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Formulation and Assessment of Tooth Spray

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Formulation and Assessment of Tooth Spray

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Formulation and Assessment of Tooth Spray

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Dedication

I dedicate this work to my beloved family, whose unwavering support, encouragement, and love have been my greatest strength throughout this journey.

Especially to my dear father, who devoted his life to me and my education.Your sacrifices and endless care have been the foundation of everything I've achieved.

To my dear friends, thank you for standing by me with patience, motivation, and faith.

Your presence has made this achievement meaningful.

Declaration

I certify that this thesis which is submitted to the Deanship of Graduate Studies to get the degree of master in on filed Nursing Management, this is my own research and my own work and it doesn't submit to any other universities or any institutions.

Bashaer Zaher Mustafa Abd Nabi

Signed: 

Date: 2025/4/26

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Abstract

Nowadays, essential oils are commonly utilized to treat and prevent a number of conditions linked to dental illness, such as tooth decay, gingivitis, and halitosis.

The purpose of this study was to develop and assess a tooth spray in a novel formulation and dosage form, as well as to examine the antibacterial qualities of essential oils and herbal tooth sprays against microorganisms that cause dental disease. Three essential oils clove, peppermint, and sage were used as active components in equal amounts to create a dental spray formulation for this investigation. In order to guarantee stability and usefulness, the formulation also contained the proper excipients. To determine the product's quality and efficacy, a number of tests were carried out, including stability, chemical, physical, and antibacterial ones. The outcomes confirmed the dental spray's acceptability as a natural oral care product by showing that it is stable, homogenous, effective, and safe to use at room temperature. Because of its stability, ease of use, and ability to transport active chemicals straight to the mouth cavity, a dental spray was selected as the dosage form. Compared to alternative dose forms like rinses or gels, sprays offer a quicker start of action, consistent dispersion throughout the targeted region, and better patient compliance. Additionally, this approach minimizes systemic exposure and any adverse effects by enabling targeted therapy. Tooth spray was formulated as a new dosage form not available in the market. The studies suggested acceptability criteria, which include being consistent, flexible, non-sticky, and devoid of odors and stains, can be met by tooth sprays. The sprayed component exhibits antibacterial and antifungal properties, according to incubation-based microbiological investigations.
When the pH of the mouth starts to fall below 5.5, tooth decay may result. In tooth spray, bases are used to counteract oral acid. The tooth spray was therefore created with a pH of 8.5. The stability study results confirmed the spray's effectiveness based on the comparison between zero-time data and the results obtained after two months.

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Abbreviation

Mm	millimeter
µl	microliter
McF	MacFarland
NaCl	Sodium chloride
CEO	Clove essential oil
PEO	Peppermint essential oil
SEO	Sage essential oil
W/v	Weight /volume
ρ	Density
Conc	Concentration
η	Viscosity
V	Sample volume
NaHCO3	Sodium Bicarbonate
D	Density
T	Time
Cps	centipoise
DMSO	Dimethyl Sulfoxide
MHB	Mueller Hinton Broth
^{0}C	Celsius
Hr	Hour
W/V	Weight/ volume
#	Number
EO	Essential Oil
TDL0	Toxic Dose low
H2SO4	Sulfuric Acid
PPM	Parts Per Million
PTFs	Primary trial formulations
STFs	Successful trial formulations
QC	Quality Control
RH	Relative Humidity
McF	McFarland Standard
ZOI	Zone of Inhibition
GBD	Global Burden of Disease
WHO	World Health Organization
H2S	Hydrogen Sulfide
NH3	Ammonia
pH	Potential of Hydrogen
LDH	Lactate Dehydrogenase
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic Acid
DCFH-DA	2, 7-Dichlorodihydrofluorescein Diacetate
GC-MS	Gas chromatography-mass spectrometry
TEM	Transmission electron microscopy
LD50	Lethal Dose 50
DEJ	Dentine-enamel junction
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
EOCs	Essential oil components
GC	Gas chromatography
SEM	Scanning electron microscope
GC/FID	Gas Chromatography Flame Ionization Detector

RH	Relative Humidity
SI	Scientific Instruments
H2O2	Hydrogen peroxide
CO_2	Carbon Dioxide
EOs	Essential Oil

Chapter One:

Introduction:

One major challenge in creating a patient-friendly dosage form is the palatability of a drug's active component. When choosing a medication from the many generic versions on the market that have the same active ingredient, organoleptic qualities such as taste and odor and natural rather than chemical products are crucial considerations. The majority of people prefer to use natural components when choosing between buying chemical or natural oral care products. When tooth enamel demineralizes more quickly than it remineralizes, dental decay occurs. Acidogenic bacteria, which consume carbohydrates and generate weak organic acids, are the main cause of demineralization. When plaque builds up on a tooth's surface, it transforms free sugars into acids that gradually erode the tooth, causing dental caries. Natural tooth care items could reduce exposure to chemicals: Avoiding artificial coloring, flavoring, and preservatives lowers the amount of chemicals the person is exposed to. Sustainable Choices: Many companies prioritize eco-friendly packaging and sustainable sourcing in order to attract environmentally conscious consumers (Koch & Graetz, 2021). Considering the analogy between turbulence generation caused by the collapse of the cavitation bubbles inside the injection nozzle and acoustic atomization, the ratio of the nozzle length to its diameter is an important factor influencing the form and placement of nozzle geometrical configurations. Tooth sprays reach all parts of the teeth and areas that cannot be brushed if using the traditional method to clean teeth due to direct contact with teeth and high flow rate. The agreement between the predicted droplet size and the corresponding measurements in the developed spray has validated the validity of the predicted probability density function for the initial droplet distribution. Achieving the right atomization is essential to fulfilling application requirements. The properties and design of the atomization mechanism, as well as the atomized liquid, all influence one another. Understanding and, consequently, being able to simulate the atomization process is essential to fine-tuning the atomization mechanism and generating the appropriate atomization properties (Mohamed et al., 2021). One of the most widely used atomization techniques is mechanical atomization, which entails applying pressure to the liquid against a tiny nozzle. Understanding this basic atomization process can help one to better understand the process of creating sprays (Kramm et al., 2023). The

creation of a tooth spray as a novel cosmetic oral hygiene product is the goal of this thesis. Some studies indicate that erosion is increasing across all age groups, with the highest frequency occurring in children and teenagers (National Institute of Dental and Craniofacial Research, 2021). It was suggested that this was due to the recent increase in consumption of fruit juices, soft drinks, and power drinks. Cola is one of the most popular acidic drinks, with a pH of 6.3, the lowest of the foods and drinks they examined (Polyakova et al., 2024). Plots showing the pH reading shift following the rinse are known as "Stephan curves." Its well-known erosive effect on teeth causes the most change in the tooth's surface hardness. Following an initial decline, the acid pH measurements progressively returned to baseline levels. The decrease in plaque pH to below 5.5-5.7, which was believed to be the acidity threshold necessary for enamel demineralization, was very significant (Bowen, 2013). According to the clinical stage of caries, which included aggressive, advanced lesions, apparent lesions, caries-free, and small caries, there were notable differences in plaque acid responses. The post-glucose rinse's acidity remained above pH 5.5–5.7 in cavity-free individuals, suggesting that the enamel had not demineralized (Bowen, 2013). In contrast, aggressive caries had lower initial resting plaque acidity than was necessary for demineralization, and after the glucose challenge, it further dropped to less than a pH of 4 (Spatafora et al., 2024). America's use of sweetened beverages has increased dramatically over the last 35 years, with carbonated soft drinks being the most popular. Children, teens, and young adults are the main consumers of these beverages (Reddy et al., 2016). This is a significant issue for public health (Pitts et al., 2017). Oral diseases are caused by a variety of causes, such as smoking cigarettes, drinking alcohol, and consuming sugary foods (Chi & Scott, 2019). Early treatment and prevention are possible for most oral disorders. To take care of the teeth, products are employed. Some of the characteristics of tooth spray, such as its ease of use and convenient pump action nozzle, make it a viable option for dental issues. Additionally, it creates an antimicrobial barrier that shields the teeth and gums. It can be applied directly to the gums and teeth by teeth. Because of its spray action, it targets particular parts of the mouth that could be hard to reach otherwise. Use essential oils to protect tooth enamel without the negative effects of fluoride. The prevalence and severity of oral diseases are continuously correlated with socioeconomic status (income, occupation, and educational attainment) (Almajed et al., 2024). Over the course of their lives, oral diseases and afflictions disproportionately affect the poor and vulnerable members of society, which frequently include those with low incomes, people with disabilities, refugees, and socially excluded groups. Dental cavities are more common in children with special needs, including those with autism, cerebral palsy, and disabilities (Almajed et al., 2024). Since essential oils (EOs) are holistic, integrative approaches to well-established medical therapies, many Americans have been using them in place of other prescription medications during the past 10 years (Amorati et al., 2013). Numerous essential oils and their constituents have been researched for their antibacterial properties against specific bacteria and fungi (de Sousa et al., 2023). Essential oils typically consist of 20 to 60 different compounds in different ratios, while some may have more than 300 different chemicals. However, compared to other components that are present in trace amounts, two or three components are usually found in greater amounts (20–70%). Essential oil constituents have distinct basic metabolic precursors and are made through a variety of biosynthetic processes. They can be divided into two main groups: terpenoids (the most common type) and non-terpenoids (mostly phenylpropanoid). They can be in many different forms and are all hydrocarbons and their oxygenated derivatives. The chemical classes include oxides, esters, amines, amides, phenols, aldehydes, ketones, alcohols, nitrogen and sulfur compounds, and heterocyclic (de Sousa et al., 2023). Essential oils, sometimes referred to as ethereal or volatile oils, are naturally occurring volatile organic molecules that are produced in significant quantities from the raw materials

of plants or their organs, such as flowers, seeds, buds, and leaves, as well as from twigs, roots, bark, lumber, and fruits. A mixture of more than 300 distinct compounds may be present in these oils. Usually, their molecular weight is under 300 Da. These chemicals can be classified chemically into a number of classes, such as amines, alcohols, phenols, ethers, and carbonyl compounds, including aldehydes, ketones, amides, and esters. Among the chemical constituents of essential oils are terpenes and Phenylpropanoids (Masyita & Sari, 2022). If kept in dark, closed glass bottles away from light and heat, many essential oils will survive for two to five years. Increasing the oils' shelf life is quite easy, just follow the following instructions: Keep them sealed and store them in dark amber bottles. Keep them out of direct sunshine and heat sources (Xu et al., 2021).

1.1 Oral health

We learn early on that maintaining healthy teeth is crucial to overall oral health. Brushing and flossing are the fundamental routines that keep our "pearly whites" in good condition, but oral health is much more than just having clean teeth (Peres et al., 2019). The condition of the gums, teeth, and entire oral-facial region—which enables speech, smiles, and chewing—is referred to as oral health. Tooth decay, dry mouth, mouth ulcers, tooth erosion, tooth discoloration, and periodontal disease comprise the bulk of disorders affecting the oral cavity (Peres et al., 2019). Factors that contribute to tooth caries in general, include poor brushing practices, sugar-containing medications, sweet drinks, and foods that stimulate bacterial growth and produce acid that erodes tooth enamel; when decayed teeth occur, bacteria are produced in the mouth, acid builds up on the surface of teeth, and it can cause pain and inflammation (National Institute of Dental and Craniofacial Research, 2021). Bacteria in meals turn sugar into acids that can lead to tooth decay. Cavities and tooth decay are the world's most significant health issues. It affects adults, children, and teenagers (National Institute of Dental and Craniofacial Research, 2021). Cavities are places where your teeth's hard coating has been damaged. These dental decay spots develop into tiny holes that may cause tooth loss and serious cavities. Cavities can be caused by a variety of factors, such as oral bacteria, sugar-containing diets, and poor tooth hygiene. Cavities can damage deeper layers of your teeth and cause major issues if left untreated (Featherstone, 2008). The best defense against cavities is adherence to a daily dental care regimen, which includes brushing your teeth often and seeing a dentist once every six months (National Institute of Dental and Craniofacial Research, 2021**).**

1.1.1. Recent studies on teeth caries

One of the most significant oral health issues in the world is tooth decay. In children, adults, and adolescents, tooth decay is four to five times more common than asthma. 37% of children aged 2 to 8 have primary tooth decay, per the 2011–2012 National Health and Nutrition Examination Survey (Dye et al., 2011). Approximately 58% of teenagers between the ages of 12 and 19 have dental caries in their permanent teeth (Heng, 2016). About 15% of tooth disease in children and adolescents that went untreated spread, while 90% of individuals aged 20 and older had dental caries. Nonetheless, tooth decay was prevalent in 27% of persons aged 20 to 64 who had not had dental caries treated (Heng, 2016). The global burden of sugar-related tooth decay is based on data from 168 nations that provide advanced facts that validate the link between tooth decay and excessive sugar consumption and record the anticipated monetary losses brought on by tooth decay (Meier et al., 2017). In most countries across the world, untreated dental cavities are a major public health hazard. Untreated caries was the most prevalent issue in permanent teeth (Featherstone, 2008).

Of all the conditions evaluated in the GBD 2010 survey, caries in deciduous teeth, which affects 621 million children worldwide, was the tenth most prevalent, affecting 2.4 billion individuals. Furthermore, we discovered that there will be 15 new cases of tooth decay in primary teeth and 27 new cases in permanent teeth annually based on 100 people who were followed up. Dental caries is the fourth most expensive chronic illness to treat, according to the WHO (Petersen 2008). In 2014, dental disease treatment in the US alone was estimated to have cost $122 billion (Petersen 2008).

1.2. Tooth decay Stages

The deterioration of teeth occurs in stages. Plaque, a sticky bacterial film that covers teeth, causes the enamel layer, the tooth's outermost layer, to weaken in the first stage (Featherstone, 2008). If the proper procedures are not followed to avoid tooth decay, plaque turns into tartar. Enamel and dentine degradation, pulp damage, abscess formation, tooth loss, discomfort, and inflammation are the following stages. When dental roots lack the protective enamel coating and decay spreads into enamel cavities, dentine degradation results (Fejerskov, 1997). The four primary layers of teeth are pulp, dentine, cementum, and enamel, enamel is the outermost layer, as seen in **Figure 1**. The toughest part of the dentine is enamel, a translucent coating that covers it. The dentine, a less mineralized layer that preserves the integrity of the enamel, protects both the pulp and the enamel. The enamel and dentine contact line are known as the enamel-dentine junction (DEJ). The pulp of the tooth contains the nerves and blood supply. The Cementum is the layer that covers the dentine in the root section. The intersection of the crown and root is also known as the enamel-cementum (Sarkhouh, 2014).

Figure 1: Structure of teeth (Koch & Graetz, 2021).

1.2.1. Diet and dental caries

One of the most important factors influencing the risk of tooth decay is diet (Fejerskov, 1997). Too little fiber in the diet may be linked to a high tooth decay index; dental decay is caused by eating a lot of carbohydrates. Therefore, the ratio of fiber to carbs in meals is crucial (Palacios et al., 2016). Patients with diabetes are more prone to dental deterioration.

The worst sugar that causes tooth decay is sucrose, which bacteria digest into acids and then convert to polysaccharides (Mohamed et al., 2021).

