl, 2003). For

instance, using 30% of the active ingredient (neem oil) absorbed over 15% silica and then combining it with 40% of acids/acid salts and bicarbonates in definite proportion resulted in an effervescent tablet with all the physicochemical parameters within the limits (Iqbal et al., 2021). The tablet production should be in an environment with low humidity (25°C) because moisture can damage the tablets (Stahl, 2003). Drying agents, such as silica gel, are incorporated into storage tubes to help maintain this condition (Stahl, 2003).

The control parameters of effervescent tablets are similar to conventional tablets such as weight, hardness, pH, disintegration rate and friability (Aslani & Jahangiri, 2013; Monrle, 1980). Hardness was determined by measuring the tablet's resistance to crushing or rupture under continuous pressure using a hardness tester (Aminuddin & Azhar, 2023). The hardness test is conducted to determine the tablet's resistance to mechanical shocks during handling, shipping, storage, and transportation (Preethi et al., 2017).

2.9 Agar well diffusion test

The agar well diffusion method is widely used to evaluate the antimicrobial activity of plant or microbial extracts (Balouiri et al., 2016; Magaldi et al., 2004; Valgas et al., 2007). Similar to the disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire surface (Balouiri et al., 2016). A hole with a diameter of 6 to 8 mm is then aseptically punched with a sterile cork borer or tip, and a volume (20 to 100 µL) of the antimicrobial agent or extract solution at the desired concentration is introduced into the well (Balouiri et al., 2016). The agar plates are then incubated under suitable conditions for the test microorganism (Balouiri et al., 2016). Following incubation, a dense bacterial growth covering the surface is to be observed (Valgas et al., 2007). The antimicrobial agent diffuses through the agar medium, inhibiting the growth of the tested microbial strain (Balouiri et al., 2016). The inhibition of bacterial growth is quantified in millimetres (Valgas et al., 2007).

Based on a previous study, bacterial strains, including *Streptococcus mutans* ATCC 25175, *Streptococcus sanguinis* ATCC 10556, *Staphylococcus aureus* ATCC 6538, and *Escherichia coli* ATCC 25922, were cultured in brain heart infusion (BHI) agar (Difco, Detroit, USA) in a CO_2 incubator at 37 °C for 24 hours (Kaypetch et al., 2023). Meanwhile, *Candida albicans* ATCC 10231 was grown in Sabouraud dextrose agar (SDA; Difco, Detroit, USA) in an incubator at 37 °C for 48 hours (Kaypetch et al., 2023). These cultures were then used for further experiments. The well variant of the diffusion method was proven to be more sensitive compared to the disc variant when testing a diverse range of natural products from plants, fungi, and lichens against two bacterial species, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 (Valgas et al., 2007).

CHAPTER 3: METHODOLOGY

3.1 Ethical consideration and funding requisition

Ethical approval was not required as this study does not involve any human or animal subjects. This study was funded by the Dental Postgraduate Research Grant (DPRG), Faculty of Dentistry, University of Malaya; Grant Number: UMG023E – 2024.

3.2 Study design

This is a pilot, in vitro investigation study conducted in a controlled laboratory environment.

3.3 Preparation of tablet

3.3.1 Materials

Details of the materials used in this study are summarised in Table 3.1.

Table 3.1: Materials used in the study

Chemical	Grade	Brand	Lot number
Silicon Dioxide	Analytical Reagent (AR)	Chemiz, UK	2201221
Sodium Bicarbonate	Analytical Reagent (AR)	Chemiz, UK	2311111
Magnesium Stearate	Analytical Reagent (AR)	Alfa Aesar, USA	A0446366
Citric Acid	Analytical Reagent (AR)	Chemiz, UK	2207201
Tartaric acid	99%	Acros Organics, USA	A0454791
Eugenol	99%	Alfa Aesar, USA	10240458

3.3.2 Eugenol-based effervescent and placebo tablet preparation

The eugenol-based effervescent tablets were prepared according to published protocol (Iqbal et al., 2021). Initially, 0.75 grams of silica were weighed and transferred into a ceramic mortar. A volume of 1.8 ml of eugenol was then measured with a sterile glass pipette and added to the silica while mixing simultaneously with a pestle. This specific amount of eugenol was determined to be optimal for 0.75 grams of silica

according to the preliminary trial conducted. Adding more than 1.8 ml of eugenol resulted in the aggregation of silica particles and adversely affected their flowability. After impregnating the silica with eugenol, other effervescent ingredients were added sequentially: first, 1.1 grams of sodium bicarbonate, followed by 0.4 grams of citric acid and 0.5 grams of tartaric acid. Once all ingredients were incorporated, they were thoroughly mixed. Finally, 0.5 grams of magnesium stearate, serving as a lubricating agent, was added and uniformly mixed in the ceramic mortar. The mixed powdered ingredients, totalling approximately 5 grams, were then sieved to ensure uniform mixture is obtained. Additionally, another batch of the mixture was prepared with the exact same amount of the other ingredients, except for the eugenol, to produce placebo tablets.

After sieving, the uniformly powdered ingredients were weighed into equal 0.5 grams portions and transferred into the funnel of a manual tablet compaction machine (MTCM-I/Globepharma, INC). The equally divided portions were then all compressed with a 10 mm punch set, applying 10 psi of hydraulic pressure uniformly. The compressed tablets were subsequently stored in airtight containers with silica gel. Steps taken in tablet preparation are shown in Figure 3.1.

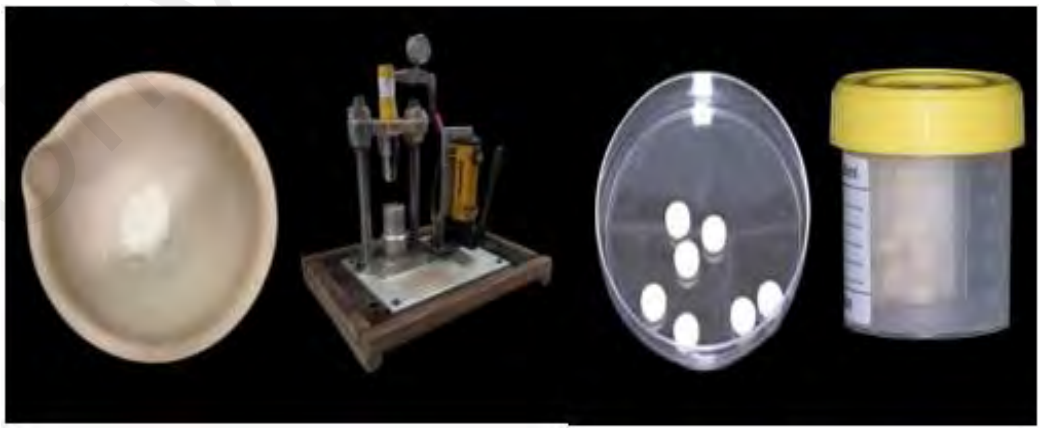

Figure 3.1: Steps of tablet preparation

3.4 Tablet analysis

After the compression process, several physicochemical tests were conducted to evaluate the hardness, dimensions, pH and effervescence time of the novel eugenol-based denture cleansing tablet, placebo tablet and Polident® tablet. Additionally, content analysis of the novel eugenol-based denture cleansing tablet was conducted to determine the eugenol concentration.

3.4.1 Hardness and thickness of the tablet

The hardness of the tablet was measured by a hardness tester (Electrolab EBT-2PL) and expressed in Newton (N). The thickness was measured using the features of this machine (Dhahir & Al-Kotaji, 2019). These measurements were taken in triplicate on three tablets randomly, and the average was calculated with standard deviation for the novel eugenol-based denture cleansing tablet, placebo tablet, and Polident® tablet.

3.4.2 Tablet dimension

The diameter of the novel eugenol-based denture cleansing tablet, placebo tablet, and Polident® tablet was measured on three randomly selected tablets using a 15-centimeter (cm) scale (Iqbal et al., 2021). The average was calculated with standard deviation.

3.4.3 pH measurement

Dissolved a novel eugenol-based denture cleansing tablet in 200 ml water and pH of the solution was measured by the pH meter (Aquasearcher™ AB33PH, OHAUS). This test was conducted similarly for placebo tablet and Polident® tablet. The pH measurement test was done in triplicates for each tablet and the average was calculated with standard deviation (Aslani & Jahangiri, 2013).

3.4.4 Effervescence time

In this evaluation, a novel eugenol-based denture cleansing tablet was placed in 200 ml of distilled water and gently stirred with a glass rod for a specified period. Time taken for complete disintegration of the tablet was indicated by the absence of any residue and recorded by stopwatch (Iqbal et al., 2021).This test was repeated three times and average calculated with standard deviation for placebo and Polident® tablet.

3.4.5 Content analysis of the tablet by UV-VIS spectrophotometer

The amount of eugenol in the tablet was evaluated through a direct method whereby the novel eugenol- based denture cleansing tablet was dissolved in 200 ml of distilled water and the solution was analysed using a UV-VIS spectrophotometer (Shimadzu UV-VIS/ UV-1900i) by measuring absorbance at 282 nm wavelength. Prior to the assessment of the eugenol content, a standard calibration curve was set by using concentration ranges of 20 to 70 µg/ml against mean absorption at 282 nm and regression equation was determined (Jagtap & Paradkar, 2021). Stock solution of eugenol prepared with dilution ratio of 10mg per 100ml methanol as proposed by previous study (Jagtap & Paradkar, 2021).The amount of eugenol released in the dilution was determined based on the regression equation. The percentage of eugenol content in each tablet was calculated according to the following equation.

$$\text{Percentage of eugenol (\%)} = \text{Amount of eugenol measured} / \text{Amount of eugenol added} \times 100$$

3.5 Culture media preparation

Firstly, Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB), Brain Heart Infusion (BHI) Agar and Brain Heart Infusion (BHI) broth (OXOID Ltd, Basingstoke, Hampshire, UK) was prepared according to manufacturer instruction as summarised in Table 3.2. The media which comes in powder form was appropriately

weighed as directed for the preparation, dissolved in the distilled water, boiled and later sterilised at 121°C for 15 min in an autoclave. The prepared SDA and BHI agar were later poured into agar plates. The prepared agar plates and broth media were kept refrigerated at 4°C for later use.