1.2.2. Yeast and dental caries

Due to its uric acid, thick biofilm formation, fermentation of dietary carbohydrates, and production of enzymes that break down collagen—a crucial component in the development of dental caries—*Candida albicans* may be a significant contributor to dental caries. According to studies, the proportion of fungi in the mouth is larger than that of the bacteria that cause tooth decay (Kramm et al., 2023).

1.3. Bacteria and Dental caries

When sucrose is fermented, plaque bacteria create acids that lower the pH below 5.0 (Bowen, 2013). Even though s*taphylococci*, a common Gram-positive bacterium, have long been recognized as part of the oral flora, their importance in oral health and disease is still up for debate (McCormack et al., 2015). Isolation rates of *Staphylococcus aureus* vary by demographic; in healthy adult dentate oral cavities, carriage rates range from 24% to 84%, whereas in the denture-wearing group, the prevalence is 48%. These findings suggest that *S. aureus*, a prevalent gram-positive bacterium, is still often isolated in the oral and perioral canals. The oral cavity must be considered a source of *S. aureus* in order to prevent cross-infection and spread to other parts of the body (McCormack et al., 2015). Certain bacteria that adhere to the surface of teeth and form dental plaque or bacterial colonies, produce acid that irreversibly dissolves tooth minerals, causing dental decay(Roberts, Mangum, & Schneider, 2022). Plaque bacteria can produce a range of compounds (H_2S, NH_3, amines, toxins, enzymes, antigens, etc.) that induce periodontal tissue and pocket disease in addition to producing a protective inflammatory response. A caries lesion becomes excruciatingly painful as it approaches the tooth pulp. The tooth surface usually loses some of its mineral content after eating foods that contain fermentable carbohydrates because of the acid that plaque bacteria create (Featherstone, 2008). In between meals, saliva usually replenishes this mineral. However, regular ingestion of fermentable foods causes a net loss of minerals from the tooth and keeps the pH of the plaque is low. because they can release acid and hold onto polysaccharides for a long time after food has been eaten (Roberts, Mangum, & Schneider, 2022). Within three months following radiotherapy, new deteriorated lesions become visible, and the patient may experience one or more new degraded surfaces on average per month following the procedure. as deterioration is developing. Acids from bacterial metabolism seep into enamel and dentine, eroding the mineral and causing dental caries, a contagious bacterial disease process (Edit et al., 2019). When the bacterium in question breaks down fermentable carbohydrates, organic acids are produced as a byproduct. Demineralization starts at the atomic level at the crystal surface inside the enamel or dentine and can continue unless stopped, leading to cavitation. The caries process is a continuum that is the result of numerous cycles of demineralization and remineralization. There are numerous ways to stop or reverse the progression of the lesion. In subsurface lesions that persist after demineralization, remineralization—the natural repair process for non-cavitated lesions—relies on calcium and phosphate ions with fluoride assistance to create a new surface on the crystal remnants that are already there (National Institute of Dental and Craniofacial Research, 2021). Because they are significantly less soluble than the original mineral, these remineralized crystals are resistant to acid. In this study, we examined the presence of *S. aureus* in dental caries specimens. Moreover, in light of the above- indicated circumstances, sensitivity (Roberts, Mangum, & Schneider, 2022). When bacteria break down fermentable

carbohydrates, they produce organic acids as a byproduct. The processes of demineralization and remineralization repeatedly result in the continuum known as the caries process. Dental caries is a bacterial illness that spreads easily and dissolves minerals in enamel and dentine due to the acids produced by bacterial metabolism. Demineralization can continue until cavitation happens at the atomic level at the crystal surface inside the enamel or dentine if it is not halted. There are a number of methods to become involved in this continuous process and halt the growth of the lesion (Edit et al., 2019). For non-cavitated lesions, fluoride aids calcium and phosphate ions in forming a new surface on any residual crystal remnants during the natural healing process known as "remineralization" (Roberts, Mangum, & Schneider, 2022).

1.3.1. Spray absorption

When compared to alternative dose forms (such as gel, mouthwash, foam, etc.), the spray form offers advantages. Direct spraying onto the afflicted area, rapid absorption because of the delivery system, efficient targeting, lightweight, immediate effect, no irritant effect, and ease of application. It has a pump mechanism; spray it on the afflicted region, massage it in well with a toothbrush for a minute to allow it to absorb, and then wash. The product's cooling effect, which can provide immediate pain relief, is an additional advantage (Kim, Lee, & Hwang, 2019). They have a range of physical characteristics, such as textures, looks, and rheological properties, as shown in **Table 1**. All of these elements are intended to give the customer a satisfying experience in addition to making tooth spray storage inside tubes easier. The complex composition of toothpaste produces interesting rheological properties. This section looks at a handful of these characteristics as well as the pharmacokinetics of some of the active ingredients. The pH level of your mouth indicates how acidic it is. The pH scale has values between 0 and 14. A material's pH value increases with its alkalinity. Lower numbers, on the other hand, indicate that the substance has more acidity. pH is a crucial factor for all medications manufactured in aqueous liquid forms because it influences the solubility of the molecule, which in turn influences the molecule's activity, the formulation's pharmacological tolerability, and the medication's stability.

A nozzle is defined by its spray dispersion in addition to its flow rate, spray shape, and spray angle.

Table 1: Ideal Properties for Tooth Spray (National Institute of Dental and Craniofacial Research, 2021) .

Parameter	Ideal Properties
PH	Basic
Price	Acceptance
Viscosity	Moderate-low
Density	Moderate-low
Color	Safe/compatible with ingredients
Safety	Nontoxic
Effect	Prolonged effect
Staining	Not leave stains on teeth
Spreading rate(cm)	High

1.3.2. Clevenger's apparatus

In 1928, this apparatus was given the name Clevenger in honor of its creator, Joseph Franklin Clevenger. As seen in **Figure 2**, the method of action relies on the extraction of essential oils from plant sources *via* steam distillation. The device's mechanism of action Water and plant samples are added to the flask and allowed to boil. After ascending to the condenser, the steam is gathered in the burette. Oils are kept from degrading by the steam; they float on the water and slowly return to the flask. It is simple to gather the oil in the burette following a 4-hour extraction. A flask and my adapted glass—a vertical tube with a condenser and a glass stopcock burette—make up this apparatus. In accordance with the method outlined in the European Pharmacopoeia (McCormack et al., 2015), the essential oils were separated by hydro distillation for four hours using a Clevenger-type device.

Figure 2: Hydro-distillation Clevenger apparatus (Polyakova et al., 2024).

1.3.3. Incubator

An incubator is a device used in laboratories to cultivate and maintain cultures and microorganisms. An incubator provides the proper quantity of moisture, pressure, and temperature to promote the growth of microorganisms. Based on the principle of preserving an environment that is conducive to the growth of microorganisms, incubators have a heating system that allows the temperature to be adjusted according to the type of organism being grown within. In a similar vein, they are given modifications to maintain the CO_2 content in order to balance the pH and humidity needed for the organisms' growth (Lagier et al., 2015).

1.3.4. Solubility of essential oils

Essential oil components (EOCs) are used to inhibit infections at much higher concentrations in complex food matrices than undergrowth settings due to their limited water solubility (Chen, Davidson, & Zhong, 2014). Essential oils are soluble in ether, alcohol, and fixed oils but insoluble in water. These volatile oils are usually colorless and liquid at room temperature. With few exceptions, they have a density of less than unity, are usually liquid at room temperature, and have a noticeable odor. Moreover, EOs contain insecticidal or antifungal, or deterrent qualities. All parts of fragrant plants, including fruits, flowers, leaves, and seeds, can contain essential oils (Dhifi et al., 2016).

1.3.5. Microbiology test

The disk diffusion test measures activity in relation to a particular microorganism. Microbiology techniques are methods for studying microorganisms (such as bacteria and fungi). Microbiologists use five basic laboratory techniques for microbial identification, staining, engineering, surveying, culture, and manipulation in order to study and characterize microorganisms. Among the techniques employed for this purpose are inoculation, incubation, isolation, examination, and identification. The antibacterial efficacy of the tooth spray against *Staphylococcus aureus* and yeast must be assessed. The tooth spray should be left to diffuse in an incubator set at 35°C for a day. The results of antibacterial activity will depend on the diameter of the zone of inhibition (Lagier et al., 2015; Turek & Stintzing, 2013).

1.4. Essential oils and oxidization

An oil's composition changes as it comes into contact with heat, light, and oxygen; the oxygen bonds that hold molecules together are changed to carbon bonds (Xu et al., 2021). Since oxidation breaks down the chemical structure of essential oils over time, they lose their power and efficiency. For this reason, most essential oils are marketed in amber-colored bottles, which offer better protection from ultraviolet light due to the darker glass. Not at all, Essential oils undergo a process of oxidation that starts as soon as the container is opened and the oil is exposed to oxygen in the air, but they don't expire in the same way that food or other goods "go bad." Oxidation is also accelerated by heat and light (Turek & Stintzing, 2013).

1.4.1.Clove essential oil

The essential oil which is extracted from the clove plant has, analgesic, antibacterial, and antifungal activities are found in clove oil, an essential oil that is extracted from the clove plant. Clove essential oil is a general stimulant and analgesic with strong antibacterial properties that are used to treat herpes, mycoses, neuritis, viral infections, and infections of the oral cavity (Pathirana et al., 2019). To separate volatile ingredients, dried clove flower buds were exposed to steam distillation. Clevenger's apparatus was used to distill 50 g of clove with 150 ml of water; the apparatus was carefully closed to prevent oil evaporation (Ferhat et al., 2006). To prevent oxidation, it was packed in a refrigerator and kept in dark containers (National Institute of Dental and Craniofacial Research, 2021). Clove oil is made *via* steam distillation, which employs steam to gently extract the oil from the dried flower buds of clove trees. After the water condenses and the oil is scraped from the top, the essential oil is produced. This extraction technique is non-invasive and frequently yields the most oil (Moon, Kim, & Cha, 2011). Other methods, like cold pressing or using alcohol to extract the oil, can also be used, although sometimes they might cause more damage and produce less oil than is required. The resulting pale yellow, translucent liquid had a strong clove scent and yielded 12.8% (v/w) of essential oil. The chemical makeup of the essential oil was analyzed using GC and GC-MS, and the findings were shown. A total of 22 essential oil components were identified (Xu et al., 2016). Many authors have already studied the essential oil content of cloves. As seen in **Figure 3**, clove bud oil included eugenol (70.1%), caryophyllene (4.8%), IX-humulene (0.55%), IX-terphenyl acetate (0.1%), methyl eugenol (0.2%), humulene epoxide (0.2%), and chavicol (0.3%). Nevertheless, a wide range of

compounds are present in trace levels, such as alcohols and oxides, methyl ketones, aliphatic alcohols, esters, and sesquiterpenic hydrocarbons (Muchalal & Crouzet, 1985). In **Figure 3**, as stated Eugenol, the primary ingredient, accounts for at least 50%. The remaining 10–40% is composed of eugenyl acetate, α-humulene, and β-caryophyllene.(Spatafora et al., 2024)

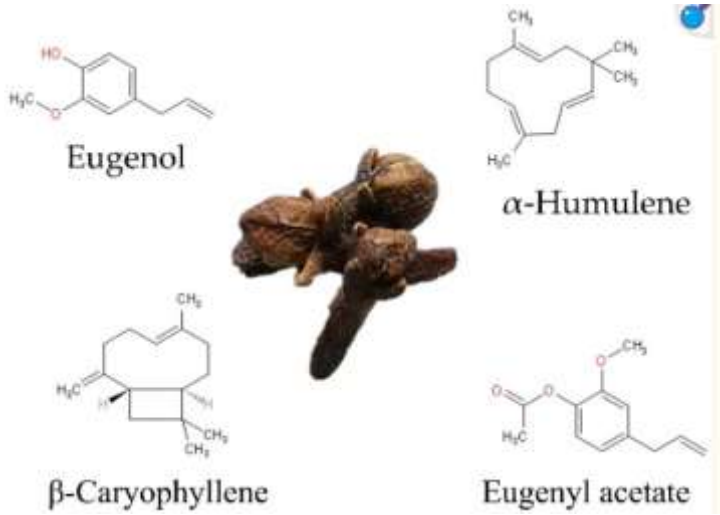

Figure 3: The primary chemical components of clove essential oil.(Pitts et al., 2017)

Clove oil's main active ingredient, eugenol, has strong antibacterial and antifungal properties via a number of mechanisms. By reacting with lipid bilayers, it breaks down microbial cell membranes, increasing permeability and allowing vital internal components to flow out. Eugenol also produces reactive oxygen species (ROS), which lead to oxidative stress and harm to biological components such as proteins, lipids, and nucleic acids. Additionally, by changing sodium and calcium ion channels, eugenol disrupts cellular communication and metabolic processes, interfering with ion homeostasis. **Figure 4** shows the SEM images of the treated and untreated bacteria. The treated strains' surfaces saw some morphological changes in comparison to the untreated controls. In contrast to treated cells, which became pitted, shriveled, and uneven (**Figure 4, A1**), untreated cells were spherical, uniform, and smooth on the surface **(Figure 4**, **A0**). Figure 4 similarly showed the TEM image of *S. aureus* after treatment. In S. aureus micrographs, the cytoplasm was uniformly distributed, and the control cell wall and membrane remained intact (**Figure 4**, **B0**). Nevertheless, after being treated with essential oil, some cells lost their normal spherical shape and displayed partial lysis along with abnormalities in their cell wall and cytoplasmic membrane (**Figure 4**, **B1**). Furthermore, some of the results of the SEM and integrity of cell membrane tests were found to be consistent with the broken cell wall and the disruption of the outer membrane structure due to membrane sloughing and breaching, which might cause the cell content to leak out (Xu et al., 2016), providing short-term respite. Since the EPA classified clove oil and eugenol as minimal-risk pesticides, products containing these substances are exempt from FIFRA requirements. The oral LD50 of clove oil is 3597.5 mg/kg, and it has been demonstrated in numerous acute and chronic toxicity trials to have no adverse effects in subchronic toxicity testing, with NAOEL values ranging from 900 to 2000 mg/kg/day. It was discovered that the oral LD50 of eugenol ranged from 2650 to 3000 mg/kg b.wt. Eugenol was also shown to be rapidly absorbed, metabolized in the liver, and eliminated in less than twenty-four hours when taken orally. Clove essential oil may be a good external antibacterial agent because it is not greatly affected by organic waste or deactivated by dilution. Clinical trial design will

assess efficacy in dental and medical practice (Nuñez & Aquino, 2012). Clove oil has demonstrated potent germicidal effects against a variety of periodontal pathogens, including superinfections such as *Candida albicans*, *Pseudomonas aeruginosa*, *S. aureus*, and *E. coli.* The fact that clove oil is commonly included in toothpaste is evidence that clove buds can reduce mouth germs by 70%. Eugenol's anti-inflammatory qualities have been linked to its ability to reduce the formation of prostaglandins, neutrophil/macrophage chemotaxis, and the expression of the cyclooxygenase II enzyme. By lowering macrophage cytokine production, eugenol dimers have also shown chemopreventive advantages (Gawish et al., 2024).

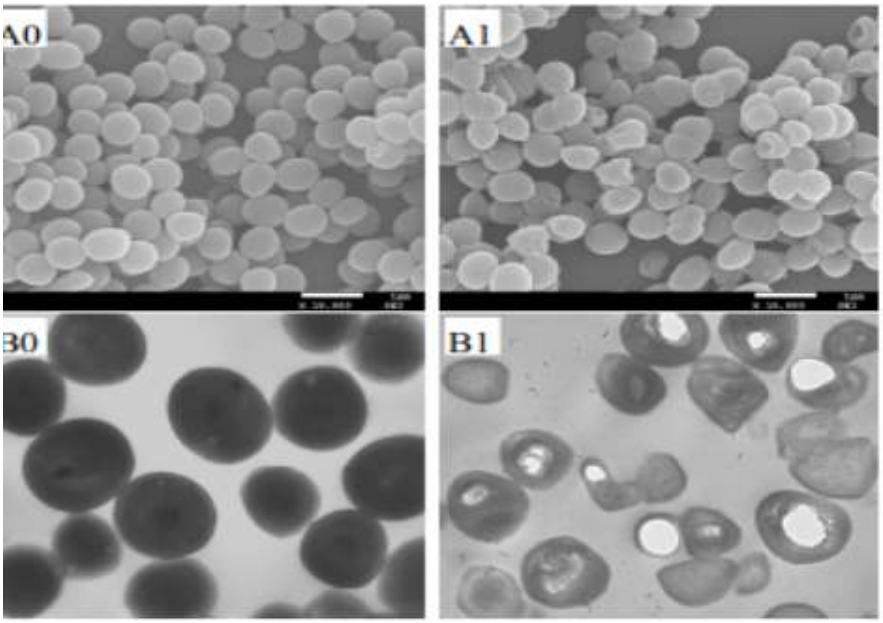

Figure 4: *S. aureus* TEM and SEM images. Bacteria A0 and B0 were left untreated, whereas bacteria A1 and B1 were exposed to the essential oil at a 1time MIC (Bowen, 2013).

1.4.2. Eugenol

Eugenol is a weakly acidic phenolic compound with the chemical formula $C_{10}H_{12}O_2$

(Chi & Scott, 2019).Eugenol and clove oil are commonly used by dentists due to their antibacterial and anti-inflammatory properties. It can also be used on the gums to get rid of bacteria and reduce pain from dental treatments, including fillings, root canals, and denture extractions. Eugenol is one of the world's most potent antioxidants. It is also a naturally occurring antibacterial and antibiotic that eliminates bacteria, viruses, and other dangerous substances. One study found that eugenol stops more than 25 harmful germs, including *salmonella* and *candida*, from growing. Eugenol is a phenolic, somewhat acidic compound with the formula $C_{10}H_{12}O_2$**.** (Ulanowska & Olas, 2021). the allelic substitute for guaiacol is eugenol, a light-yellow, oily liquid. It has a pleasant, spicy, clove-like scent and is one of the most important compounds in cloves. The substance eugenol (4-allyl-2-methoxyphenol) has antioxidant, antibacterial, and antiseptic qualities. The main source of the phenolic aromatic compound eugenol is clove oil. Its well-known antibacterial, antiviral, antifungal, anticancer, and anti-inflammatory properties have led to its long-standing usage in pharmacology, medicine, and cosmetics (Didehdar, Chegini, & Shariati, 2022). However, large amounts

could be dangerous. A dosage of 2.5 mg/kg body weight is regarded as safe. This paper reviews fresh research on eugenol and its derivatives, as well as their activities and applications. This analysis was based on current research and information on eugenol characteristics from PubMed papers. Eugenol's multifarious properties continue to pique researchers' curiosity as a potential component of drugs and other products with potential for treating a range of illnesses. More significantly, clove oil has strong bactericidal effects due to its high eugenol content (Nuñez & Aquino, 2012). Furthermore, there is ample evidence of the synergistic effects of phytochemicals. It's interesting to note that prior research has demonstrated that the MIC of pure eugenol is higher than that of whole clove oil. The antibacterial activity of cloves is attributed to eugenol Phyto complexes and the minor components in **Figure 5** (Marchese et al., 2017). Carbohydrates typically make approximately 90% (w/w) of the cell wall of *Candida albicans*, with proteins accounting for 3–6% and lipids for 2%. As a result, polysaccharides and proteinaceous components make up the majority of *Candida albicans'* cell walls in a 9:1 ratio. Mannoproteins, a phospholipid bilayer, beta-glucans (both 1, 3- and 1, 6-linked), and a small quantity of chitin are the constituents of the cell wall. The cell wall of *Candida albicans* has attracted attention because of its function in adhesion, colonization, antigenic components, and released products. Therefore, new antifungal medications may target these compounds and the enzymes that generate and degrade them. Most of the time, the cell membrane may protect the bacteria from damage caused by antibiotics. Certain cytoplasmic components are produced when this essential structure is damaged. Proteins, reducing sugars, and nucleic acids, among others, are released. The findings demonstrated that eugenol significantly increased the amount of LDH, protein, and nucleic acids that leaked from *S. aureus* cells in a time- and dose-dependent manner. One of the primary sources of intracellular oxidation that may endanger bacterial viability is the overabundance of ROS, which include dioxide, hydrogen peroxide, nitric oxide, and hydroxide. In particular, an explosion of ROS can harm proteins and DNA and create lipid peroxides in cell membranes by reacting with unsaturated fatty acids in phospholipids. DCFH-DA was used as a molecular probe for ROS detection in order to investigate the impact of eugenol on ROS generation in *S. aureus* cells. Following eight hours of eugenol exposure, the quantity of ROS generated in the experimental groups' bacterial cells rose quickly.

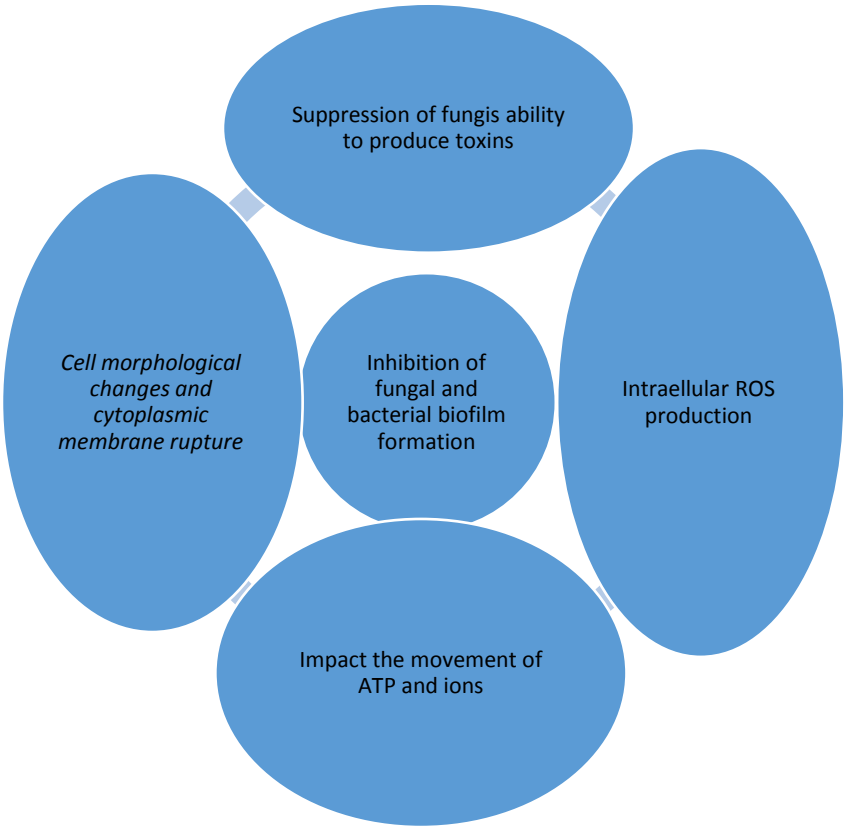

Figure 5: Antibacterial and antifungal activity of eugenol (Reddy et al., 2016)

1.4.3. Sage essential oil

Sage oil is among the earliest therapeutic oils. It has a green hue, and the oil that is produced from its leaves using the Clevenger device has a white to yellowish hue. After adding sage leaves to the flask, they submerged it in water, then extraction process started (Đurović et al., 2022; Porte Godoy & Maia-Porte, 2013). SEO is a great therapeutic agent with antiviral, antibacterial, and antifungal properties. Antibacterial, anti-inflammatory, and antioxidant. Triterpenoids, diterpenes, phenylpropanoid glycosides, flavones, phenolic acids, tannins (salviatannin), and essential oils (camphor (26.12%), alpha-thujone (40.90%), 1,8-cineole, alpha-pinene (5.85%), beta-thujone (5.62%)(Almajed et al., 2024). Monoterpenes (such as camphor, 1,8-ineole, and α- and β-thujone), diterpenes (such as carnosic acid), triterpenes (such as ursolic and oleanoic acids), and phenolic compounds (such as rosmarinic acid) are the most common metabolites having therapeutic use. Because of its constituents, sage oil has the capacity to eliminate bacteria and fungi (Porte Godoy & Maia-Porte, 2013). The presence of flavones, phenolic acids, phenylpropanoid glycosides, triterpenoids, diterpenes, tannins (salviatannin), and essential oils (alpha-, beta-, and camphor-thujone, 1,8-cineole, and camphor) in sage (*Salvia officinalis*) leaves has been linked to the antibacterial qualities of sage oils. Spanish sage (*Salvia lavandulaefolia*) is a related plant with similar components, even if its thujone content is lower. The results of the study also indicate that camphor and 1,8-cineole are the primary sources of the antifungal activity of the strains that were evaluated. Examine the oil's antifungal and anti-inflammatory properties at levels that won't harm mammalian cells. Sage (*Sage officinalis*) is a member of the Lamiaceae family and comes in two primary varieties: *S. officinalis* and *S. triloba*. The main chemical components of oil from *S. officinalis* are cineole, camphor, borneol, and pinene, but cineole, camphor, and caryophyllene are also present. Essential oils produced from sage include antiviral,

antifungal, antibacterial, and antiseptic properties. *S. officinalis* also possesses antioxidant, antimicrobial, and free radical scavenging qualities. Because of their medicinal properties, sage leaves are frequently employed (Gawish et al., 2024).
MWD 200 W had the largest concentration of borneol, whereas D 200 W had the highest concentration of camphor. The same pattern was shown in the Thu-Jones example, where D 200 W showed the highest values. All EO samples included both α-caryophyllene (1.44–2.44%) and β-caryophyllene (0.90–1.60%), with α-caryophyllene being present in greater concentrations across all six samples (Amorati et al., 2013).As seen in **Figure 6**, many dietary botanical supplements and herbal therapeutic preparations include thujone, a monoterpene ketone, in varying levels since it is found in a wide variety of plants. In nature, thujone is found as a fluctuating combination of α-thujone and β-thujone. Thujone is a naturally occurring component of many plants' essential oils that are utilized for culinary and/or therapeutic reasons.
The detection of a- and b-thujone in a range of matrices has been accomplished through the widespread use of gas chromatography methods.

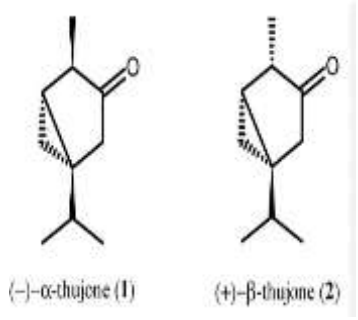

Figure 6: Chemical structure of α-and β-thujone (de Sousa et al., 2023).

1.4.4. Peppermint essential oil

The peppermint plant thrives in Europe and North America (Fatmawati, Wachyuni, & Azhary, 2022). Both peppermint essential oil and peppermint leaves have been used medicinally. Peppermint oil is the essential oil that is extracted from the leaves and flowering parts of the peppermint plant. The components that give a plant its unique flavor or aroma are found in essential oils, which are incredibly concentrated oils (Prasad et al., 2019). In addition to being widely used as a flavoring in food and beverages, peppermint oil is utilized as a fragrance in soaps and cosmetics. It appears to be safe to apply peppermint oil topically or take it orally in the recommended amounts. Peppermint oil has been used without incident in a number of clinical research studies. When taken orally, peppermint oil might lead to heartburn, nausea, dry mouth, and stomach pain. Peppermint oil can occasionally cause allergic reactions. The peppermint plant's leaves and flowers are used to make a medicinal herb. Its primary ingredient is 35–65% menthol (Fatmawati, Wachyuni, & Azhary, 2022). Menthol possesses antimicrobial and analgesic properties (Fatmawati, Wachyuni, & Azhary, 2022). It is present in toothpaste and mouthwash, among other dental products. Oil is extracted *via* the steam distillation technique. Because of its cooling properties and capacity to eradicate the bacteria that cause bad breath, they are widely utilized in oral care products. One of the best antivirals, antifungals, and antibacterial botanicals is PEO. Within the Lamiaceae family, there are two primary types of sage (*S. officinalis*): *S. triloba* and *S. officinalis*. While cineole, camphor, and caryophyllene are the main chemical components of *S. officinalis* oil, cineole, camphor, borneol, and pinene are also present. Essential oils

produced from sage include antiviral, antifungal, antibacterial, and antiseptic properties. *S. officinalis* also possesses antioxidant, antimicrobial, and free radical scavenging qualities. Because of their medicinal properties, sage leaves are frequently employed (Gawish et al., 2024). Based on several of studies. Peppermint oil works well against facultative and obligatory anaerobes. It also has bactericidal effects on at least 20 common enteric pathogens, such as *Salmonella typhi*, *Escherichia coli*, *Helicobacter pylori*, *Klebsiella sp.*, *Shigella boydii*, and *Shigella flexneri*. Menthol has been shown in more recent research to inhibit gram-negative bacteria's ability to use quorum sensing. Peppermint oil also appears to be useful in treating infections caused by fungi. In rinse-off formulations, peppermint oil is utilized at a concentration of less than or equal to 3%. Menthone and menthol make up the majority of peppermint oil. Menthofuran, pulegone, and limonene are other possible ingredients. Most of the safety test results are related to peppermint oil. Since the oil is considered to give the "worst-case scenario" because of its many components, data on it were considered relevant to the full collection. Peppermint oil was found to have a modest negative impact in acute oral tests. Rats given dosages of peppermint oil, including pulegone, pulegone alone, or substantial quantities (>200 mg/kg/day) of menthone in both short-term and sub-chronic oral studies, displayed cerebellar lesions that resembled cysts. It is also known that there are hepatotoxins such as pulegone. After receiving repeated intradermal dosages of peppermint oil, rabbits had moderate to severe reactions, despite the oil's apparent lack of phototoxicity. A mouse and peppermint oil did not pass the Ames test. When looking for approved drug items, the maximum potency per unit dose is 0.04 percent as an inactive ingredient. GC/FID and GC-MS were employed to examine the essential oil of peppermint. The main components were menthol (40.7%) and Menthone (23.4%). Other components were 1, 8-cineole, limonene, beta-pinene, beta-caryophyllene, and (+/-)-methyl acetate. high level of tannin (Beigi, Torki-Harchegani, & Pirbalouti, 2018). The data was tabulated and statistically assessed using Fisher's exact test and the chi-squared test. Statistical significance was defined as $P < 0.05$ (Prasad et al., 2019). Peppermint leaves, peppermint oil and peppermint extract are all produced from the Mentha piperita plant. In 1998, peppermint oil was used in 102 cosmetic items as a scent element. Peppermint extract was used in 35 recipes as a flavoring and fragrance ingredient. Peppermint was used in two formulations (Nair, 2001). Furthermore, it has been shown that certain plant extracts can stop *Candida albicans* from growing biofilms. Furthermore, EOs hold potential for use as preservatives and to increase product shelf life in the food industry, especially those with proven antibacterial qualities (Saharkhiz et al., 2012). examined in the next portion of this study. Methyl acetate (4% to 14%) and trace amounts of cineole and other terpenes are also present in peppermint oil. Other components identified include limonene, pinene, phellandrene, cadinene, acetaldehyde, amyl alcohol, methyl esters, and dimethyl sulfide (Dooms-Goossens et al. 1977; Andersen 1978 (Nair, 2001)). Additionally, some trace elements, such as β-pinene, p-menthane, sapinene, terpinolene, ocimene, gamma-terpinene, and fenchene, were identified by Baslas, Singh, and Baslas (1973) and Lawrence (1972) (Nair, 2001).