Table 3.2: Manufacturer instruction of powder to liquid ratio for culture media preparation

Culture media	Weight (per litre)
Sabouraud dextrose agar (SDA)	65 g
Sabouraud dextrose broth (SDB)	65 g
Brain heart infusion (BHI) agar	47 g
Brain heart infusion (BHI) broth	37 g

3.5.1 Cultivation of *Candida albicans*

The *Candida albicans* strain (ATCC 14053), obtained from the American Type Culture Collection (ATCC) in Manassas, USA was cultured in SDB (OXOID Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours. The turbidity of this suspension was adjusted to an optical density of 0.144 at 550 nm using spectrophotometer (Shimadzu UV-1700, Japan). This optical density corresponded to a concentration of 1×10^6 cells/ml, standardised by 0.5 McFarland standard.

3.5.2 Cultivation of *Streptococcus mutans* and *Escherichia coli*

The strains of *Streptococcus mutans* (ATCC 25175) and *Escherichia coli* (NCTC 12900) obtained from the American Type Culture Collection in Manassas (ATCC), USA and National Collection of Type Cultures (NCTC), UK were cultured separately in BHI broth (OXOID Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for 24 hours (Kaypetch et al., 2023). After incubation, the turbidity of each suspension was adjusted and standardised to an optical density of 0.144 at 550 nm using spectrophotometer (Shimadzu UV-1700, Japan). This optical density corresponded to a concentration of 1×10^8 cells/ml, standardised by 0.5 McFarland standard.

3.6 Denture cleansing solution preparation

Tablet Polident® was diluted with 150 ml distilled water as per the manufacturer's instruction. Novel eugenol-based denture cleansing tablet was diluted with 0.5 ml distilled water to produce a concentrated solution. Placebo tablet was diluted with 0.5 ml distilled water to produce a concentrated solution. 0.12% Chlorhexidine (Orodex®), used as a cleansing solution. Distilled water, preparation was done by sterilisation at 120°C for 20 min in an autoclave. The denture cleansing solutions were freshly prepared simultaneously before the experiment as described in Table 3.3.

Table 3.3: Denture cleansing solution preparation

Denture cleansing solution	Preparation
Polident® solution (experimental control)	1 Tablet of Polident® & 150 ml of distilled water (according to manufacturer instructions)
0.12% Chlorhexidine (positive control)	0.12% Chlorhexidine (Orodex® mouthwash)
Distilled water (negative control)	Distilled water
Novel eugenol-based denture cleansing solution	1 Tablet of novel eugenol-based denture cleansing tablet (0.5g) & 0.5 ml of distilled water
Placebo tablet solution	1 Placebo tablet (0.5g) & 0.5 ml of distilled water

3.7 Agar well diffusion test

Methods to conduct this agar well diffusion test have been adapted and modified from previous study (Balouiri et al., 2016). Firstly, SDA and BHI Agar (OXOID Ltd, Basingstoke, Hampshire, UK) were prepared according to the manufacturer. The freshly prepared suspension of 100 µl *Candida albicans* (ATCC 14053), 1×10^8 cells/ml, was seeded onto SDA surfaces in petri dishes using the swabbing method with a sterile cotton swab. Uniform strokes were applied to ensure even distribution of the suspension. Five wells were then made with a diameter of 6 mm using a sterile cork borer on the agar surface (Balouiri et al., 2016). Prepared cleansing solutions were poured into each well

using sterile pipette tips and an electronic pipette. Each well received 60 µl of Polident® solution and 60 µl of 0.12% chlorhexidine as the positive control. Additionally, 60 µl of distilled water was added as the negative control. Finally, 60 µl of novel eugenol-based denture cleansing solution and 60 µl of placebo tablet solution without eugenol were added to separate wells. This entire procedure was conducted in triplicate (Iyer et al., 2017). The plates were left to stand for 10 minutes to allow the solutions to diffuse as shown in Figure 3.2 (Iyer et al., 2017). The agar plates were subsequently closed and sealed, then incubated at 37°C for 24 hours. After incubation, the diameter of zones of inhibition was measured with a digital calliper and recorded.

Similarly, the freshly prepared suspension of 100 µl *Streptococcus mutans* (ATCC 25175) and *Escherichia coli* (NCTC 12900), both at a concentration of 1×10^6 cells/ml, was swabbed with a sterile cotton swab onto BHI agar surfaces. Five wells were created on each agar plate using a sterile cork borer. Each set of wells received 60 µl of placebo tablet solution, 60 µl of Polident® solution, 60 µl of 0.12% Chlorhexidine, 60 µl of distilled water and 60 µl of novel eugenol-based denture cleansing solution. This process was repeated in triplicate for both bacterial strains. After setting up the wells, the plates were left to stand for 10 minutes to allow the solutions to diffuse as shown in Figure 3.3 and 3.4. Subsequently, the agar plates were sealed and incubated at 37°C for 24 hours. Following the incubation period, the zones of inhibition around the wells were measured with a digital calliper and recorded.

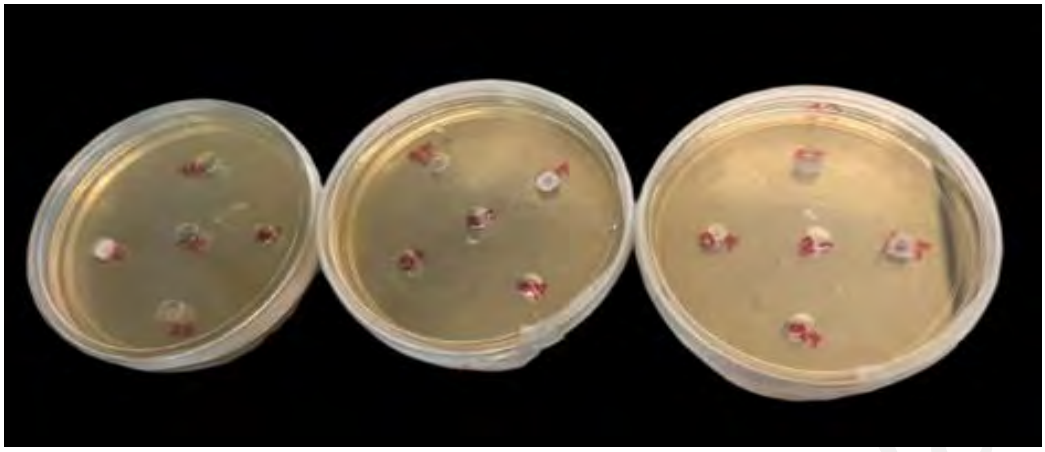

Figure 3.2: Triplicate agar plates of *Candida albicans* for incubation

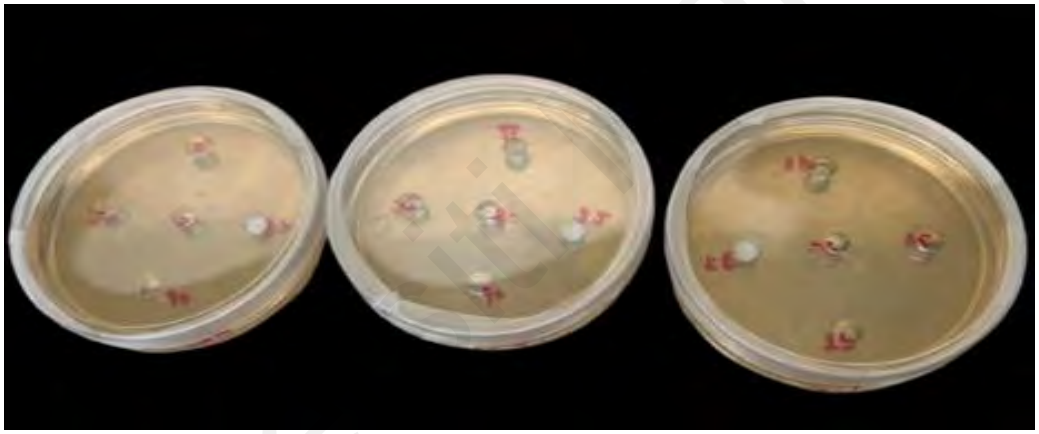

Figure 3.3: Triplicate agar plates of *Streptococcus mutans* for incubation

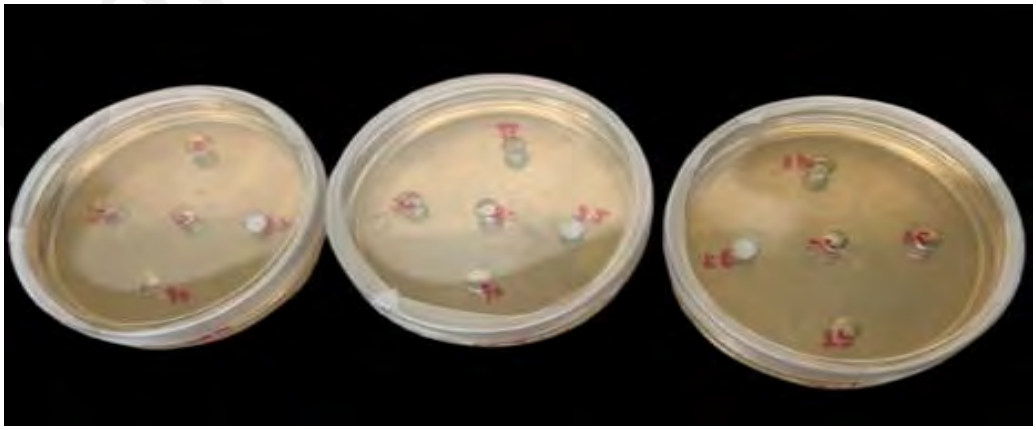

Figure 3.4: Triplicate agar plates of *Escherichia coli* for incubation

3.8 Statistical Analysis

The data were analysed using SPSS version 29 (SPSS Inc., IBM, USA) software, on descriptive statistics to determine the mean values and standard deviation. This qualitative analysis technique was employed to determine the antifungal and antibacterial efficiency, offered a summary and forming the basis for further statistical analyses.

3.9 Research Workflow

The brief workflow of methodology is outlined in Figure 3.5 below.

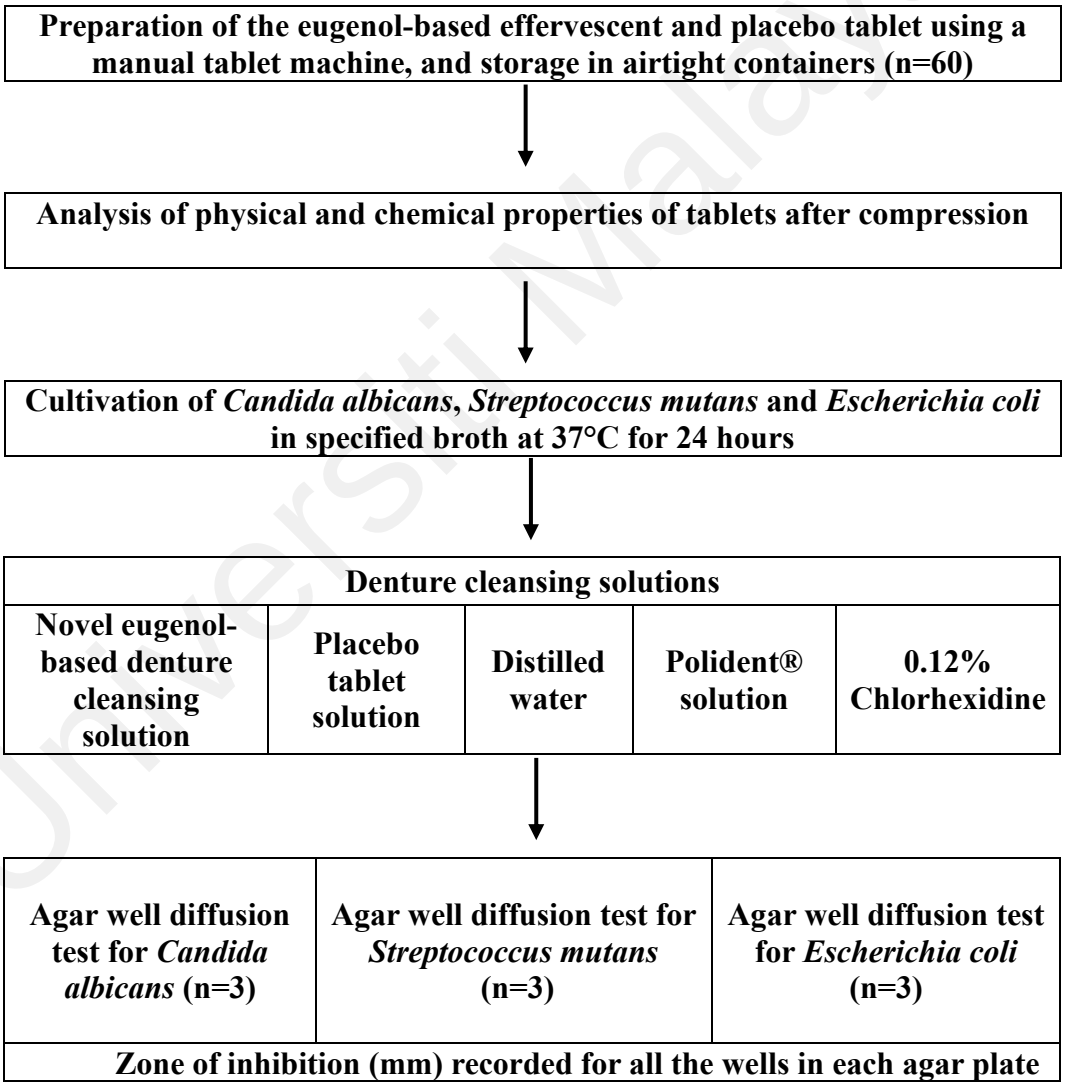

Figure 3.5: Brief workflow of methodology

CHAPTER 4: RESULTS AND DATA ANALYSIS

4.1 Physicochemical analysis

The physicochemical properties of the novel eugenol-based denture cleansing tablet, placebo tablet and Polident® tablet are summarised in Table 4.1 below in mean values and standard deviation.

Table 4.1: Physicochemical evaluation of the novel eugenol-based denture cleansing tablet, placebo and Polident ® tablet

Tablet Analysis	Hardness (N)	Thickness (mm)	Diameter (mm)	pH	Effervescence time (min)
Novel eugenol-based denture cleansing tablet	3.79 ± 0.01	10.06 ± 0.00	10.00 ± 0.00	4.97 ± 0.10	3.22 ± 0.03
Placebo tablet	6.54 ± 0.01	7.03 ± 0.01	10.00 ± 0.00	6.80 ± 0.05	3.36 ± 0.01
Polident® tablet	175.92 ± 0.02	3.90 ± 0.01	23.50 ± 0.00	6.63 ± 0.02	3.31 ± 0.03

4.1.1 Content analysis by UV-VIS Spectrophotometer

Standard calibration curve was set using eugenol concentration ranges of 20 - 70 µg/ml against mean absorption at 282 nm wavelength as shown in Figure 4.1 below. The curve demonstrated good linearity of $R^2 > 0.99$.

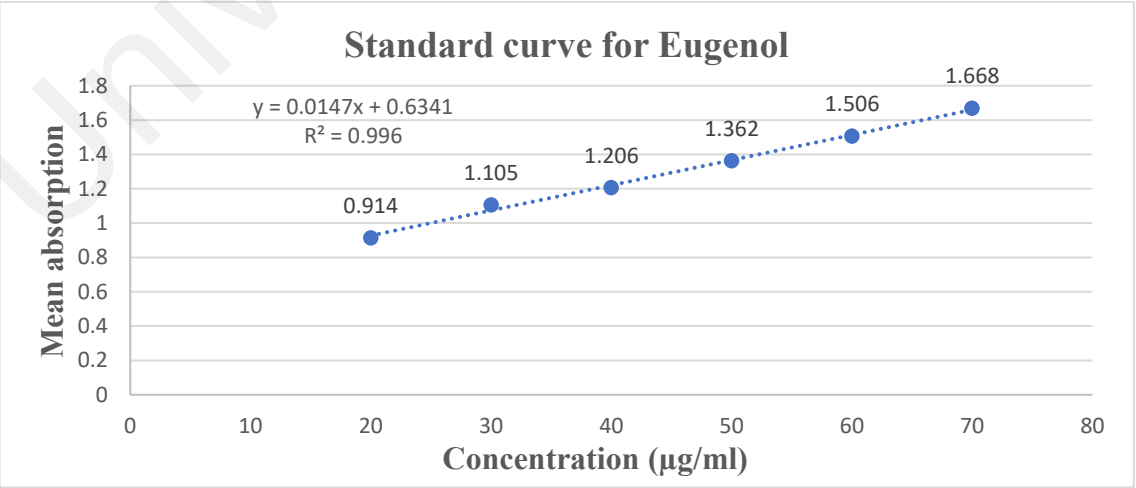

Figure 4.1: Standard calibration curve of eugenol

Mean absorption was determined for a novel eugenol-based denture cleansing tablet in 200 ml of distilled water using a UV-VIS spectrophotometer in triplicate followed by determination of amount of concentration of eugenol release in the sample by utilising regression equation from the standard curve (y = 0.0147x + 0.6341). The percentage of eugenol in each tablet was calculated according to the following equation.

$$\text{Percentage of eugenol (\%)} = \text{Amount of eugenol measured} / \text{Amount of eugenol added} \times 100$$

Table 4.2: Summarised measurements and values of eugenol release in novel eugenol- based tablet

Measurement	Value
Mean absorption + Standard deviation	1.459 ± 0.001
Concentration of eugenol release (µg/ml)	56.116
Amount of eugenol released in 200 ml distilled water (mg)	11.223
Average amount of eugenol added in a novel eugenol-based tablet	0.2ml / 212mg
Percentage of eugenol concentration in a novel eugenol-based tablet (%)	5.294

4.2 Zone of inhibition (mm)

The diameter of the zone of inhibition around the five wells was measured with a digital calliper and recorded after 24 hours of incubation period. The inhibition of bacterial growth is quantified in millimetres (mm). Preliminary data collection was done and attached in Appendix B. Descriptive statistics was used to determine the mean value and standard deviation for *Candida albicans* group, *Streptococcus mutans* group and *Escherichia coli* group as below.

4.2.1 *Candida albicans* Group

Table 4.3: Descriptive statistics for the *Candida albicans* group

Descriptive statistics					
Denture cleansing solution	**n**	**Minimum (mm)**	**Maximum (mm)**	**Mean (mm)**	**Std. Deviation**
Novel eugenol-based denture cleansing solution (T.E)	3	19.83	23.82	21.26	2.22
0.12% Chlorhexidine (+C)	3	11.17	13.88	12.53	1.36
Placebo tablet solution (T.P)	3	0.00	0.00	0.00	0.00
Distilled water (-S)	3	0.00	0.00	0.00	0.00
Polident® solution (+P)	3	0.00	0.00	0.00	0.00