1.4.5. Vegetable glycerin

The first accidental discovery of glycerol was made in 1779 by a Swedish scientist named K. W. Scheele (Izyan et al., 2019). He discovered a sweet-tasting, water-soluble molecule—later identified as glycerol—through a chemical reaction between lead monoxide and olive oil. Scheele's discovery of glycerol as the "sweet principle of fat" was the first reported chemical isolation of the substance. Pelouze, a French scientist, introduced $C_3H_8O_3$ as the empirical formula of glycerol in 1836 (Izyan et al., 2019), and the structural formula of $C_3H_5(OH)_3$ was approved in 1886 based on the research of two scientists, Berthelot and Lucea. Glycerin can

be found naturally in palm, soy, and coconut. Candles and animal-fat soaps were common sources of glycerin in the 18th and 19th centuries. Plant-based oils are increasingly being used to make glycerin. Because glycerin is a humectant, which draws water to itself, it helps keep toothpaste from drying out in the tube. Glycerin is also a component of many other skincare products and cleansers, including my hydrating homemade foamy hand soap, to keep them (and you) from drying out. Because of its emollient properties, glycerin can also be utilized as a lubricant and is a good choice for tooth spray (Izyan et al., 2019). Glycerol's water solubility and three alcoholic hydroxyl groups provide it hydrophilicity and hygroscopicity, making it a highly effective humectant in cosmetics. The main factors influencing glycerol's versatility are its physical and chemical properties. Through a number of catalytic routes, glycerol's polyhydroxy structure enables the synthesis of several beneficial compounds, such as lactic acid, acrylic acid, dihydroxyacetone, glycerol carbonate, and 1, 3-propan. The most sought-after platform chemicals in the glycerol derivatives previously addressed are lactic acid and its ester (Shreyas & Anbalagan, 2022). When looking for approved drug items, the maximum potency per unit dose is 0.15% as an inactive ingredient. Since it is non-toxic, glycerin is probably beneficial for your teeth. When consumed, glycerol has virtually no toxicity. Rat oral LD50: 12600 mg/kg Oral LD50 in mice: -4090 mg/kg the flavor of human TDLo (oral): 1428 mg/glycerin is sweet (Izyan et al., 2019). Because it makes your toothpaste taste better, you and your kids are more likely to brush with it. A typical component of toothpaste, glycerin, serves as a binder: In order to ensure a consistent texture and prevent component separation, glycerin helps to bind all of the toothpaste's ingredients. It increases the overall stability of the toothpaste formula. Humectant: Glycerin helps keep the toothpaste wet by acting as a humectant. When the toothpaste is exposed to air when the tube is opened, it retains its smooth consistency, preventing it from drying out and becoming difficult to use. Texture enhancer: Glycerin helps give toothpaste its creamy, smooth texture, which makes it simpler to apply to teeth and spread on a toothbrush. Additionally, it contributes to a comfortable mouth feel when brushing. Glycerin is completely dissolved in both alcohol and water (Shreyas & Anbalagan, 2022).

1.4.6. Stevia

The perennial plant *Stevia rebaudiana* is a member of the Asteraceae family. Native to Argentina, Brazil, and Paraguay, this sweetening plant is also known as honey leaf, sweet weed, sweet leaf, and sweet herbs. Stevia leaves are sweeter than sugar and contain no calories (Samuel et al., 2018). Steviol, a diterpenoid glycoside derivative produced by this plant, is sweeter than sucrose and safer to use as a sweetener (Samuel et al., 2018). For diabetics and obese people with hyperglycemia who are unable to follow a strict diet, Stevioside can be used as an alternative sweetener. The plant possesses cardiotonic, hypotensive, antibacterial, anti-inflammatory, hypoglycemic, diuretic, antifertility, and antiseptic properties (Samuel et al., 2018). Stevia plants contain secondary metabolites called diterpene glycosides, which are about 300 times sweeter than sucrose (Samuel et al., 2018). With the Japanese, there are no negative repercussions. We carried out over 40,000 clinical studies that demonstrated Steviol glycosides' safety. In comparison to non-herbal (conventional) dentifrices, herbal dentifrices are safer, have no negative side effects, and are more effective at lowering plaque and gingivitis. used to make meals sweeter. *P. stevia* plants were cultivated in Europe (Samuel et al., 2018).

Methylparaben (MP)

Methylparaben (CAS No. 99-76-3) is a methyl ester of *p*-hydroxybenzoic acid. This stable, non-volatile material has been used as an antimicrobial preservative in foods, medicines, and cosmetics for over 50 years (Hagel, Chen, & Facchini, 2019). Methylparaben is readily and completely absorbed through the skin and digestive tract. Following hydrolysis to *p*-hydroxybenzoic acid, it undergoes conjugation and is promptly removed from the body by urine. No accumulation is evident. Acute reports that methylparaben is generally non-toxic when taken orally or intravenously. The carboxyl group of 4-hydroxybenzoic acid reacts with methanol to form Methylparaben, a 4-hydroxybenzoate ester. It is the most often used antibacterial preservative in cosmetics. It is found naturally in many foods, including blueberries. It serves as a plant-metabolite food preservative, antifungal, and antibacterial. 0.05% is the maximum potency per dosage. $C_8H_8O_3$ is the molecular formula. 152.15 g/mole is the molecular weight (Hagel, Chen, & Facchini, 2019).

1.4.8. Volatile solvent

Class 3 residual solvents include volatile solvents, which are advantageous. since it poses little harm to human health and is not very poisonous. This class of solvents includes ethanol, which has excellent oil solubility. Because of its excellent ability to solubilize oils and its minimal toxicity and health risks, ethanol might be the best option to be used in tooth spray. Increased contact time is beneficial for medication penetration since it can improve it through a variety of processes. can improve the solubility of drugs (Beaulieu et al., 2017).

1.5. Viscosity

The resistance of a fluid to flow is measured by its viscosity. Low viscosity is ideal for tooth spray in order to improve formulation. Spreadability on the teeth, precise dosage administration, and improved patient adherence. A device known as an Ostwald–Cannon–Fenske viscometer can be used to measure the viscosity of Newtonian liquids. The formula for a liquid with known density (ρ1) and unknown viscosity (η1). Is set to fall naturally. The liquid's flow time (t1) between two timing markers is compared to the flow time (t2) of another liquid (often water) with known density (ρ2) and viscosity (η2). The unit of measurement is either centipoise (cp) or dyne sec/cm². Dynamic viscosity is measured in units of 1/1000 of a poise (Beaulieu et al., 2017).

The abbreviation is cPs. It can be calculated by using Eq#1.

η1/ η2= ρ1t1/ ρ2 t2……Eq. #1D=weight/volume

4.98/5=0.996g/ml

Stopwatch

Trial 1=21.15
Trial 2=21.22
Trial 3=21.15
Average= (21.15+21.22+21.15)/3= 21.173
V1/V2=d1*t1/d2*t2
(1*5.1)/0.996)) *(21.173) =0. 2418mpa.s)

1.5.1. Ostwald Viscometer

Determine the absolute and relative kinematic viscosities of liquids with Newtonian flow properties. The SI Analytics Manual Ubbelohde Viscometer comes with ring markings to ensure that manual measurements can be used to confirm the results. A U-shaped piece of glassware, the Ostwald viscometer features a measuring bulb with a reservoir on one side and a capillary on the other. A liquid is pulled *via* the capillary and measuring bulb after entering the reservoir. By letting the liquid return through the measuring bulb and timing how long it takes for it to pass between two calibrated markings, the viscosity may be determined. The Ubbelohde gadget has a third arm that protrudes from the end of the capillary and is open to the surroundings. In this way, the pressure head only depends on a specified height rather than the entire volume of liquid (Zimmerman, 1967).

1.5.2. McFarland Standards

Using McFarland Standards as a guide, the microbiology lab modifies the turbidity of the liquid/bacterial suspension in the vial or tube. Maintaining and/or ensuring that the number of bacteria will decrease within a given range is made easier by standardizing microbiological testing. The cell count density varies with the concentration, and the McFarland standard can be produced at concentrations between 0.5 and 4. However, the most commonly used concentration for evaluating culture media performance and antibiotic resistance is the 0.5 McFarland standard, which is usually used in microbiological labs. Before swabbing on MHA media, the bacterial solution is compared to Standard McFarland as part of the antimicrobial susceptibility testing process. Verifying and modifying the densities of bacterial suspensions that can be utilized for susceptibility and identification tests is a component of quality control (Zapata & Ramirez-Arcos, 2015). The first McFarland standards were made by mixing sulfuric acid and barium chloride in specific amounts. The solution becomes murky when the two chemicals are mixed together because a precipitate of barium sulfate is produced. A 0.5 McFarland standard is made by mixing 0.05 mL of 1.175% barium chloride dehydrate ($BaCl_2 \cdot 2H_2O$) with 9.95 mL of 1% sulfuric acid (H_2SO_4). It was developed to quantify specific amounts of colony-forming units of bacteria per milliliter (CFU/ml) and to estimate concentrations of the Gram-negative rod-shaped bacterium. Even while McFarland turbidity standards are commonly used, they become unclear when working with other organisms since different mold, yeast, and bacterial species differ in size and shape (Zapata & Ramirez-Arcos, 2015).

.1.6 Objectives and significance

1.6.1. Significance of the study

Nowadays, tooth decay is seen as a worldwide problem. These days, numbers are all around us. We hardly ever give these numbers any weight most of the time. However, for those of us engaged in giving dental treatment, encouraging improved oral health, or carrying out. According to oral health studies, 3.5 billion people globally suffer from severe periodontitis, edentulism, and untreated tooth caries. Since they have a direct connection to oral health, two concepts in the 2015 GBD update are very important. First, the global burden of infectious diseases declined between 1990 and 2015 (Dye, 2017). Tooth decay raises the risk of tartar, foul breath, pain, inflammation, and tooth loss, among other possible oral health issues. The antibacterial properties of essential oils in spray were explained by terpenes and terpenoids, which are all distinguished by a low molecular weight, aliphatic, and aromatic group. In general, tooth sprays can be categorized as either medicinal, cosmetic, or a mix of the two

(Peres et al., 2019). Essential oils found in dental spray create a powerful barrier that eliminates inflammation and microorganisms, strengthening teeth and gums against germs and yeast that cause oral illness. Oils are recognized to have fewer negative effects and to be less hazardous. Natural dental sprays are free of dangerous chemicals and fluoride conventional approaches to tarter control, including fluoride treatments, frequently have issues with patient compliance, safety, and efficacy (Ramsey et al., 2020). While fluoride plays a significant role in preventing tooth decay, excessive fluoride consumption can result in tooth fluorosis (Browne, Whelton, & O'Mullane, 2005). The brown- to- white discoloration of teeth is known as fluorosis. Teeth are not stained by the natural oils in tooth spray (Browne, Whelton, & O'Mullane, 2005). Therefore, it is crucial to use natural dental products to combat dental decay in order to get around these restrictions, which include tooth discoloration; the permissible level of fluoride in cosmetics is limited to 1500 ppm (1000–1500 ppm), but it can reach 5000 ppm in therapeutic dental products; artificial sweeteners, such as sucrose, have adverse effects, such as laxatives and stomach pain. Stevia is an example of a natural botanical sweetener. have no negative side effects, are safe, and works better at preventing gingivitis and plaque than non-herbal (conventional) dentifrices. in the role of a sweetener. The 1,500-ppm fluoride (F–) content limit set for European cosmetic toothpaste must be evaluated in light of both efficacy and toxicity. There is strong evidence that the dose-response relationship between F-concentration (25–30% caries) and caries prevention is present. Reduce with a 1,000 ppm F-dentifrice. The therapeutic significance of elevated F-concentration has not been thoroughly established. Low F– toothpaste (1500 ppm F–) has been pushed to urge young children to eat less F– in order to reduce the occurrence of dental fluorosis. Their effectiveness is still unknown. Dental professionals should conduct evaluations of caries risk and fluoride intake, and individual modifications should be made to topical fluoride prescriptions (Bloch-Zupan, 2001). These products' makers use a variety of herbal compounds that they say replicate the advantages of conventional toothpaste, including their capacity to remove plaque, improve breath, and stave off gum disease. Customers' desire to "go natural" has led to a rise in demand for these products; many seem to choose them for cultural reasons because they are free of animal testing, have no negative effects, don't use animal products, are vegan-friendly, and don't include artificial colors or flavors. In many areas, more herbal products are sold than toothpaste with fluoride (Ramsey et al., 2020).

1.6.2 Objectives

- To formulate and develop a tooth spray aimed at enhancing oral hygiene -
- To assess the tooth spray's beneficial effects
- The creation and description of the tooth spray
- Creating a microbiological test to precisely assess the essential oils' activity in tooth spray
- To evaluate how well tooth spray works to prevent dental cavities since it spreads easily and penetrates easily between teeth.
- To evaluate the antimicrobial properties of herbal tooth spray and essential oils against dental disease-causing microorganisms
- To assess the efficacy of herbal oral products relative to conventional oral products

Chapter Two:

Methodology:

Several formulas were used in the preparation of tooth spray. The materials, equipment, and tools used for there are summarized in Table (2+3). The total volume of the sample used in this study was 10 ml.

2.1. Formulation materials, equipment, and tools

2.1.1. Materials

Table 2 contains a list of all the materials used in the formulation.

Table 2: Materials used for formulation trials and their functions.