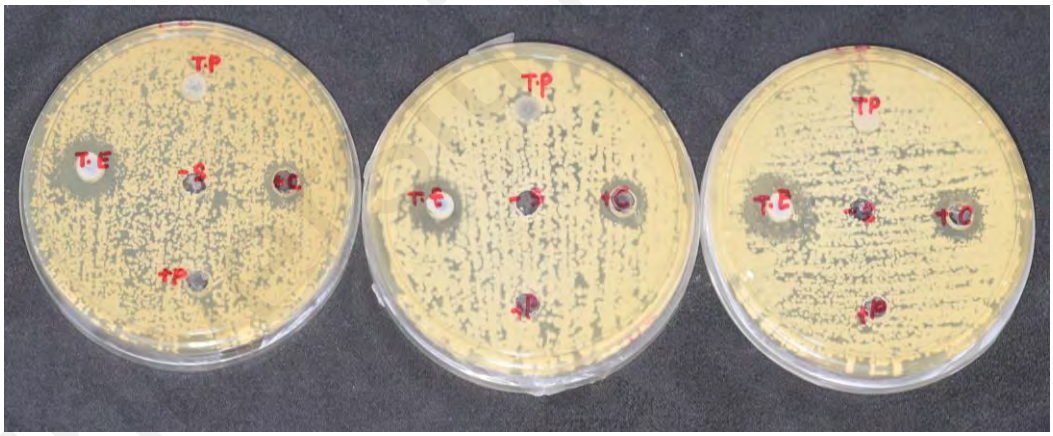

Figure 4.2: Triplicate agar plates of *Candida albicans* after incubation

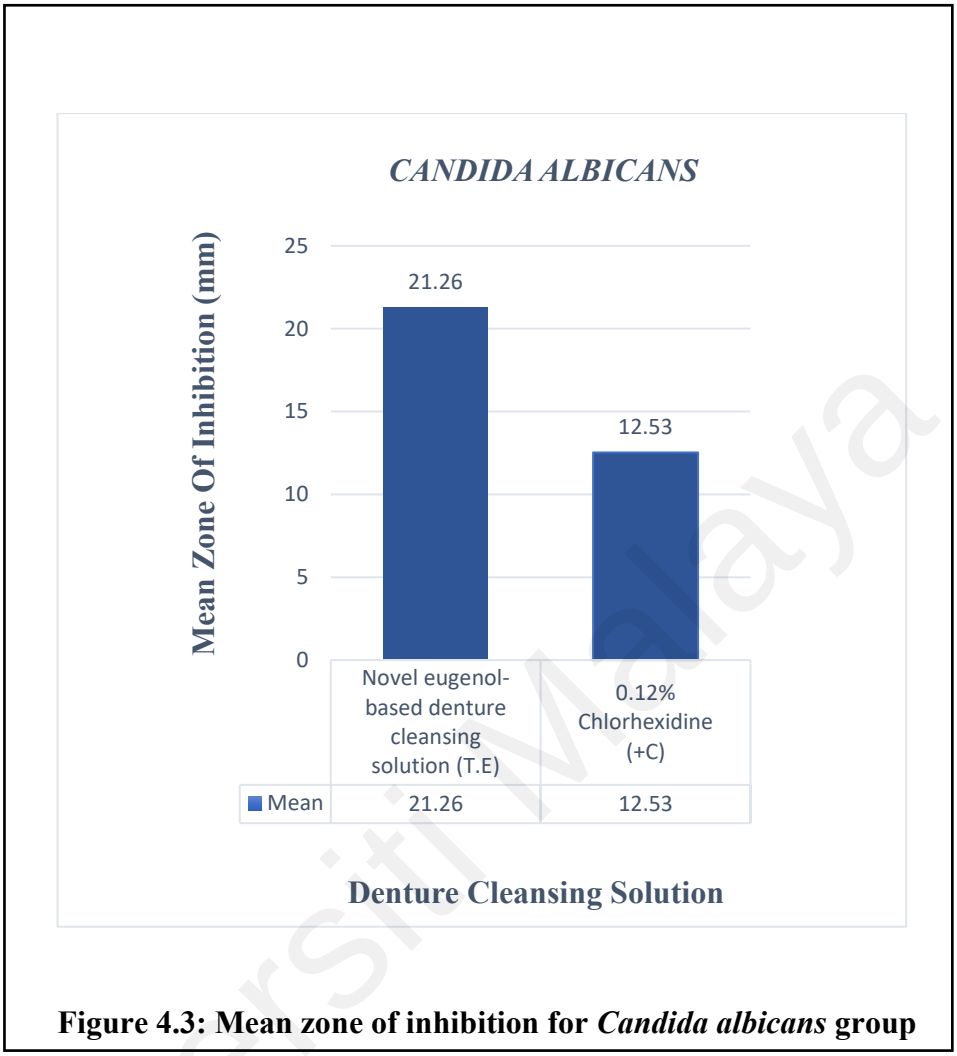

Figure 4.3: Mean zone of inhibition for *Candida albicans* group

The novel eugenol-based denture cleansing solution (T.E) had three observations with a minimum value of 19.83 mm and a maximum value of 23.82 mm, resulting in a mean of 21.26 mm and a standard deviation of 2.22. The 0.12% Chlorhexidine (+C) also had three observations, with a minimum value of 11.17 mm and a maximum value of 13.88 mm. This solution had a mean of 12.53mm and a standard deviation of 1.36.

The placebo tablet solution (T.P), distilled water (-S), and Polident® solution (+P) showed no sensitivity, with all values being zero across the three observations, resulting in a mean and standard deviation of 0.00.

In summary, the novel eugenol-based denture cleansing solution (T.E) and 0.12% Chlorhexidine (+C) displayed measurable values and variability against *Candida albicans*, whereas the other solutions (T.P, -S, and +P) consistently showed no effect.

4.2.2 *Streptococcus mutans* Group

Table 4.4: Descriptive statistics for the *Streptococcus mutans* group

Descriptive statistics					
Denture cleansing solution	**n**	**Minimum (mm)**	**Maximum (mm)**	**Mean (mm)**	**Std. Deviation**
Novel eugenol-based denture cleansing solution (T.E)	3	7.59	8.67	8.20	0.55
0.12% Chlorhexidine (+C)	3	19.65	19.90	19.79	0.13
Placebo tablet solution (T.P)	3	0.00	0.00	0.00	0.00
Distilled water (-S)	3	0.00	0.00	0.00	0.00
Polident® solution (+P)	3	0.00	0.00	0.00	0.00

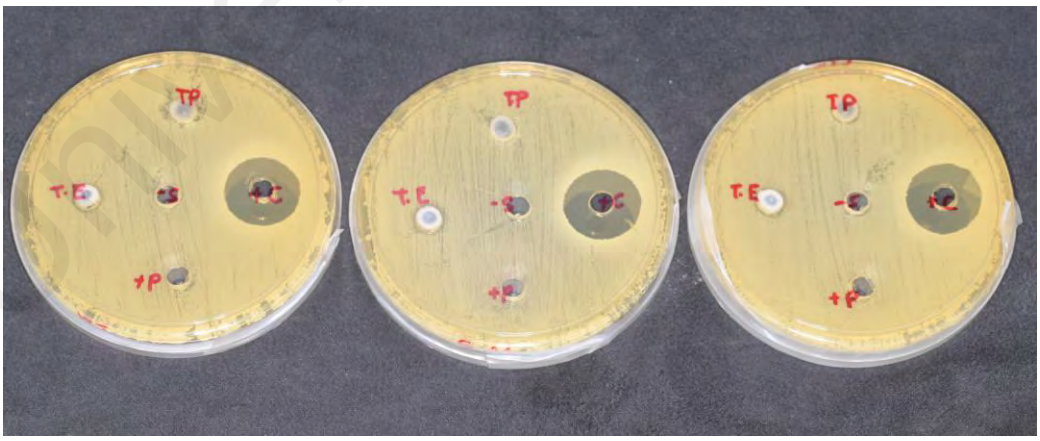

Figure 4.4: Triplicate agar plates of *Streptococcus mutans* after incubation

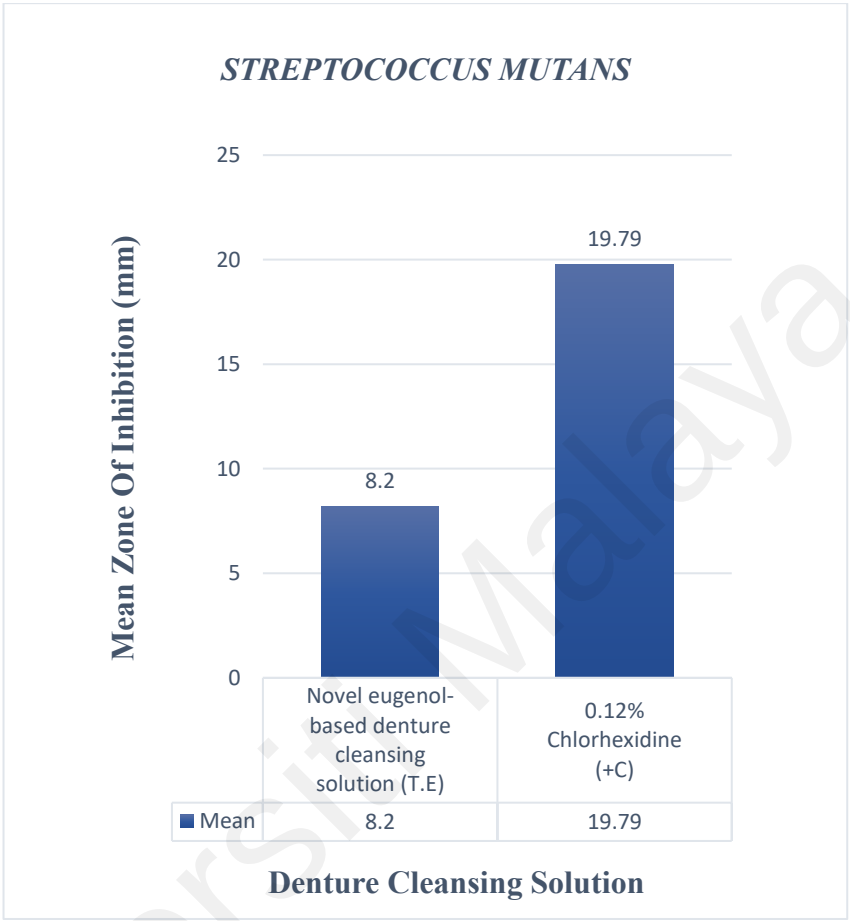

Figure 4.5: Mean zone of inhibition for *Streptococcus mutans* group

The novel eugenol-based denture cleansing solution (T.E) had a range of inhibition with a minimum value of 7.59 mm to a maximum value of 8.67 mm, resulting in a mean inhibition zone of 8.20 mm and a standard deviation of 0.55. The 0.12% Chlorhexidine (+C) also had a slightly higher range of inhibition, with a minimum value of 19.65 mm to a maximum value of 19.90 mm resulting in a mean of 19.79mm and a standard deviation of 0.13.