Ingredients	**Source**	**Functions**	**Quantities (%w/w)**
Clove oil	Chemistry Department Laboratory at Al-Quds University using steam distillation	Active ingredient	5%
Peppermint oil	Chemistry Department Laboratory at Al-Quds University using steam distillation	Active ingredient	5%
Sage oil	Chemistry Department Laboratory at Al-Quds University using steam distillation	Active ingredient	5%
98%v/v alcohol	Sigma-Aldrich CAS No: 64-17-5	Solvent	5 ml
Glycerin	Sigma-Aldrich CAS No:56-81-5	Emollient	15%
Stevia	Sigma-Aldrich CAS No:91722-21-3	Sweetening agent	0.1%
Sodium bicarbonate	Sigma-Aldrich CAS No:144-55-8	Balance PH level	0.8%
Methylparaben	Sigma –Aldrich CAS No:99-76-3	Preservative	0. 1%
Distilled water	Sigma-Aldrich CAS No:7732-18-5	Vehicle	2 ml

2.1.2. Equipment and tools.

Table 3 is a list of all the instruments and equipment utilized in formulation and analysis.

Table 3: Equipment and tools used in the experiments.

No.	Name of Equipment/Tool	Used for
1	Incubator	Provide controlled environmental conditions, especially for the cultivation of microorganisms.
2	Magnetic stirring bar	Mixing
3	Refrigerator	Stability and storage
4	PH meter	PH adjustment
5	Micropipette	Formulation
6	Viscometer	Viscosity test
7	Stop watch	Viscosity test
8	Digital laboratory weighing scale	Weight sample
9	Clevenger apparatus	Oils Extraction
11	Metric ruler and caliber	Measure the diameter of the zone of inhibition
12	Volumetric flask	For precise dilutions and preparation of standard solutions.
13	Volumetric and graduated pipette	For the transfer and dispense of a single, specific quantity of liquid to a very high degree of accuracy.
14	Conical flask	Use for mixing
15	Wire brush	For cleaning bottles, pipettes, beakers, graduated cylinders, and flasks
16	Cotton swab	To take microbiological cultures.
17	Tape	Close the oil bottles tightly.
18	Vernier caliper	Measure the diameter of the zone of inhibition
19	stirrer	To mix or disperse fluids in a container(homogenization)
20	Filter paper	To separate bacteria or other microorganisms from liquids
21	Glass vials	to store medicines or laboratory samples
22	Agar plate	To culture (grow) bacteria and fungi in the lab
23	Sterilized loop	To transfer bacteria and yeast
24	Petri dish	To grow bacteria and fungi in the microbiology laboratory
25	Vortex mixer	Mixing
26	Muller-Hinton agar	Bacteria/Yeas growth test
27	Beakers	Formulation

2.2. Methods:

A formulation was prepared by combining selected essential oils with specific solvents. The oils were mixed in defined ratios, and the final volume of each sample was adjusted to 10 mL. To evaluate the antimicrobial efficacy, essential oils were diluted using the following substances: DMSO, Paraffin Oil, 70% Ethyl Alcohol, 95% Ethyl Alcohol, and 98% Ethyl Alcohol. The following concentrations were prepared: 5%, 2%, 1%, and 0.5% of essential oil, with the quantities used for each solvent as follows:

-DMSO: 50 µL of essential oil and 950µL of DMSO at 5% concentration,20µL of essential oil and 980µL of DMSO at 2% concentration,10µL of essential oil and 990 µL of DMSO at 1%,5µL of essential oil and 995 µL of DMSO at 0.5%

-Paraffin Oil: 50µL of essential oil and 950µL of Paraffin oil at 5% concentration,20µL of essential oil and 980µL of Paraffin oil at 2% concentration,10µL of essential oil and 990µL of Paraffin oil at 1% ,5 µL of essential oil and 995µL of Paraffin oil at 0.5%

-70% Ethyl Alcohol: 50µL of essential oil and 950µL of 70%Ethyl Alcohol at 5% concentration,20µL of essential oil and 980µL of 70%Ethyl Alcohol at 2% concentration,10µL of essential oil and 990µL of 70%EthylAlcoholat 1% ,5 µL of essential oil and 995µL of 70%EthylAlcohol at 0.5%

-95% Ethyl Alcohol: 50µL of essential oil and 950µL of 95%Ethyl Alcohol at 5% concentration,20µL of essential oil and 980 µL of 95%Ethyl Alcohol at 2% concentration,10 µL of essential oil and 990µL of 95%EthylAlcohol at 1% ,5µL of essential oil and 995µL of 95%EthylAlcohol at 0.5%

-98% Ethyl Alcohol: 50µL of essential oil and 950 µL of 98%Ethyl Alcohol at 5% concentration,20µL of essential oil and 980µL of 98%Ethyl Alcohol at 2% concentration,10µL of essential oil and 990µL of 98%EthylAlcohol at 1% ,5µL of essential oil and 995µL of µL98%EthylAlcohol at 0.5%

The materials DMSO, Paraffin oil, 70% Ethyl Alcohol, and 95% Ethyl Alcohol were excluded from use in the study as they showed no antimicrobial activity. 98% Ethyl Alcohol was used in the tooth spray formula as it demonstrated effective antibacterial and antifungal activity.

Formulation Method of the Tooth Spray:

1. The oil was extracted using a Clevenger apparatus via steam distillation.

2. 98%Ethanol used was obtained by diluting 99.5%ethanol to 98%and then added directly to the extracted oil to ensure complete solubility.

3. The preservative was then added with continuous stirring until fully dissolved.

4. Glycerin was gradually added while maintaining constant stirring for proper homogenization.

5. The natural sweetening agent is added followed by the gradual addition of sodium bicarbonate to adjust the pH of the final formulation.

6. Water was added gradually with continuous stirring to achieve a uniform final mixture.

7. The final formulation was stored in opaque containers at room temperature to maintain stability.

In **Figure 7,** decayed teeth were soaked with hydrogen peroxide, and its effectiveness was proven to whiten the teeth, as shown in the figure before and after using hydrogen peroxide. In **Figure 8**, hydrogen peroxide at rates of 3%, 2%, 1%, and 0.1% was used as an excipient to whiten teeth. Real teeth full of pigmentation was used. Hydrogen peroxide showed strong results as a teeth whitener, but it was excluded because the diameter of the zone of inhibition of H_2O_2 is three times greater than the diameter of the zone of inhibition of essential oils. In **Figure 9**, the zone of inhibition of H_2O_2 on yeast and bacteria at concentrations of 0.1% shows that the diameter of the zone of inhibition of H_2O_2 on bacteria is higher than the diameter of the zone of inhibition of H_2O_2 on yeast. This difference can be attributed to several structural and biochemical factors. Bacterial cells, especially Gram-positive types, have relatively simpler cell walls that are more susceptible to oxidative agents. In contrast, yeast cells possess a more complex and rigid cell wall composed of chitin and β-glucans, which act as a protective barrier against external stressors such as H_2O_2. Moreover, yeast cells often have more efficient antioxidant defense systems, including catalase and glutathione peroxidase enzymes, which help neutralize reactive oxygen species like hydrogen peroxide. Bacteria, on the other hand, may lack these robust enzymatic defenses or possess them at lower levels, making them more vulnerable to oxidative damage. Additionally, bacterial cell membranes tend to be more permeable, allowing H_2O_2 to penetrate and damage intracellular components more readily. These factors collectively explain the greater sensitivity of bacteria to H_2O_2 observed in the experiment.

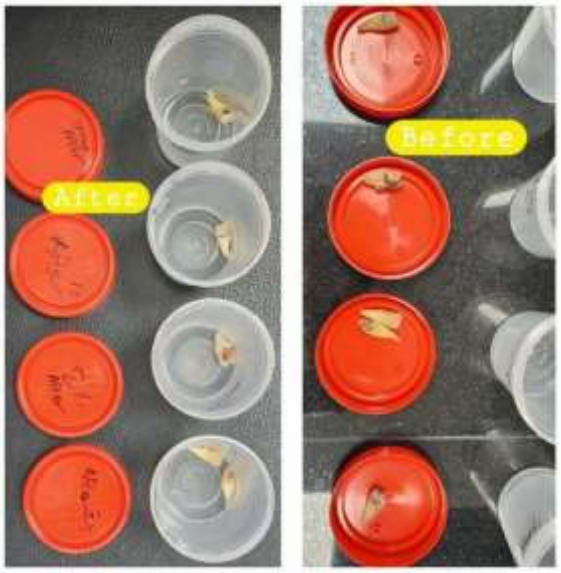

Figure 7: The effect of hydrogen peroxide whitening on teeth was evaluated using hydrogen peroxide concentrations of 1%,2%,and3%

Figure 8: Zone of inhibition of H_2O_2 on yeast and bacteria at concentrations of 1%, 2%, and 3%

Figure 9: Zone of inhibition of H_2O_2 on yeast and bacteria at concentrations of 0.1%

2.1.3. Analytical Microbiology Materials

Table 4 lists the analytical tools used in the microbiology analysis and their functions.
Table 4: Materials used in microbiology analysis.

No.	**Name**	**Source**	**Function**
1	Bacteria *S. A*	Al-Ahli Hospital Lab (ATCC 25923)	Measure antibacterial activity
2	*Candida albicans* fungus	Al-Ahli Hospital Lab (ATCC 10231)	Measure antifungal activity
3	NaCl 0.9%	Sigma-Aldrich CAS No:7647-14-5	To keep microorganisms alive
4	Alcohol 98%	Sigma-Aldrich CAS No:64-17-5	Diluent for oils

2.2.1. Culture media

Using the following culture media, antibacterial and antifungal activity tests were performed: Mueller, Hinton Broth (MHB). A culture is a test sensitivity test used to discover bacteria or fungi that might cause tooth decay. It determines which natural essential oils, such as peppermint, sage, and clove oil, will be most effective in treating tooth decay caused by bacteria and fungi. A Mueller-Hinton agar is inoculated with a liquid spray sample for a culture. Large "zones of inhibition" that encircle the discs indicate that the essential oils are inhibiting the current bacterial or fungal strain.
The absence of a "zone of inhibition" is a clear sign of antibiotic resistance. We used, 15% vegetable glycerine was combined with 5% clove oil, 5% sage oil, and 5% peppermint oil to make a final volume of 10 ml, which applied to culture media.

A. Serial dilution of a Stock solution preparation:

To precisely determine the quantity of germs present, the original sample must be diluted using a procedure known as serial dilution. Only after the substance is sufficiently diluted to produce distinct individual colonies can a computation be carried out. The successful identification, isolation, growth, and characterization of microorganisms depend on the capacity to precisely measure their concentration. Microbiologists have been accurately measuring the bacterial and virus load in clinical, industrial, pharmaceutical, and academic laboratory settings for more than a century using serial dilution and other plating techniques.
In **Figure 10**, one colony of *S. aureus* cells suspended in two milliliters of 0.9% NaCl makes up Solution 0. The new solution (solution 1) would contain half of the original concentration

of *S. aureus* in 1 mL of solution 0 were taken out and transferred to tube 1, which already contains 1 mL of normal saline. Solution 2, which only contains one *Aureus cell*, would be produced by repeating this procedure with 1 mL of solution 1 removed and added to another 1 mL of regular saline. We can determine that the dilution factor for this reduction is 50% because each new solution (1 mL of diluent plus 1 mL of solution) comprises a total of 2 ml.
Tube 0: 2 ml 0.9% NaCl, a single colony of bacteria, Tube 1: 1 ml 0.9% NaCl, Tube 2: 1 ml 0.9% NaCl, Tube 3: 1 ml 0.9% NaCl, Take 1000 µl from tube 0 to tube 1. Take 1000 µl from tube 1 to tube 2. Take 1000 µl from tube 2 to tube 3.

Figure 10: Serial dilution of a Stock solution preparation

B. Protocol for inoculating bacterial and fungal cultures and method

The fundamentals of bacterial cultivation and culture media have been discussed. Additionally, the various types of microbial culture media are described along with how to sterilize them. Using a marker, draw a line on the agar plate to indicate the division of a single colony of *Staphylococcus aureus* bacteria and *Candida* fungi. Mueller Agar plates are created by pouring Hinton agar into sterilized petri dishes and allowing it to solidify. Distributing bacteria or fungi onto the plates and allowing them to develop into separate colonies of that specific bacterium is conceivable. A particular bacterium would compete for nutrients in the broth or agar with other contaminating bacteria if it were to be developed. Certain bacteria can be dangerous and can skew test results for the effectiveness of antibiotics or other antimicrobial medicines, just like diseases.
For a zone of inhibition test, one million cells from a single bacterial or fungal strain are spread out onto an agar plate using a sterilized brush. The plate is then incubated with the antimicrobial item present. If the bacterial or fungal strain is responsive to the antimicrobial treatment, a distinct zone of microbial growth suppression will appear on the agar plate. If it is resistant to the antimicrobial treatment, growth is unaffected, and there is no observable clean zone. Therefore, before using any petri dishes or agar, it is imperative to disinfect them and inoculate the plates using aseptic techniques. An inoculating loop is one way that bacteria can propagate. It is sanitized by using a Bunsen flame to heat it to a red-hot temperature both

before and after use. To inoculate the agar, lift the cover of the Petri dish and tilt it. Don't take the lid off entirely or place it on the desk since it prevents airborne bacteria from contaminating the culture and vice versa. Bacterial development is influenced by a wide range of factors, such as temperature, pH, moisture content, and oxygen level. Nonetheless, cultural media has been sterilized; four effective steps are required to do this. The many sterilizing methods, such as filtration, high pressure, irradiation, and chemicals, could be summed up as follows. After inoculation, the Petri dish cover should be securely fastened with strips of adhesive tape. The sample must be labelled and dated. In a laboratory, inoculated agar plates are incubated for 24 hours at 35°C. This encourages the growth of cultural media. After streaking *S. aureus* ATCC 25923 on a blood agar plate, it was incubated for 24 hours at 35°C. A single bacterial colony was then spread out in blood agar broth and shaken for 24 hours at 35°C. To prepare it for future studies, the bacterial suspension was diluted to 0.5, 0.25, 0.125, and 0.0625 CFU mL^{-1}, placed in Molar-Hinton agar, and a disc of essential oils in varying concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%) was added. The *Staphylococcus genus* of bacteria, of which *Staphylococcus aureus* is the most common type, is responsible for *staph* infections.

The main difference is that while non-MRSA frequently reacts well to a variety of treatments, MRSA may only react to a limited number of them, making treatment more challenging. The following procedures are necessary since disk diffusion and MIC approaches both employ the phenotypic identification of susceptibility. By selecting colonies that have undergone total isolation and utilizing a bacterial culture to produce a standardized inoculum, the bacterial suspension—also known as the inoculum—is standardized using McFarland standards. The bacterial solution is diluted exclusively for the MIC technique.

The bacterial suspension is inoculated using one of the methods listed below. A specific growing media, like disk diffusion MHA or Mueller Hinton Agar Microorganisms, are examined for their capacity to grow visibly in broth (broth dilution) or on a succession of agar plates (agar dilution) that contain dilutions of the antimicrobial agent. In **Figure 11**, *Staphylococcus aureus subsp. aureus* ATCC 25923 is commonly used as a control strain for susceptibility testing to antibiotics and as a quality control strain for commercial products. We present the completed genome sequence for the strain, consisting of the chromosome and a 27.5-kb plasmid.

Figure 11*:* *S. aureus* ATCC 25923

C. Prepare filter paper discs:

The size of the disk filter paper used in the filter paper disk diffusion technique in microbiology is often measured in milliliters rather than inches. The 6 mm size of these filter paper disks is the most commonly utilized. The diameter of the filter paper disk is important because it determines the amount of test material that can be applied to the disk, which in turn affects the size of the zone of inhibition that develops when the disk is placed on an agar plate that has been infected by bacteria. Creating dried filter paper discs Using Whatman filter paper no. 3, discs with a diameter of roughly 6 mm are created, placed in a petri dish, and then sterilized in a hot air oven. The antibiotic delivery loop is made of 20-gauge wire and has a diameter of 2 mm. Antibiotics total 0.01 millilitres (10 microliters) per disk. Sterile discs were placed approximately 5 mm apart in petri dishes. Using a mechanical pipette, a fixed volume of 10 µl was applied to each disk separately, being cautious to maintain the pipette tip barely touching the disc.
Without covering the petri dishes, the discs were allowed to dry for 10 to 15 minutes at 35°C in a sterile incubator.

D. Measurement of Zone Diameters

Place the plates in the reading boxes after removing them from the incubator, being careful to align the tops. Beginning with the upper left-hand zone, measure the diameter of each zone on the medium's surface using needle-point calipers, as seen in **Figure 12**

.

Figure 12: Measuring the diameter of zones using vernier scale calipers.

2.2.3. Formulation development

Several primary trial formulations (PTFs) were prepared as part of the formulation development process. After testing and characterization, these PTFs were found to satisfy the acceptance requirements. The pH and viscosity of the successful trial formulations (STFs) were determined.

2.2.3.1. General method of preparation

Each component was weighed into a volumetric flask for every sample formulation. The antibacterial and antifungal properties of peppermint, sage, and clove oils were used to make the medicated tooth spray. Peppermint oil helps to avoid bad breath. To dissolve the oils, 98% ethyl alcohol was added. A complete breakdown of the oil was ensured by sonication and shaking. First, it was sonicated in the evaporating solvent to dissolve the oils. Oil preparation can be aided by the use of a magnetic stirrer. After the other ingredients were added and dissolved, vegetable glycerine was added as a humectant.
Stir and add slowly. Next, put Methylparaben as a preservative and stevia as a sweetener in a mixer. Once a homogenous solution has formed, gradually add 70% alcohol and the water

last, stirring at a slow speed to reduce foaming, and combine. To guarantee total homogeneity, it is sonicated once more for a few minutes at the end, kept in cool, dry, dark and well-sealed closed container.

2.2.3.2. The primary trial formulations (PTFs)

Generally, the trial formulations are composed of three active ingredients among the following:

- Active ingredients (clove oil, sage oil and peppermint oil)

-Solvent (98% ethyl alcohol)

- Excipients (Stevia, Glycerin, Methylparaben, $NaHCO_3$)

A. Characterization of the PTFs

Table 5 shows the acceptable criteria of PTFs, which have been evaluated.

Table 5: Film acceptance criteria of PTFs.

Acceptance criteria	
Stickiness	Not sticky
Staining	Not stain
Cosmetic appearance	Clear
pH	Basic
Viscosity	Moderate-Low

A. Step 1:

All of the formulations were determined to have good pH, good spreadability, viscosity characteristics, and cosmetic appearance attributes during our physiochemical evaluation investigations, which were conducted using a graduated syringe to spread an exact volume of liquid preparation.

A.1. Spread ability property:

In **Figure 13**, shows the packaging extrudability, Spreadability test, which should be, substrate application ease, appropriate dosage transfer to the desired location, and, most importantly, consumer preference. We covered the sterile gauze with 0.5 cc of the substance, utilizing the graded needle. In a matter of seconds, the product dispersed with a toothbrush and did not leave any trace and spread swiftly.

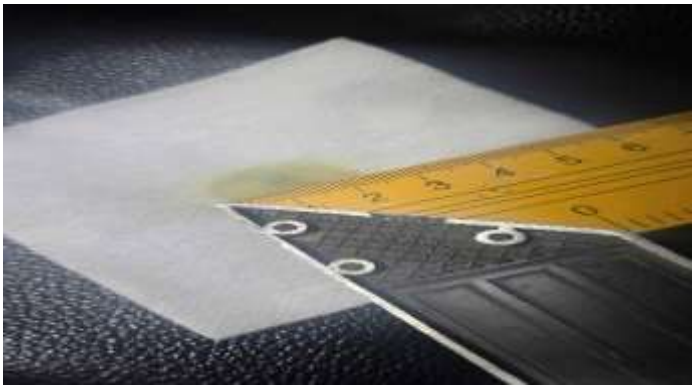

Figure 13: Spreadability test

A.2. Cosmetic appearance:

The resulting spray was homogeneous, clear, and had a pleasing color and scent, which was deemed acceptable.

A.3. Packaging

The product was stored for each formula in a dark container at room temperature.

2.2.3.3. The successful trial formulations (STFs)

The STFs were chosen based on the results of the test in step 1. Tests for pH, viscosity, and spreadability were performed on these compositions.

A. pH test: As a quality control (QC) test, pH was assessed. Using 27 ml of filtered water was used to dilute 3 ml of STF. Using a lab pH strip and a pH meter at room temperature, the pH of the supernatant was determined, and found to be 8.5(basic)

B. Viscosity test: We determined each STF's density (ρ1) by dividing its weight by its volume. We were sure that the Ostwald-Cannon-Fenske viscometer (**Figure14**) was dry, clean, and particle-free before beginning the test. To achieve equilibrium, the flask holding the STF and the viscometer were submerged in a water bath at 25°C ± 1 for five minutes. The reservoir was filled with the STF, and the viscometer was suspended vertically on a retort stand. In **Figure 14 (**Ostwald-Cannon-Fenske Viscometer), the parts are (1) the reservoir, (2) the pre-run sphere, (3) the measurement sphere, (A) the capillary tube, (B) the venting tube, and (C) the upper and lower time marks. From the venting tube (A). The capillary (B) is used to suck the liquid out of the tube so that it reaches the pre-run sphere above the upper time mark (C). After that, the suction is stopped, allowing the liquid to flow freely. The time it takes for the meniscus to move between (C) and (D) is measured using an accurate stopwatch. The experiment was conducted three times (n=3) for each STF, and the average time (t1) was recorded. The Viscometer was cleaned twice with acetone and then completely dried before being used again.

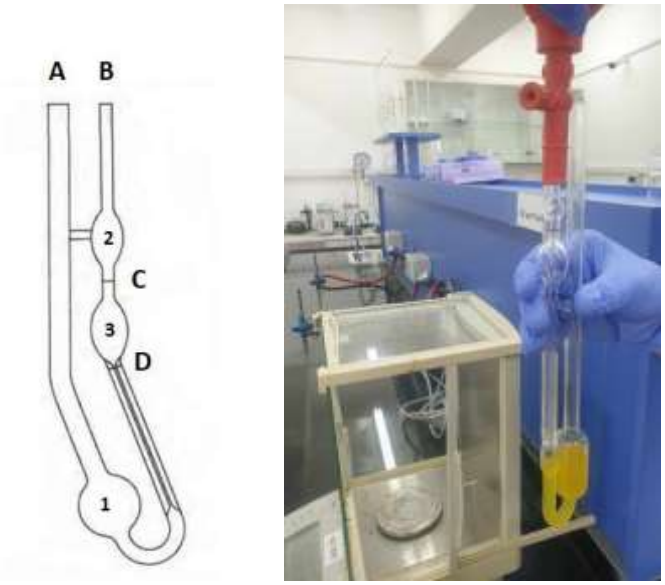

Figure 14: Ostwald-Cannon-Fenske viscometer (Sinko, 2013).

2.2.3.4. The primary formulations

Numerous trial formulations (with different ingredients) were chosen from the STFs in order to produce the primary formulations loaded with natural essential oils. To determine whether the final product would still meet the acceptance requirements, as mentioned in step 1, after tests were conducted on these formulations.

2.2.4. Stability Studies

The stability of the successful formulation was studied under two storage conditions at zero time and after incubation for 2 months (**Table 6**). The sample was taken from the chamber, and tests again were done, such as pH, viscosity, and microbial tests. Protected from light for each time period and for each storage conditions.

Table 6: The incubation period of stability tests under specific storage conditions.

Study	**Storage condition**	**Incubation period(months)**
Long term	**25°C ± 2°C/60% RH ± 5%**	**2**
Accelerated	**40°C ± 2°C/75% RH ± 5% RH**	**2**

Chapter Three:

Results and discussion:

As shown in **Table7**, we conducted six experiments with the aim of diluting essential oils. When we tested the diluted oils using the selected substances, when we applied the oil directly to the cultured bacteria and fungi, no results were observed- there was no antibacterial or antifungal activity. Therefore, these substances were excluded from further testing. Six trials of experiments were conducted to test the effectiveness of diluted essential oils. Only experiment number 6 showed positive antibacterial and antifungal effects. Therefore, the substances that showed no activity were excluded from further testing. In the initial experiment, dimethyl Sulfoxide (DMSO) was used. Although this approach successfully solubilized the oil, DMSO was excluded from the final formulation due to its unsuitability for oral use. DMSO possesses high mucosal and transdermal permeability, which allows it to transport dissolved compounds directly into the bloodstream through the oral tissues. This characteristic poses a potential health risk when used with bioactive ingredients such as essential oils, which may cause irritation or systemic toxicity if absorbed in significant amounts. Additionally, DMSO has an unpleasant odor and taste, making it incompatible with oral hygiene applications. In the second experiment, paraffin oil was used as a solvent to dilute essential oils for oral spray application. Despite being miscible with essential oils, paraffin oil proved unsuitable for this formulation; its high viscosity and complete immiscibility with water prevented proper dispersion in the aqueous phase of the spray, leading to phase separation and an unstable emulsion. Additionally, paraffin oil left an oily residue in the oral cavity and was unsuitable for oral use. Therefore, this method was considered a failure and excluded from further development. In the third experiment, 70% ethyl alcohol (ethanol) was used to dissolve essential oils for incorporation into a tooth spray formulation. While ethanol is generally considered safer than methanol and is widely used in pharmaceutical and cosmetic products, the formulation was not successful. At this concentration, the essential oils were only partially miscible, and the solution showed signs of phase separation after standing, indicating poor long-term stability. Additionally, the high water content (30%) in 70% ethanol is for hydrophobic essential oils. In the fourth experiment, 95% ethyl alcohol (ethanol) was used to dissolve essential oils in the preparation

of a tooth spray formulation. Although the oils appeared to dissolve initially, the formulation failed due to several issues. The high volatility of 95% ethanol led to rapid evaporation, which compromised the stability and consistency of the spray. Additionally, when microbiological tests were performed, the formulation showed no significant antibacterial or antifungal activity. In the fifth experiment, we used 98% ethyl alcohol as a solvent to dissolve essential oils for a tooth spray formulation. This approach was successful in terms of solubility, and the resulting formulation exhibited clear antibacterial and antifungal activity during microbiological testing. However, the experiment was not considered valid due to a procedural error: the Mueller-Hinton agar plates, on which the alcohol-dissolved oils had been inoculated, were accidentally left outside the incubator for 24 hours prior to incubation. This led to the appearance of abnormal inhibition zones (double zones), which made the antimicrobial results unreliable. Although 98% ethanol proved to be the most effective solvent in terms of oil solubility and initial biological activity, the experiment will be repeated under proper conditions to confirm the validity of the results. The sixth experiment was the successful formulation, in which 98% ethyl alcohol was used as the solvent to dissolve the essential oils. Initially, microbiological tests were conducted using 0.5 McF bacterial suspensions, and the antimicrobial activity observed was moderate. To enhance accuracy, serial dilution of bacteria was performed, reducing it to 0.25, 0.125, and 0.0625 McF units. At these lower bacterial densities, the essential oils demonstrated strong antibacterial and antifungal activity, proving to be the most effective and reliable among all tested preparations. 98% ethanol provided better solubility and stability, resulting in consistent antibacterial and antifungal effects. Additionally, the use of DMSO (dimethyl Sulfoxide) and paraffin oil as solvents was also unsuccessful. While both agents were able to dissolve the essential oils, they failed to produce any significant antimicrobial activity. This is likely due to their incompatibility with aqueous environments and poor dispersion in aqueous microbiological media, which limits the diffusion of active compounds toward microbial colonies. Moreover, paraffin oil's high viscosity and DMSO's potential toxicity in oral formulations further contributed to the rejection of these solvents for mouth spray development. Both DMSO and paraffin oil were able to dissolve the essential oils due to their chemical compatibility with hydrophobic compounds. However, solubility alone does not guarantee effective distribution within the formulation or the microbiological testing medium. In the case of paraffin oil, its high viscosity and immiscibility with aqueous environments hindered the proper diffusion of active compounds, preventing them from reaching microbial cells. Similarly, while DMSO is a powerful solvent, its limited compatibility with oral formulations and potential toxicity made it unsuitable for use in a mouth spray. As a result, despite their solubilizing capacity, both solvents failed to support antimicrobial activity and were excluded from further consideration. Method No. 6 was the successful experiment after 5 failed experiments. 98% alcohol was used to dilute the oils, and satisfactory results emerged that proved the effectiveness of natural essential oils in eliminating bacteria and fungi. As shown in **Table 8**, the formula was prepared using the three essential oils tested as active ingredients: clove oil, peppermint oil, and sage oil. Each oil was prepared in six different concentrations to evaluate its antimicrobial efficacy. The dilutions were as follows: 100% concentration: 50 µL of pure essential oil; 5% concentration: 950 µL ethanol + 50 µL essential oil; 3% concentration: 970 µL ethanol + 30 µL essential oil; 2% concentration: 980 µL ethanol+20 µL essential oil, 1% concentration: 990 µL ethanol + 10 µL essential oil; 0.5% concentration: 995 µL ethanol + 5 µL essential oil. Excipients were then added to achieve a tooth spray with the desired chemical and physical activity.

3.1. Table7: Preparation of essential oil dilutions using different solvents

Material	**5% Oil(µL)**	**2% Oil(µL)**	**1% Oil(µL)**	**0.5% Oil(µL)**	**Total Volume(µL)**	**Antimicrobial Activity**
DMSO	50	20	10	5	1000	No antimicrobial activity
Paraffin Oil	50	20	10	5	1000	No antimicrobial activity
70%Ethyl Alcohol	50	20	10	5	1000	No antimicrobial activity
95% Ethyl Alcohol	50	20	10	5	1000	No antimicrobial activity
98% Ethyl Alcohol	50	20	10	5	1000	Used in tooth spray formula(antifungal/antibacterial activity)

3.2. Table8: Tooth spray formula

No.	**Ingredient**	**Quantity**
1	Clove oil	5%
2	Peppermint oil	5%
3	Sage oil	5%
4	70%v/v alcohol	5ml
5	Glycerin	15%
6	Saccharine	0.1%
7	Sodium bicarbonate	0.8%
8	Methylparaben	0.1%
9	Distilled water	2 ml
	Sample Size	**10 ml**

3.3. Microbiological analysis

The Inhibition Zone Test Overview. To produce a specific strain of bacteria or fungus, pure culture is utilized. A sterile swab is used to evenly apply a suspension of the pure culture to the face of a sterile agar plate. The antimicrobial agent is injected into the center of the agar plate to stop it from spreading outward around the plate. A hole can be poked into the center of an agar to represent a liquid. For24 hours, the agar plate is incubated at a temperature suitable for the test microbe. If the antimicrobial agent penetrates the agar and stops growth, a clear area known as the zone of inhibition encircles the test product. The size of the zone of inhibition usually corresponds to the level of antimicrobial activity in the sample or product; a larger zone of inhibition usually denotes a more potent antibacterial. Zone of inhibition testing is quick and inexpensive when compared to other laboratory tests for antimicrobial activity. Zone of inhibition assays can be used to assess the potential of water-soluble

antimicrobials to halt the growth of germs, albeit qualitatively. Many samples quickly assessed for antibacterial properties using this test method. It was possible to investigate the generation of a zone of inhibition by liquids, coated antimicrobial surfaces, and solid objects impregnated with antimicrobials. In **Figures 15**, the zone of inhibition by clove oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%) is graphically represented by the zone of inhibition at 0.5, 0.25, 0.125, and 0.0625 McF by clove oil.