The placebo tablet solution (T.P), distilled water (-S), and Polident® solution (+P) showed no sensitivity, with all values being zero across the three observations, resulting in a mean and standard deviation of 0.00. In summary, the novel eugenol-based denture cleansing solution (T.E) and 0.12% Chlorhexidine (+C) displayed measurable values and variability against *Streptococcus mutans*, whereas the other solutions (T.P, -S, and +P) consistently showed no effect.

4.2.3 *Escherichia coli* Group

Table 4.5: Descriptive statistics for the *Escherichia coli* group

Descriptive statistics					
Denture cleansing solution	**n**	**Minimum (mm)**	**Maximum (mm)**	**Mean (mm)**	**Std. Deviation**
Novel eugenol-based denture cleansing solution (T.E)	3	7.32	11.60	9.21	2.18
0.12% Chlorhexidine (+C)	3	13.80	14.84	14.21	0.55
Placebo tablet solution (T.P)	3	0.00	0.00	0.00	0.00
Distilled water (-S)	3	0.00	0.00	0.00	0.00
Polident® solution (+P)	3	6.32	7.19	6.66	0.45

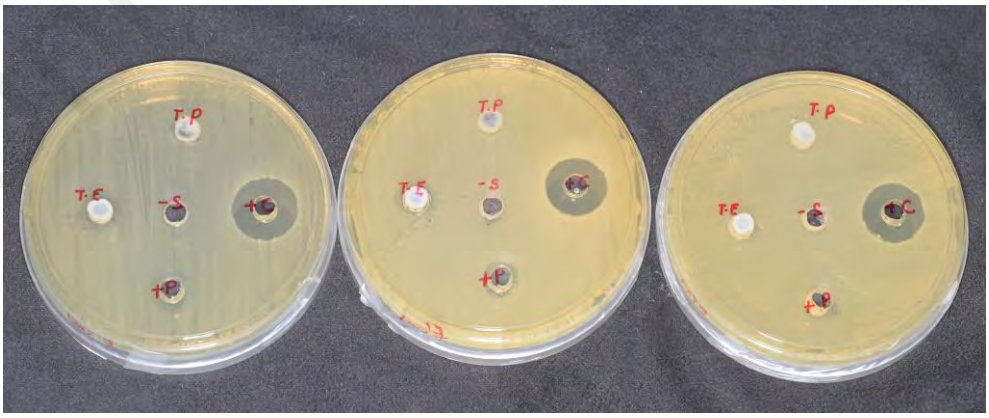

Figure 4.6: Triplicate agar plates of *Escherichia coli* after incubation

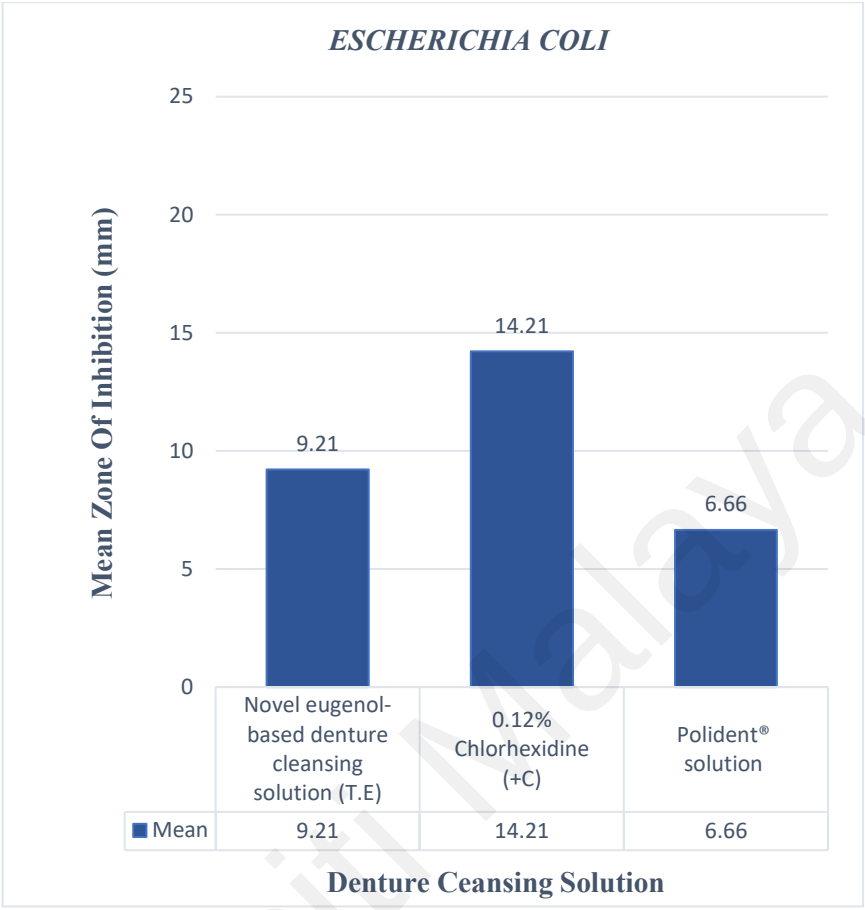

Figure 4.7: Mean zone of inhibition for *Escherichia coli* group

The novel eugenol-based denture cleansing solution (T.E) demonstrated a range of inhibition from a minimum value of 7.32 mm to a maximum value of 11.60 mm, with a mean inhibition zone of 9.21 mm and a standard deviation of 2.18 mm. The 0.12% Chlorhexidine (+C) showed a higher range of inhibition, from a minimum value of 13.80 mm to a maximum value of 14.84 mm, resulting in a mean inhibition zone of 14.21 mm and a standard deviation of 0.55 mm.

Both the placebo tablet solution (T.P) and distilled water (-S) exhibited no antibacterial activity, with all observations being zero. The Polident® solution (+P)

displayed a smaller range of inhibition, from 6.32 mm to 7.19 mm, with a mean of 6.66 mm and a standard deviation of 0.45.

In summary, the novel eugenol-based denture cleansing solution (T.E), 0.12% Chlorhexidine (+C), and Polident® solution (+P) displayed measurable values and variability against *Escherichia coli*, whereas the other solutions (T.P and -S) consistently showed no effect.

CHAPTER 5: DISCUSSION

The null hypothesis was rejected as the novel eugenol-based tablet denture cleanser solution did exhibit antifungal and antibacterial properties when compared to 0.12% Chlorhexidine and commercially available denture cleansing solution **(**Polident®). The primary objective to formulate a novel eugenol-based tablet was possible with precise ratios of different materials, ensuring all physicochemical parameters remained within acceptable limits. This chapter provides an in-depth analysis of the findings from the in-vitro pilot study on the innovative eugenol-based denture cleanser tablet formulation and antimicrobial properties of the novel eugenol-based tablet denture cleanser solution using an agar well diffusion test. Lastly, it discusses the limitations encountered during the study and offers suggestions for future research.

Tablet production was based on a modified technique from a previous study to create a similar effervescent tablet using a natural extract as an oil-based substance to be diluted in water (Iqbal et al., 2021). The basic effervescent ingredients, used in exact weight ratios as in the previous study, included the adaptation of silica added to the oil and the inclusion of magnesium stearate as a lubricating agent to prevent the mixture from sticking (Iqbal et al., 2021). In addition, a preliminary trial was conducted to determine the specific amount of eugenol (1.8ml) to be optimum in 0.75 grams of silica.

The novel eugenol-based denture cleansing tablets produced in this study along with placebo and Polident® tablet, were evaluated on physicochemical parameters. The hardness of effervescent tablets is generally lower than that of conventional tablets, with the minimum acceptable hardness for uncoated tablets being approximately 40 N (Aslani & Fattahi, 2013). The hardness of the Polident® tablet was measured at 175.92 N, significantly surpassing the minimum requirement. In contrast, the hardness of the novel eugenol-based denture cleansing tablet was 3.785 N, notably less than the minimum

requirement compared to placebo and Polident® tablet. Notably, the newly developed eugenol-based denture cleansing tablet met the standards for tablet thickness of 10.06 mm, pH of 4.97 and an effervescence time of 3.22 minutes. The pH should be slightly acidic, less than 6, which is ideal for effervescence reactions (Iqbal et al., 2021). The thickness of the tablets must be 6.1 ± 0.3 mm to be considered acceptable according to pharmacopoeia standards (USP NF.2008) (Aslani & Fattahi, 2013). The effervescence time of the tablet must be less than 3 minutes (Aslani & Fattahi, 2013). The concentration of eugenol in the novel eugenol-based denture cleansing tablet was 5.294%. Given that eugenol is safe for topical application at concentrations below 5% (Opdyke, 1975), the concentration in this tablet is acceptable. The tablet will be used as a cleanser solution and thoroughly rinsed before coming into contact with the oral mucosa.

The agar well diffusion test was the choice of test to determine the antimicrobial efficiency of the novel eugenol-based tablet in this study. The agar well diffusion method is the commonly used method to assess the antimicrobial activity of plant or microbial extracts (Balouiri et al., 2016; Magaldi et al., 2004; Valgas et al., 2007). The diffusion method variant well is preferred over the variant disc due to its simplicity and minimal time consumption (Valgas et al., 2007). Using 0.12% Chlorhexidine, Polident®, and the placebo tablet as controls in this study provides a comprehensive framework to evaluate the efficacy of the novel eugenol-based denture cleansing tablet. 0.12% Chlorhexidine, a widely used antiseptic in dentistry (Kaypetch et al., 2023), offers a benchmark for antibacterial and antifungal effectiveness, known for its ability to reduce denture biofilm (de Andrade et al., 2012). Polident®, a popular peroxide-based denture cleanser in Malaysia (Tarib et al., 2018), facilitates mechanical cleaning through oxygen bubbles and surfactant action (sodium lauryl sulfate), making it relevant for commercial comparisons. The placebo tablet and distilled water serves as baselines, ensuring that observed effects are due to the active ingredients and no other factors.

The antibacterial activity of eugenol derivatives was evaluated using the inhibition zone technique, measured in millimeters (mm), to assess their potential for inhibiting microbial growth (da Silva et al., 2018). According to the literature, substances with inhibition halos of less than 7 mm are considered inactive, those with halos between 7 and 16 mm are moderately active, and those with halos greater than 16 mm are deemed to have significant antibacterial potential (da Silva et al., 2018; O et al., 2013; Ribeiro-Santos et al., 2017).