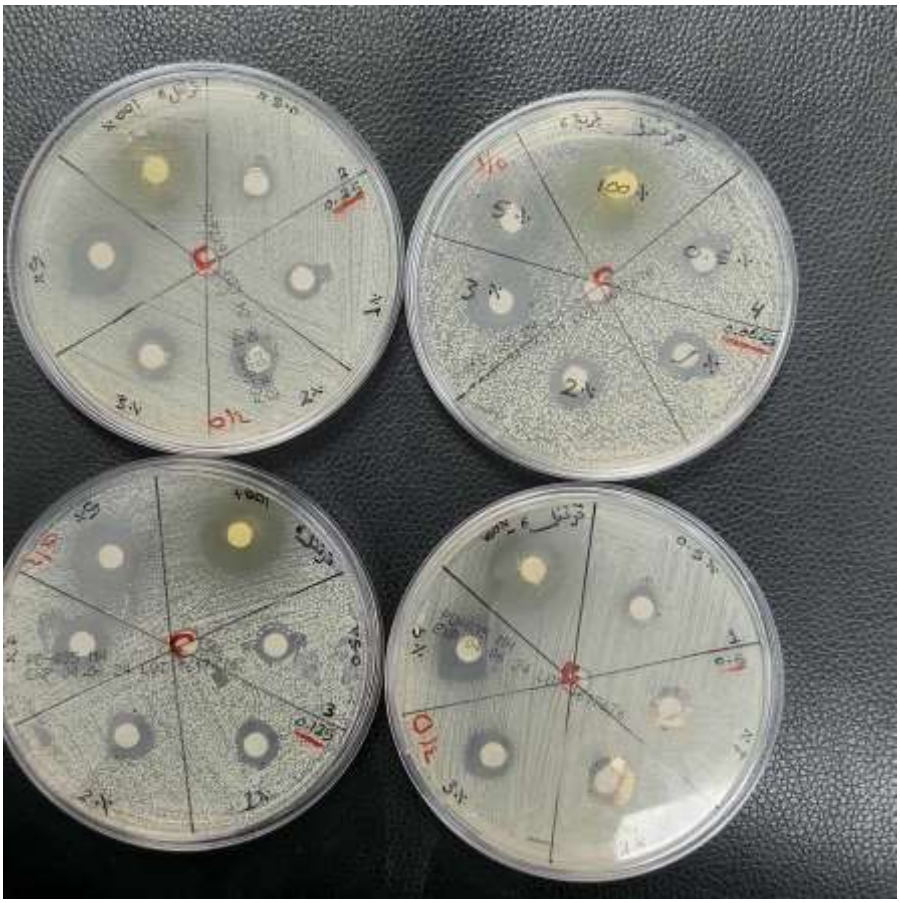

Figure 15: Zone of inhibition by clove oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%)

Table9: Diameter of zone of inhibition in (mm) by clove oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%)

Clove oil concentration	Diameter of Zone of inhibition on 0.5 McF	Diameter of Zone of inhibition on 0.25 McF	Diameter of Zone of inhibition on 0.125 McF	Diameter of Zone of inhibition on 0.0625 McF
0.5%	6mm	6mm	9mm	9mm
1%	9mm	6mm	11mm	12mm
2%	10mm	7mm	11mm	13mm
3%	12mm	11mm	13mm	16mm
5%	15mm	15mm	15mm	19mm
100%	19mm	19mm	19mm	21mm

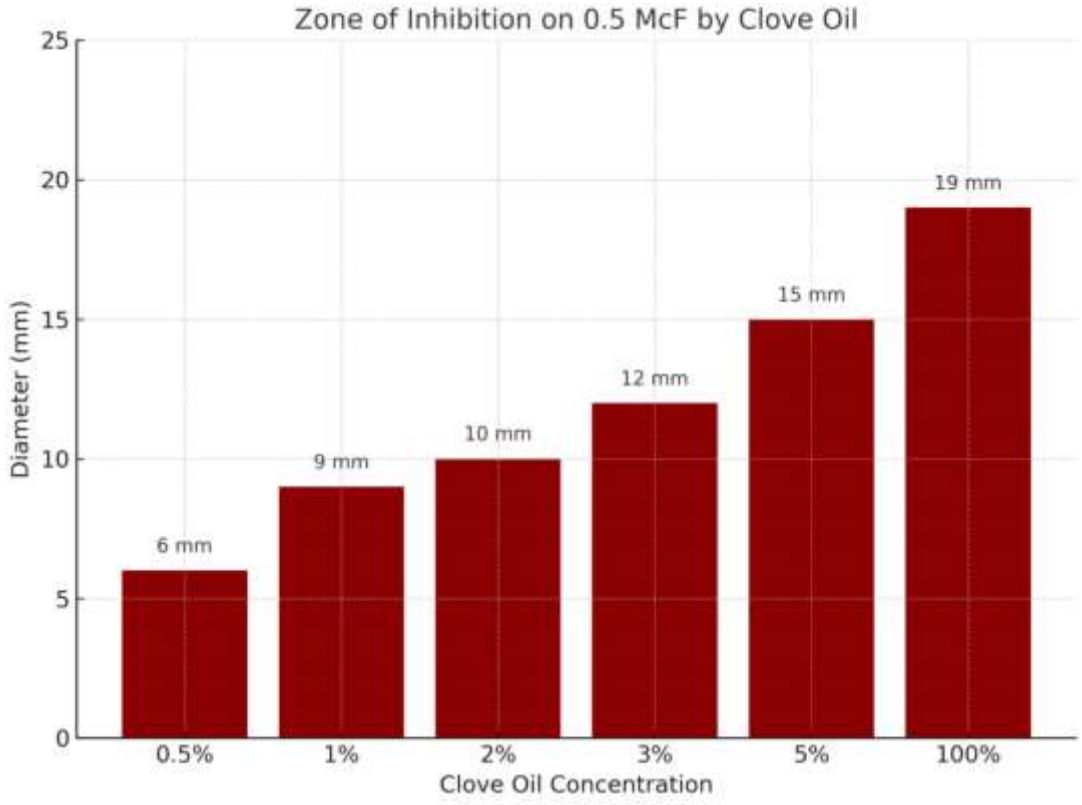

Figure 16: Graphical representation of zone of inhibition on 0.5 McF by clove oil

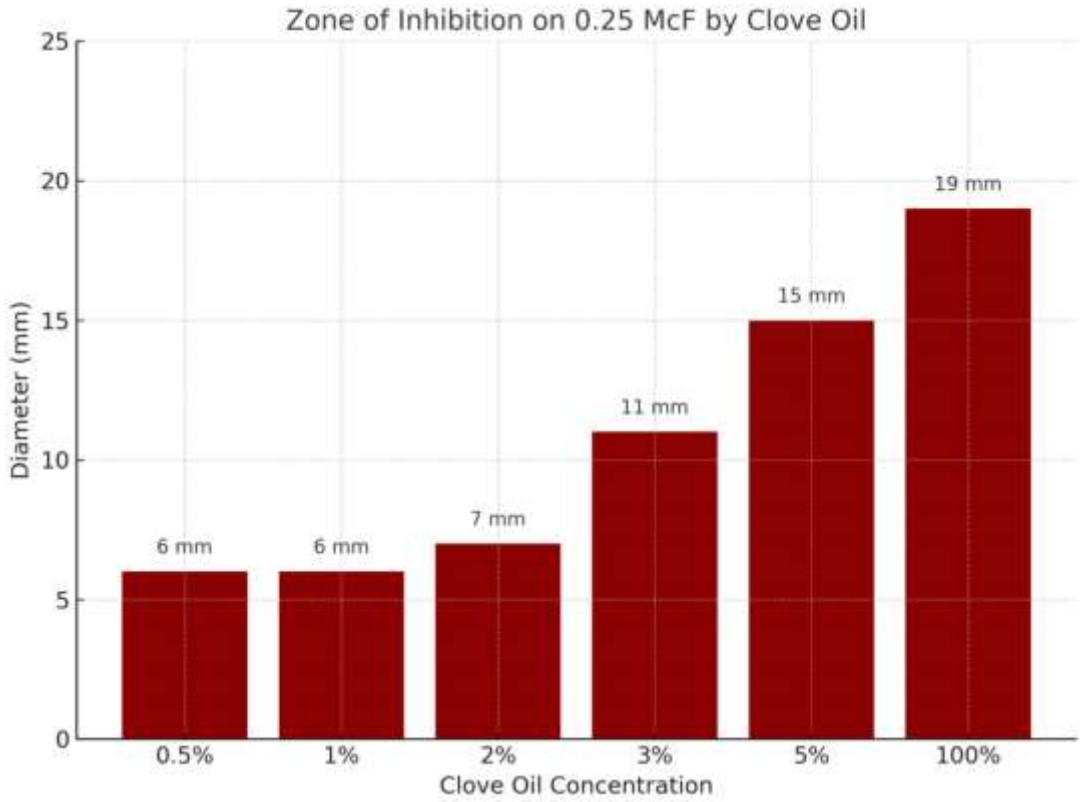

Figure 17: Graphical representation of zone of inhibition on 0.25 McF by clove oil

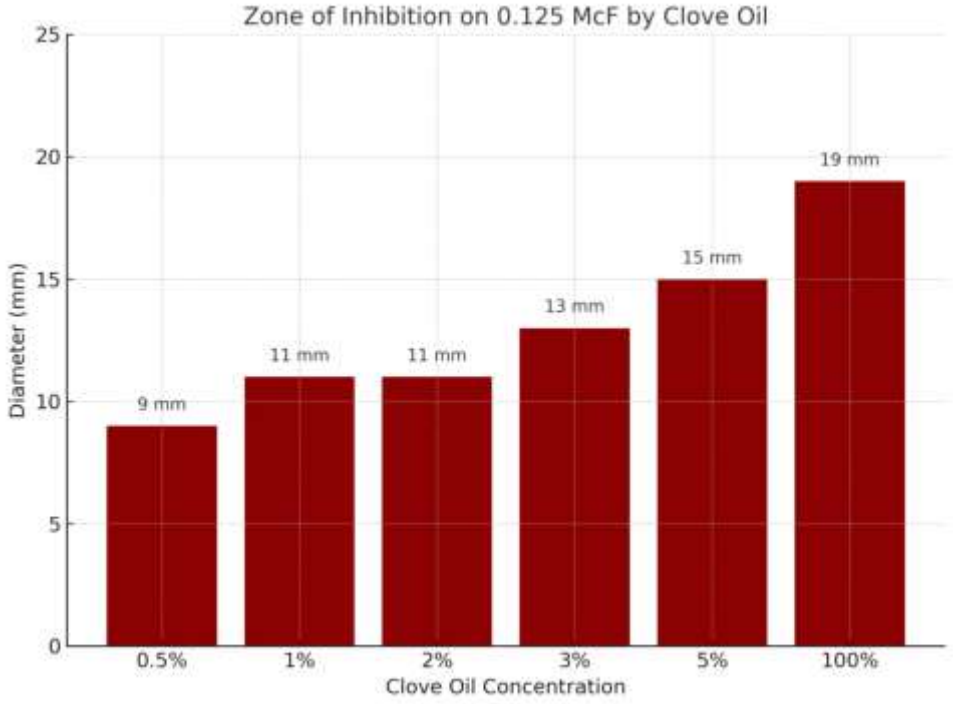

Figure 18: Graphical representation of zone of inhibition on 0.125 McF by clove oil

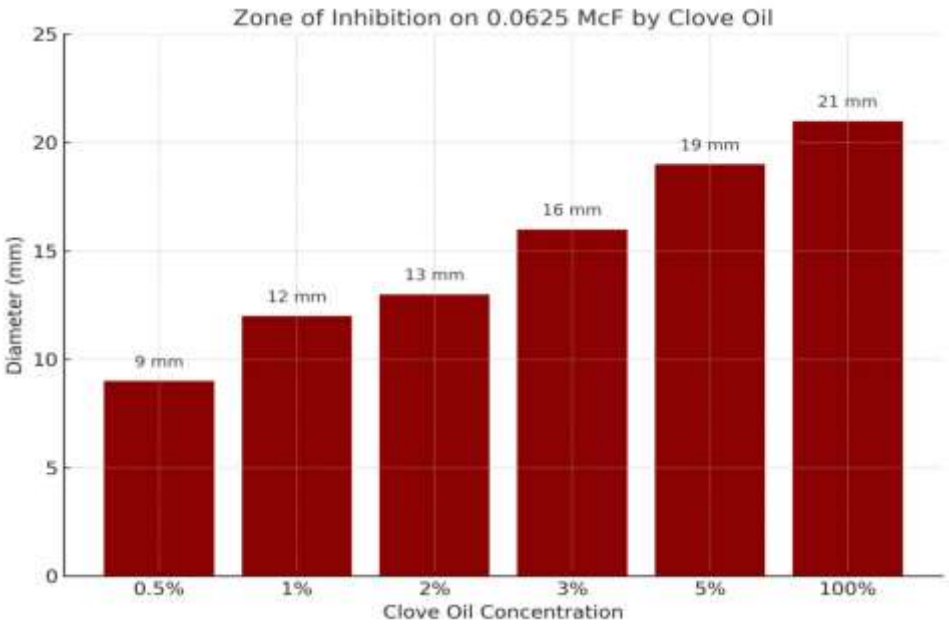

Figure 19: Graphical representation of zone of inhibition on 0.0625 McF by clove oil

Table 9 summarizes the effectiveness of clove oil against bacteria. The oils concentration affected the inhibitory zones diameter. At 100% concentration, the greatest inhibition was detected, whereas at 0.5% concentration, the least effect was noted. On discs impregnated with 0.5%,1%,2%,3%,5%,and 100%(v/v) oil, respectively, the inhibition zones measured were 6,7,9,10,11,12,13,15,19,and 21 mm. None of the tested isolates were inhibited by the oil at 0.5 %(v/v). The zone of inhibitions diameter grows as the bacterial concentration falls. The diameter of the zone of inhibition grows as the concentration of clove oil increases.