In this study, the novel eugenol-based denture cleanser solution demonstrated a mean inhibition zone of 21.26 mm, indicating superior antifungal activity, while 0.12% Chlorhexidine exhibited a mean inhibition zone of 12.53 mm, indicating moderate activity against the *Candida albicans* group. The placebo tablet solution (T.P), distilled water (-S), and Polident® solution (+P) showed no sensitivity, with all values being zero across the three observations. These findings align with previous study, confirming that novel eugenol-based denture cleanser tablet is a superior antifungal agent similar to clove - mediated denture cleansing granules (Ezhilarasan & Subha, 2019).

For the *Streptococcus mutans* group, the novel eugenol-based denture cleanser solution showed a mean inhibition zone of 8.20 mm, classifying it as moderately active. In contrast, the 0.12% Chlorhexidine displayed a s higher inhibition zone, with a mean of 19.79 mm, indicating superior antibacterial potential. The novel eugenol-based denture cleanser solution exhibited comparable antibacterial properties to 0.12% Chlorhexidine against *Streptococcus mutans*. However, it's noteworthy that at a 12.5% concentration, biological eugenol, obtained through hydro-distillation of S. aromaticum, demonstrated an inhibition zone of 16 mm while chemical eugenol derived from commercial sources, did not exhibit any antibacterial effect on *Streptococcus mutans* in a previous study,

suggesting its specific effectiveness against *Streptococcus mutans* based on source of eugenol (Fatene et al., 2021).

For the *Escherichia coli* group, the novel eugenol-based denture cleanser solution exhibited a mean inhibition zone of 9.21 mm, indicating moderate antibacterial activity. The 0.12% Chlorhexidine showed a mean inhibition zone of 14.21 mm, demonstrating superior antibacterial potential. In comparison, the commercially available Polident® solution had a mean inhibition zone of 6.66 mm, indicating it was inactive against *Escherichia coli*. *Escherichia coli* a gram-negative bacterium, has a hydrophilic cell wall made of lipopolysaccharide that blocks the penetration of hydrophobic oil components (Burt, 2004; Zinoviadou et al., 2009). Previous study has highlighted that gram-negative bacteria are generally more resistant than gram-positive bacteria because of their complex double-layer cell membrane, unlike the single membrane in gram-positive bacteria (Ribeiro-Santos et al., 2017). In contrast to this statement, novel eugenol-based denture cleanser solution exhibited comparable antibacterial properties to 0.12% Chlorhexidine with a higher mean of inhibition zone for *Escherichia coli* than *Streptococcus mutans* suggesting its high potential in antibacterial efficiency.

The standard deviation patterns observed in the study indicate that the novel eugenol-based denture cleansing solution (T.E) generally exhibited higher variability compared to the other cleansers. Specifically, for the *Candida albicans* group, the eugenol-based solution had a standard deviation of 2.22, higher than the 0.12% Chlorhexidine solution of 1.36. In the case of *Streptococcus mutans* group, the novel eugenol-based denture cleansing solution (T.E) had a standard deviation of 0.55, while the 0.12% Chlorhexidine solution showed much lower variability of 0.13. For *Escherichia coli* group, the novel eugenol-based denture cleansing solution (T. E) had a standard deviation of 2.18, higher than both the 0.12% Chlorhexidine solution (0.55) and

the Polident® solution (0.45). The small sample size of only three observations makes the standard deviation more sensitive to maximum and minimum values, leading to a less precise estimation of true variability. Additionally, variations in experimental conditions, such as measurement errors by a single operator, can contribute to higher variability in the data.

However, comparing results in published articles on the antimicrobial effects of these natural products is often difficult due to variations in non-standardised approaches, such as inoculum preparation techniques, inoculum size, growth medium, incubation conditions, and endpoint determination (Balouiri et al., 2016).

Besides that, due to the qualitative method of the agar well diffusion test, distinguishing between resistant strains and dose-dependent strains is difficult (Magaldi et al., 2004). In contrast, the National Committee for Clinical Laboratory Standards (NCCLS) micro and macro broth dilution methods are quantitative techniques that enable clear differentiation between these strains (Magaldi et al., 2004).

5.1 Limitation of the study

Firstly, the study could have profited from using eugenol in powder form, which would have allowed for the production of novel tablets with a variety of formulations and concentrations. This flexibility is crucial for a more thorough investigation into the optimal composition needed for maximum efficacy. By limiting the study to a single formulation, the full potential of eugenol in different concentrations and combinations was not explored.

Secondly, the research was constrained by the use of a single funnel size,10mm during the production of the tablets due to its only availability. A larger funnel size would have enabled the creation of tablets of varying sizes, facilitating the comparison of

different formulations and concentrations more effectively. This limitation hindered the ability to investigate how tablet size might impact the overall effectiveness of the tablet formulation.

In addition, the study primarily utilised basic analytical methods in assessing the chemical properties of the novel eugenol-based denture cleansing tablet. Incorporating more sophisticated techniques such as High-Performance Liquid Chromatography (HPLC) along with UV spectroscopy would have provided a more in-depth analysis and greater accuracy in determining the eugenol concentration within the tablets. Such advanced methods would enhance the reliability of the results and provide more detailed insights into the composition of the tablets.

Moreover, the antimicrobial evaluation was conducted on a limited range of microbial strains. Testing a broader spectrum of microbes, particularly those that closely mimic the oral environment and biofilm formation on denture surfaces, would yield more comprehensive insights into the effectiveness of the eugenol-based tablets. This would also provide a better understanding of how these tablets perform under realistic conditions.

Additionally, the in vitro nature of the study needs to fully capture the complexities of the oral environment. To study the antimicrobial effect of an agent in greater depth, time-kill tests and flow cytofluorometric methods are recommended, as they provide insights into the nature of the inhibitory effect (bactericidal or bacteriostatic), whether it is time-dependent or concentration-dependent, and the extent of cell damage inflicted on the test microorganism (Balouiri et al., 2016). In vivo testing is necessary to assess patient adaptation and potential side effects on the denture base's surface roughness or colour over time. This would provide more realistic and applicable results, ensuring the findings are relevant to real-world use.

Lastly, the study's small sample size limits the statistical power and generalisability of the findings. A larger sample size would allow for more robust statistical analysis, yielding more valid and reliable data. This would enhance the credibility of the results and support the development of more effective eugenol-based denture cleansers.

By addressing these limitations in future research, the findings can be validated and expanded, leading to a more comprehensive understanding of the potential and efficacy of eugenol-based denture cleansers.

CHAPTER 6: CONCLUSION

The following conclusions are made:

1. This study introduces a novel approach to denture care by producing an innovative eugenol-based denture cleanser tablet. Overall, the novel eugenol-based denture cleanser tablet was acceptable in maintaining most physicochemical parameters within the limits. Although the hardness of the tablet was lower than ideal, the tablet met the standards for thickness, pH and effervescence time. The eugenol concentration of 5.294% in the tablet was deemed acceptable for topical use after rinsing, aligning with safety guidelines.
2. The novel eugenol-based denture cleanser tablet demonstrated promising antifungal activity against *Candida albicans* and moderate antibacterial activity against *Streptococcus mutans* and *Escherichia coli*. This exhibits its potential as an effective denture cleanser compared to 0.12% Chlorhexidine and commercially available solutions like Polident®.

REFERENCES

Abdou, A., Elmakssoudi, A., El Amrani, A., JamalEddine, J., & Dakir, M. (2021). Recent advances in chemical reactivity and biological activities of eugenol derivatives. *Medicinal Chemistry Research*, *30*, 1011-1030.

Abuhajar, E., Ali, K., Zulfiqar, G., Al Ansari, K., Raja, H. Z., Bishti, S., & Anweigi, L. (2023). Management of Chronic Atrophic Candidiasis (Denture Stomatitis)-A Narrative Review. *Int J Environ Res Public Health*, *20*(4).

Aguayo, S., Marshall, H., Pratten, J., Bradshaw, D., Brown, J. S., Porter, S. R., Spratt, D., & Bozec, L. (2017). Early Adhesion of Candida albicans onto Dental Acrylic Surfaces. *J Dent Res*, *96*(8), 917-923.

Alpar, B., Günay, H., Geurtsen, W., & Leyhausen, G. (1999). Cytocompatibility of periodontal dressing materials in fibroblast and primary human osteoblast-like cultures. *Clin Oral Investig*, *3*(1), 41-48.

Aminuddin, N. I., & Azhar, M. A. (2023). Effect of glidant in the formulation of goat milk tablet. *Materials Today: Proceedings*.

Aslani, A., & Fattahi, F. (2013). Formulation, characterization and physicochemical evaluation of potassium citrate effervescent tablets. *Adv Pharm Bull*, *3*(1), 217-225.

Aslani, A., & Jahangiri, H. (2013). Formulation, characterization and physicochemical evaluation of ranitidine effervescent tablets. *Adv Pharm Bull*, *3*(2), 315-322.

Axe, A., Burnett, G. R., Milleman, K. R., Patil, A., & Milleman, J. L. (2019). Randomized Controlled Clinical Study to Determine the Oral and Dermal Tolerability of an Experimental Denture Wipe. *J Prosthodont*, *28*(2), 138-145.

Baena-Monroy, T., Moreno-Maldonado, V., Franco-Martínez, F., Aldape-Barrios, B., Quindós, G., & Sánchez-Vargas, L. O. (2005). Candida albicans, Staphylococcus aureus and Streptococcus mutans colonization in patients wearing dental prosthesis. *Med Oral Patol Oral Cir Bucal*, *10 Suppl1*, E27-39.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal*, *6*(2), 71-79.

Bamford, C. V., d'Mello, A., Nobbs, A. H., Dutton, L. C., Vickerman, M. M., & Jenkinson, H. F. (2009). Streptococcus gordonii modulates Candida albicans biofilm formation through intergeneric communication. *Infect Immun*, *77*(9), 3696-3704.

Baumgartner, W., Schimmel, M., & Müller, F. (2015). Oral health and dental care of elderly adults dependent on care. *Swiss Dent J*, *125*(4), 417-426.

Bilhan, H., Sulun, T., Erkose, G., Kurt, H., Erturan, Z., Kutay, O., & Bilgin, T. (2009). The role of Candida albicans hyphae and Lactobacillus in denture-related stomatitis. *Clin Oral Investig*, *13*(4), 363-368.

Brondani, M. A., & Siqueira, A. R. (2018). A critical review of protocols for conventional microwave oven use for denture disinfection. *Community Dent Health*.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*, *94*(3), 223-253.

Carrasco, H., Raimondi, M., Svetaz, L., Di Liberto, M., Rodriguez, M. V., Espinoza, L., Madrid, A., & Zacchino, S. (2012). Antifungal activity of eugenol analogues. Influence of different substituents and studies on mechanism of action. *Molecules*, *17*(1), 1002-1024.

Cavalcanti, Y. W., Morse, D. J., da Silva, W. J., Del-Bel-Cury, A. A., Wei, X., Wilson, M., Milward, P., Lewis, M., Bradshaw, D., & Williams, D. W. (2015). Virulence and pathogenicity of Candida albicans is enhanced in biofilms containing oral bacteria. *Biofouling*, *31*(1), 27-38.

Cavalheiro, M., & Teixeira, M. C. (2018). Candida Biofilms: Threats, Challenges, and Promising Strategies. *Front Med (Lausanne)*, *5*, 28.

Chittaranjan, B. (2011). Material and methods for cleaning the dentures. *Indian Journal of Dental Advancements*, *3*(1), 423-427.

da Silva, F. F. M., Monte, F. J. Q., de Lemos, T. L. G., do Nascimento, P. G. G., de Medeiros Costa, A. K., & de Paiva, L. M. M. (2018). Eugenol derivatives: synthesis, characterization, and evaluation of antibacterial and antioxidant activities. *Chem Cent J*, *12*(1), 34.

de Andrade, I. M., Cruz, P. C., da Silva, C. H., de Souza, R. F., Paranhos Hde, F., Candido, R. C., Marin, J. M., & de Souza-Gugelmin, M. C. (2011). Effervescent tablets and ultrasonic devices against Candida and mutans streptococci in denture biofilm. *Gerodontology*, *28*(4), 264-270.

de Andrade, I. M., Cruz, P. C., Silva-Lovato, C. H., de Souza, R. F., Souza-Gugelmin, M. C., & Paranhos Hde, F. (2012). Effect of chlorhexidine on denture biofilm accumulation. *J Prosthodont*, *21*(1), 2-6.

de Castellucci Barbosa, L., Ferreira, M. R., de Carvalho Calabrich, C. F., Viana, A. C., de Lemos, M. C., & Lauria, R. A. (2008). Edentulous patients' knowledge of dental hygiene and care of prostheses. *Gerodontology*, *25*(2), 99-106.

de Sousa Porta, S. R., de Lucena-Ferreira, S. C., da Silva, W. J., & Del Bel Cury, A. A. (2015). Evaluation of sodium hypochlorite as a denture cleanser: a clinical study. *Gerodontology*, *32*(4), 260-266.

de Souza, R. F., de Freitas Oliveira Paranhos, H., Lovato da Silva, C. H., Abu-Naba'a, L., Fedorowicz, Z., & Gurgan, C. A. (2009). Interventions for cleaning dentures in adults. *Cochrane Database Syst Rev*(4), Cd007395.

de Souza, R. F., Khiyani, M. F., Chaves, C. A. L., Feine, J., Barbeau, J., Fuentes, R., Borie, E., Crizostomo, L. C., Silva-Lovato, C. H., Rompre, P., & Emami, E. (2017). Improving practice guidelines for the treatment of denture-related

erythematous stomatitis: a study protocol for a randomized controlled trial. *Trials*, *18*(1), 211.

Deshpande, A., Verma, S., & Macwan, C. (2014). Allergic Reaction Associated with the use of Eugenol Containing Dental Cement in a Young Child. Austin J Dent. 2014; 1 (2): 1007. *Austin J Dent*, *1*(2-2014).

Devi, K. P., Nisha, S. A., Sakthivel, R., & Pandian, S. K. (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. *J Ethnopharmacol*, *130*(1), 107-115.

Devi, K. P., Sakthivel, R., Nisha, S. A., Suganthy, N., & Pandian, S. K. (2013). Eugenol alters the integrity of cell membrane and acts against the nosocomial pathogen Proteus mirabilis. *Arch Pharm Res*, *36*(3), 282-292.

Dhahir, R. K., & Al-Kotaji, M. (2019). Formulation of orally disintegrating tablets of cinnarizine by using direct compression method. *Int J Appl Pharm*, *11*(1), 117-123.

Dhara, L., & Tripathi, A. (2013). Antimicrobial activity of eugenol and cinnamaldehyde against extended spectrum beta lactamase producing enterobacteriaceae by in vitro and molecular docking analysis. *European Journal of Integrative Medicine*, *5*(6), 527-536.

Diaz, P. I., Xie, Z., Sobue, T., Thompson, A., Biyikoglu, B., Ricker, A., Ikonomou, L., & Dongari-Bagtzoglou, A. (2012). Synergistic interaction between Candida albicans and commensal oral streptococci in a novel in vitro mucosal model. *Infect Immun*, *80*(2), 620-632.

Dikbas, I., Koksal, T., & Calikkocaoglu, S. (2006). Investigation of the cleanliness of dentures in a university hospital. *Int J Prosthodont*, *19*(3), 294-298.

Estrela, C., Estrela, C. R., Barbin, E. L., Spanó, J. C., Marchesan, M. A., & Pécora, J. D. (2002). Mechanism of action of sodium hypochlorite. *Braz Dent J*, *13*(2), 113-117.

Ezhilarasan, D., & Subha, M. (2019). Effervescent denture cleansing granules using clove oil and analysis of its in vitro antimicrobial activity. *Indian Journal of Public Health*, *10*(11), 3687.

Faria, G., Cardoso, C. R., Larson, R. E., Silva, J. S., & Rossi, M. A. (2009). Chlorhexidine-induced apoptosis or necrosis in L929 fibroblasts: A role for endoplasmic reticulum stress. *Toxicol Appl Pharmacol*, *234*(2), 256-265.

Fatene, N., Mounaji, K., & Soukri, A. (2021). Effect of Eugenol on Streptococcus mutans Adhesion on NiTi Orthodontic Wires: In Vitro and In Vivo Conditions. *European Journal of General Dentistry*, *10*(03), 151-157.

Filgueiras, C. T., & Vanetti, M. C. D. (2006). Effect of eugenol on growth and listeriolysin O production by Listeria monocytogenes. *Brazilian archives of biology and technology*, *49*, 405-409.

Gad, M. M., & Fouda, S. M. (2020). Current perspectives and the future of Candida albicans-associated denture stomatitis treatment. *Dent Med Probl*, *57*(1), 95-102.

Gendreau, L., & Loewy, Z. G. (2011). Epidemiology and etiology of denture stomatitis. *J Prosthodont*, *20*(4), 251-260.

Harrison, Z., Johnson, A., & Douglas, C. W. (2004). An in vitro study into the effect of a limited range of denture cleaners on surface roughness and removal of Candida albicans from conventional heat-cured acrylic resin denture base material. *J Oral Rehabil*, *31*(5), 460-467.

Hundal, M., & Madan, R. (2015). Comparative clinical evaluation of removable partial dentures made of two different materials in Kennedy Applegate class II partially edentulous situation. *Med J Armed Forces India*, *71*(Suppl 2), S306-312.

Ipci, K., Öktemer, T., Birdane, L., Altintoprak, N., Muluk, N. B., Passali, D., Lopatin, A., Bellussi, L., Mladina, R., & Pawankar, R. (2016). Effervescent tablets: a safe and practical delivery system for drug administration. *ENT updates*, *6*(1), 46.

Iqbal, N., Agrawal, A., & Kumar, J. (2021). Development of effervescent tablet formulation for rapid control of mosquito problem in early stages from different breeding sites. *Arabian Journal of Chemistry*, *14*(4), 103082.

Iyer, M., Gujjari, A. K., Gowda, V., & Angadi, S. (2017). Antifungal response of oral-associated candidal reference strains (American Type Culture Collection) by supercritical fluid extract of nutmeg seeds for geriatric denture wearers: An: in vitro: screening study. *The Journal of Indian Prosthodontic Society*, *17*(3), 267-272.

Jadhav, B. K., Khandelwal, K. R., Ketkar, A. R., & Pisal, S. S. (2004). Formulation and evaluation of mucoadhesive tablets containing eugenol for the treatment of periodontal diseases. *Drug Dev Ind Pharm*, *30*(2), 195-203.

Jagtap, V., & Paradkar, O. (2021). Method Development and Validation of Estimation of Eugenol in Herbal Formulation. *International Journal of Pharmaceutical Research and Applications*.

Katsikogianni, M., & Missirlis, Y. F. (2004). Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. *Eur Cell Mater*, *8*, 37-57.

Kaypetch, R., Rudrakanjana, P., Tua-Ngam, P., Tosrisawatkasem, O., Thairat, S., Tonput, P., & Tantivitayakul, P. (2023). Effects of two novel denture cleansers on multispecies microbial biofilms, stain removal and the denture surface: an in vitro study. *BMC Oral Health*, *23*(1), 852.

Koo, H., Andes, D. R., & Krysan, D. J. (2018). Candida-streptococcal interactions in biofilm-associated oral diseases. *PLoS Pathog*, *14*(12), e1007342.

Kossioni, A. E. (2011). The prevalence of denture stomatitis and its predisposing conditions in an older Greek population. *Gerodontology*, *28*(2), 85-90.

Kulak-Ozkan, Y., Kazazoglu, E., & Arikan, A. (2002). Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. *J Oral Rehabil*, *29*(3), 300-304.

Kumar, B., Sandhu, P. K., Kumar, A. N., & Patil, C. P. (2017). A comparative study for plaque removing efficacy between commonly used denture cleansers in India. *J Indian Prosthodont Soc*, *17*(3), 295-300.

Kumar, M. N., Thippeswamy, H. M., Raghavendra Swamy, K. N., & Gujjari, A. K. (2012). Efficacy of commercial and household denture cleansers against Candida albicans adherent to acrylic denture base resin: an in vitro study. *Indian J Dent Res*, *23*(1), 39-42.

Latifah-Munirah, B., Himratul-Aznita, W., & Mohd Zain, N. (2015). Eugenol, an essential oil of clove, causes disruption to the cell wall of Candida albicans (ATCC 14053). *Frontiers in Life Science*, *8*(3), 231-240.

Lee, H.-E., Li, C.-Y., Chang, H.-W., Yang, Y.-H., & Wu, J.-H. (2011). Effects of different denture cleaning methods to remove Candida albicans from acrylic resin denture based material. *Journal of Dental Sciences - J DENT SCI*, *6*.

Lucena-Ferreira, S. C., Cavalcanti, I. M., & Cury, A. A. (2013). Efficacy of denture cleansers in reducing microbial counts from removable partial dentures: a short-term clinical evaluation. *Braz Dent J*, *24*(4), 353-356.

Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *Int J Infect Dis*, *8*(1), 39-45.

Mantri, S. S., Parkhedkar, R. D., & Mantri, S. P. (2013). Candida colonisation and the efficacy of chlorhexidine gluconate on soft silicone-lined dentures of diabetic and non-diabetic patients. *Gerodontology*, *30*(4), 288-295.

Marchese, A., Barbieri, R., Coppo, E., Orhan, I. E., Daglia, M., Nabavi, S. F., Izadi, M., Abdollahi, M., Nabavi, S. M., & Ajami, M. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. *Crit Rev Microbiol*, *43*(6), 668-689.

Marcos-Arias, C., Eraso, E., Madariaga, L., & Quindós, G. (2011). In vitro activities of natural products against oral Candida isolates from denture wearers. *BMC Complement Altern Med*, *11*, 119.

McReynolds, D. E., Moorthy, A., Moneley, J. O., Jabra-Rizk, M. A., & Sultan, A. S. (2023). Denture stomatitis-An interdisciplinary clinical review. *J Prosthodont*, *32*(7), 560-570.

Monrle, R. (1980). Effervescent tablet in: Liberman HA, Lachman I, Schwartz J. Pharmaceutical dosage form: tablets. In: New York: Marcel Dekker Inc.

Moon, S. E., Kim, H. Y., & Cha, J. D. (2011). Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. *Arch Oral Biol*, *56*(9), 907-916.

Mylonas, P., Milward, P., & McAndrew, R. (2022). Denture cleanliness and hygiene: an overview. *Br Dent J*, *233*(1), 20-26.

Nikawa, H., Hamada, T., & Yamamoto, T. (1998). Denture plaque--past and recent concerns. *J Dent*, *26*(4), 299-304.

Nisar, M. F., Khadim, M., Rafiq, M., Chen, J., Yang, Y., & Wan, C. C. (2021). Pharmacological Properties and Health Benefits of Eugenol: A Comprehensive Review. *Oxid Med Cell Longev*, *2021*, 2497354.

Nishi, Y., Seto, K., Kamashita, Y., Kaji, A., Kurono, A., & Nagaoka, E. (2014). Survival of microorganisms on complete dentures following ultrasonic cleaning combined with immersion in peroxide-based cleanser solution. *Gerodontology*, *31*(3), 202-209.

O, A. H., Mehdawi, N., Abu Taha, A., E, M. H., M, A. A.-N., & A, S. H. (2013). Synthesis and antibacterial activity of novel curcumin derivatives containing heterocyclic moiety. *Iran J Pharm Res*, *12*(1), 47-56.

Opdyke, D. L. (1975). Monographs on fragrance raw materials. *Food Cosmet Toxicol*, *13*(4), 449-457.

Palanisamy, P., Abhishekh, R., & Yoganand Kumar, D. (2011). Formulation and evaluation of effervescent tablets of aceclofenac. *Int Res J Pharm*, *2*(12), 185-190.

Papadiochou, S., & Polyzois, G. (2018). Hygiene practices in removable prosthodontics: A systematic review. *Int J Dent Hyg*, *16*(2), 179-201.

Patel, S. G., & Siddaiah, M. (2018). Formulation and evaluation of effervescent tablets: a review. *Journal of Drug Delivery and Therapeutics*, *8*(6), 296-303.

Preethi, G., Banerjee, S., Shivakumar, H., & Ravi Kumar, M. (2017). Formulation of fast-dissolving tablets of doxazosin mesylate drug by direct compression method. *Int J Appl Pharm*, *9*, 22-28.

Redfern, J., Tosheva, L., Malic, S., Butcher, M., Ramage, G., & Verran, J. (2022). The denture microbiome in health and disease: an exploration of a unique community. *Lett Appl Microbiol*, *75*(2), 195-209.

Ribeiro-Santos, R., Andrade, M., de Melo, N. R., dos Santos, F. R., de Araújo Neves, I., de Carvalho, M. G., & Sanches-Silva, A. (2017). Biological activities and major components determination in essential oils intended for a biodegradable food packaging. *Industrial crops and products*, *97*, 201-210.

Rosan, B., & Lamont, R. J. (2000). Dental plaque formation. *Microbes Infect*, *2*(13), 1599-1607.

Salles, M. M., Badaró, M. M., Arruda, C. N., Leite, V. M., Silva, C. H., Watanabe, E., Oliveira Vde, C., & Paranhos Hde, F. (2015). Antimicrobial activity of complete denture cleanser solutions based on sodium hypochlorite and Ricinus communis - a randomized clinical study. *J Appl Oral Sci*, *23*(6), 637-642.

Sarrami, N., Pemberton, M. N., Thornhill, M. H., & Theaker, E. D. (2002). Adverse reactions associated with the use of eugenol in dentistry. *Br Dent J*, *193*(5), 257-259.

Scully, C., & Felix, D. H. (2005). Oral medicine--update for the dental practitioner: red and pigmented lesions. *Br Dent J*, *199*(10), 639-645.

Senna, P. M., Vieira, A. P. C., Sotto-Maior, B. S., Silva, W. J. d., & Del Bel Cury, A. A. (2011). Influence of immersion time of denture cleansers on the surface roughness of resilient denture liners. *Revista Odonto Ciência*, *26*, 35-39.

Senpuku, H., Sogame, A., Inoshita, E., Tsuha, Y., Miyazaki, H., & Hanada, N. (2003). Systemic diseases in association with microbial species in oral biofilm from elderly requiring care. *Gerontology*, *49*(5), 301-309.

Siqueira, J. F., Jr., Favieri, A., Gahyva, S. M., Moraes, S. R., Lima, K. C., & Lopes, H. P. (2000). Antimicrobial activity and flow rate of newer and established root canal sealers. *J Endod*, *26*(5), 274-277.

Sjögren, P., Wårdh, I., Zimmerman, M., Almståhl, A., & Wikström, M. (2016). Oral Care and Mortality in Older Adults with Pneumonia in Hospitals or Nursing Homes: Systematic Review and Meta-Analysis. *J Am Geriatr Soc*, *64*(10), 2109-2115.

Stahl, H. (2003). Effervescent dosage manufacturing. *Pharmaceutical Technology Europe*, *15*(4).

Sumi, Y., Miura, H., Sunakawa, M., Michiwaki, Y., & Sakagami, N. (2002). Colonization of denture plaque by respiratory pathogens in dependent elderly. *Gerodontology*, *19*(1), 25-29.

Swamy, K., Alla, R. K., Mohammed, S., & Konakanchi, A. (2018). The role of antifungal agents in treating denture stomatitis. *Research Journal of Pharmacy and Technology*, *11*(4), 1365-1369.

Tarib, N., Gan, P., Tan, M. F., & Ahmad, M. (2018). Effect of alkaline peroxide-type denture cleaners to the microbial profile in maxillary complete denture. *Journal of Dentistry Indonesia*, *25*(1), 1-5.

Thein, Z. M., Seneviratne, C. J., Samaranayake, Y. H., & Samaranayake, L. P. (2009). Community lifestyle of Candida in mixed biofilms: a mini review. *Mycoses*, *52*(6), 467-475.

Valgas, C., Souza, S. M. d., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*, *38*, 369-380.

Walsh, S. E., Maillard, J. Y., Russell, A. D., Catrenich, C. E., Charbonneau, D. L., & Bartolo, R. G. (2003). Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. *J Appl Microbiol*, *94*(2), 240-247.

WH, H.-A., Mohd-Al-Faisal, N., & Fathilah, A. (2011). Determination of the percentage inhibition of diameter growth (PIDG) of Piper betle crude aqueous extract against oral Candida species. *Journal of Medicinal Plants Research*, *5*(6), 878-884.

Yadav, R., Yadav, V. S., Garg, S., Mittal, S., & Garg, R. (2013). Effectiveness of different denture cleansing methods on removal of biofilms formed in vivo. *J Cranio Max Dis*, *2*(1), 22-27.

Yildirim-Bicer, A. Z., Peker, I., Akca, G., & Celik, I. (2014). In vitro antifungal evaluation of seven different disinfectants on acrylic resins. *Biomed Res Int*, *2014*, 519098.

Zain, R. B., Ikeda, N., Razak, I. A., Axéll, T., Majid, Z. A., Gupta, P. C., & Yaacob, M. (1997). A national epidemiological survey of oral mucosal lesions in Malaysia. *Community Dent Oral Epidemiol*, *25*(5), 377-383.

Zanul Abidin, Z., Mohd Salleh, N., Himratul-Aznita, W. H., Ahmad, S. F., Lim, G. S., Raja Mohd, N., & Dziaruddin, N. (2023). Antifungal effects of eugenol on Candida albicans adherence to denture polymers. *PeerJ*, *11*, e15750.

Zhang, L. W., Fu, J. Y., Hua, H., & Yan, Z. M. (2016). Efficacy and safety of miconazole for oral candidiasis: a systematic review and meta-analysis. *Oral Dis*, *22*(3), 185-195.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Sci*, *82*(3), 338-345.