Table10: Diameter of zone of inhibition in (mm) by sage oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100

Sage oil concentration	**Diameter of inhibition zone on 0.5 McF**	**Diameter of Zone of inhibition on 0.25 McF**	**Diameter of Zone of inhibition on 0.125 McF**	**Diameter of Zone of inhibition on 0.0625 McF**
0.5%	6mm	6mm	6mm	6mm
1%	6mm	6 mm	6mm	6mm
2%	6 mm	6 mm	6 mm	6 mm
3%	6 mm	6 mm	6 mm	6 mm
5%	9mm	6 mm	10mm	10mm
100%	13mm	15mm	16mm	17mm

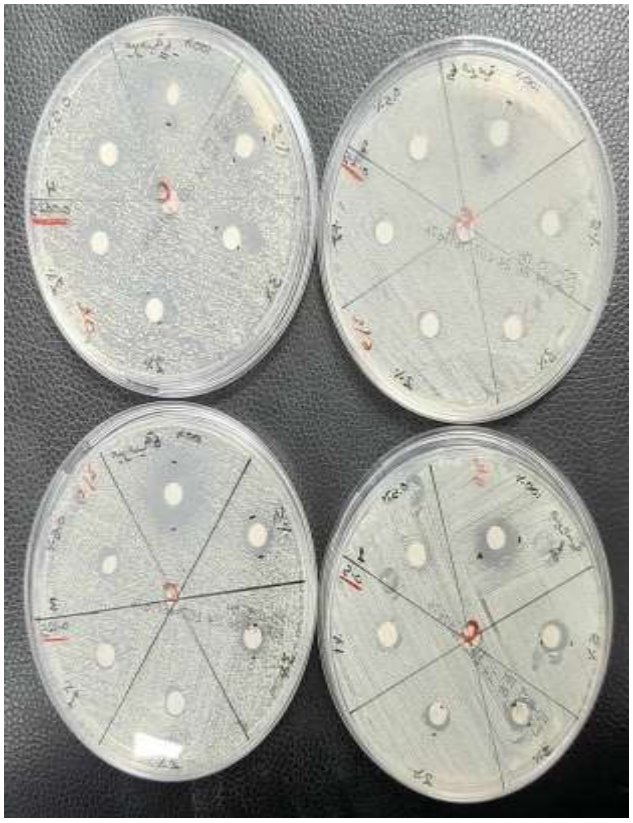

Figure 20: zone of inhibition by sage oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%)

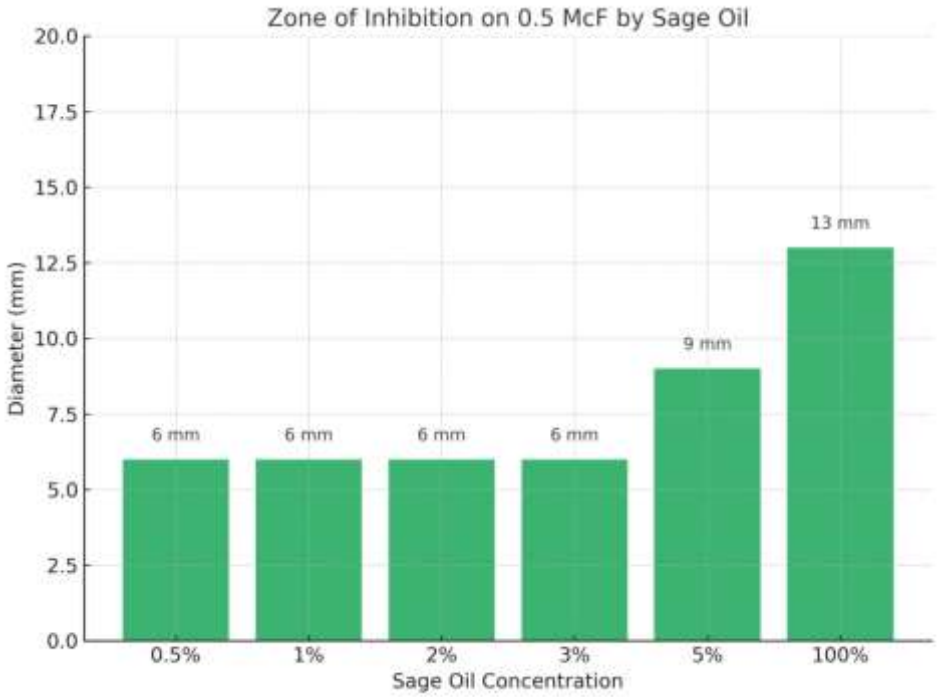

Figure 21: Graphical representation of zone of inhibition on 0. 5 McF by sage oil

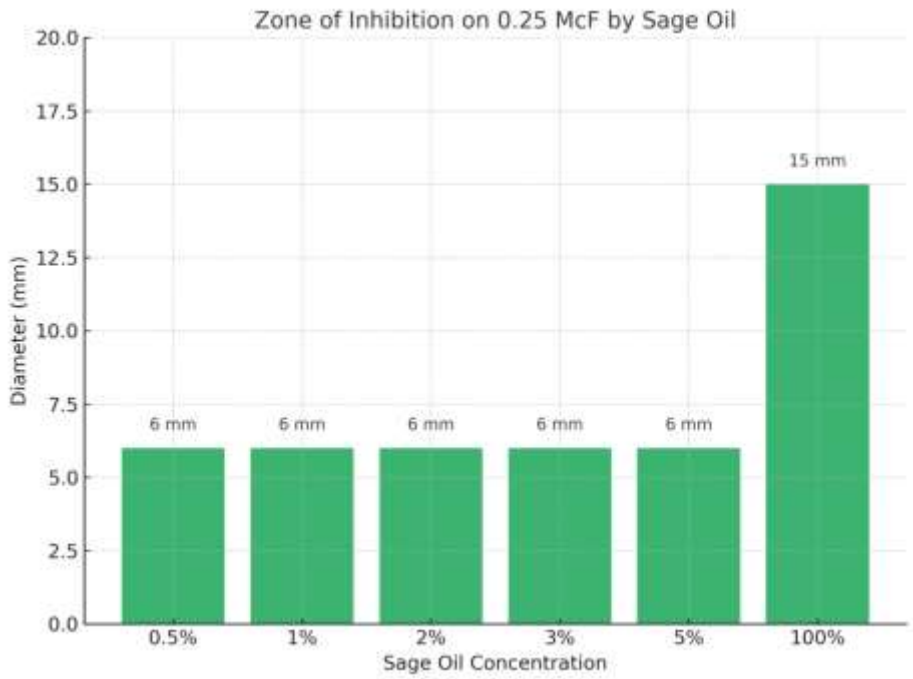

Figure 22: Graphical representation of zone of inhibition on 0.25 McF by sage oil

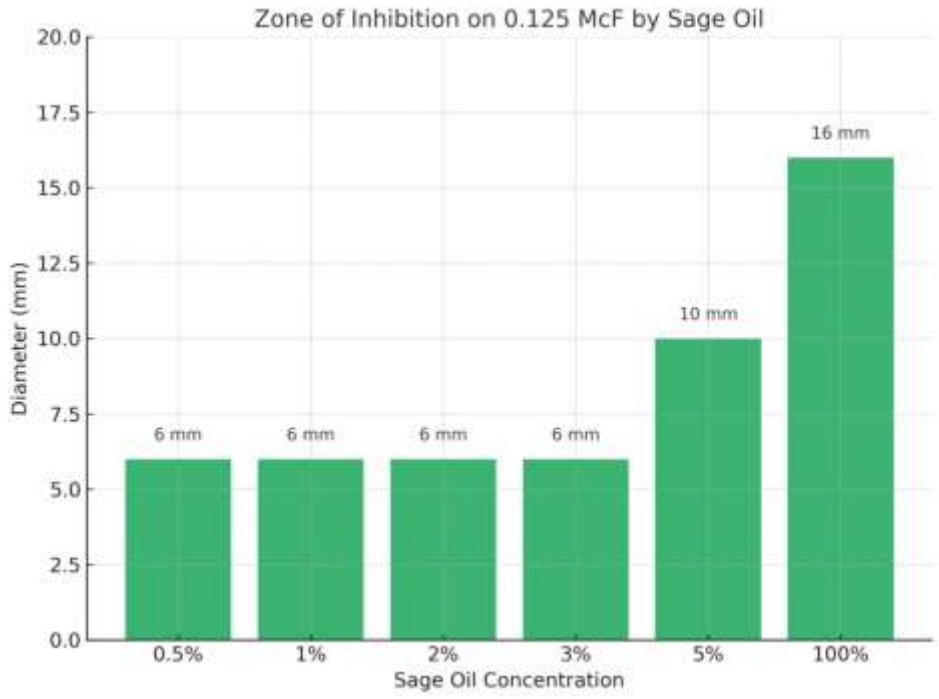

Figure 23: Graphical representation of zone of inhibition on 0.125 McF by sage oil

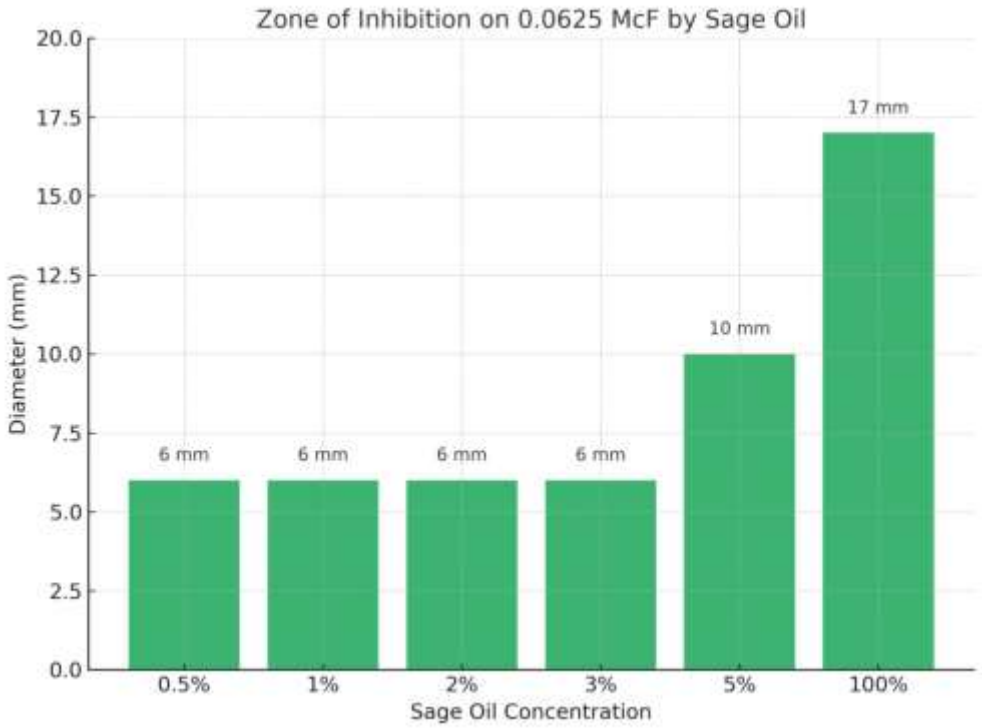

Figure 24: Graphical representation of zone of inhibition on 0.0625 McF by sage oil

Table10 summarizes the effectiveness of sage oil against bacteria. The oil's concentration affected the inhibitory zones' diameter. The concentrations of 0.5%, 1%, 2%, and 3% had the least effect, while 100% concentration showed the greatest inhibition. On discs impregnated with 0.5%, 1%, 2%, 3%, 5%, and 100% (v/v) oil, respectively, the inhibition zones measured were 9, 10, ,13, 15, 16, and 17 mm. The zone of inhibition's diameter grows as the bacterial concentration falls. The zone of inhibition's diameter grows as the sage oil concentration rises.

In **Figure20,** the zone of inhibition by sage oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%) is graphically represented by the zone of inhibition at 0.5, 0.25, 0.125, and 0.0625 McF by sage oil.

Figure25: Zone of inhibition by peppermint oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%)

Table11: Diameter of zone of inhibition in (mm) by peppermint oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%)

Peppermint oil Concentration	**Diameter of inhibition zone on 0.5 McF**	**Diameter of inhibition zone on 0.25 McF**	**Diameter of inhibition zone0.125 McF**	**Diameter of inhibition zone on 0.0625 McF**
0.5%	6mm	6mm	6mm	11mm
1%	7mm	7mm	8mm	13 mm
2%	8mm	8mm	8 mm	11mm
3%	9mm	9mm	8 mm	12 mm
5%	11mm	11mm	10mm	11 mm
100%	17mm	18mm	21mm	24 mm

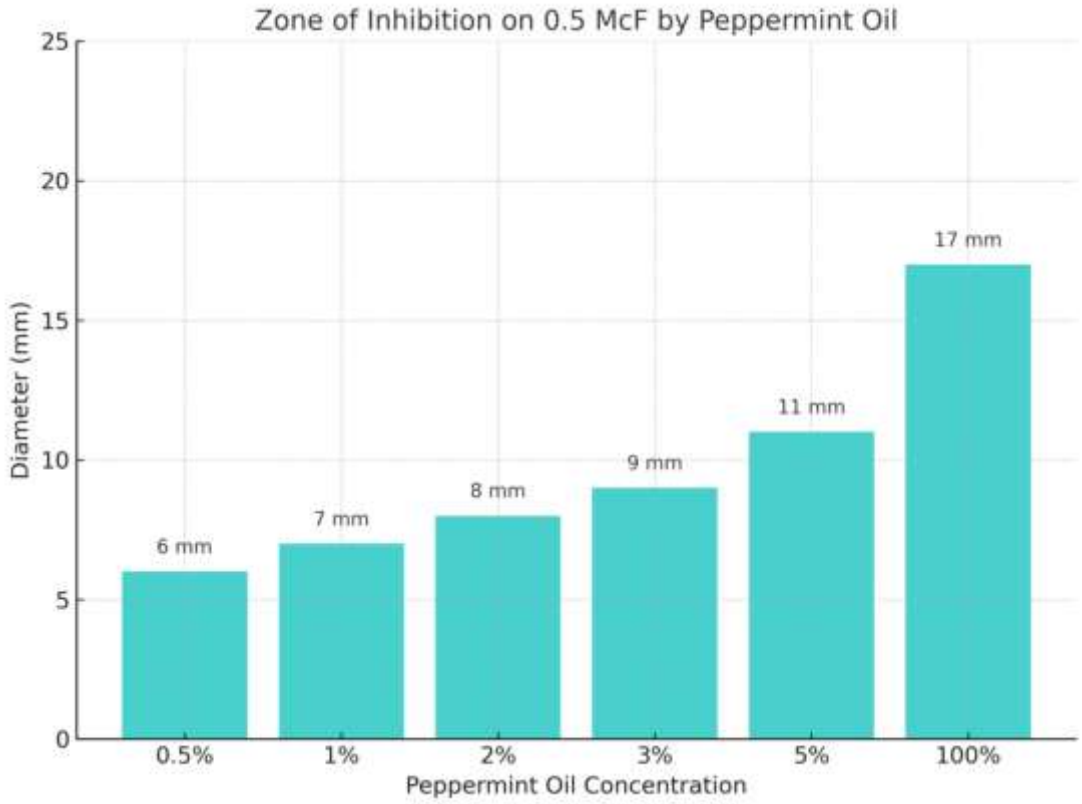

Figure26: Graphical representation of zone of inhibition on 0.5 McF by peppermint

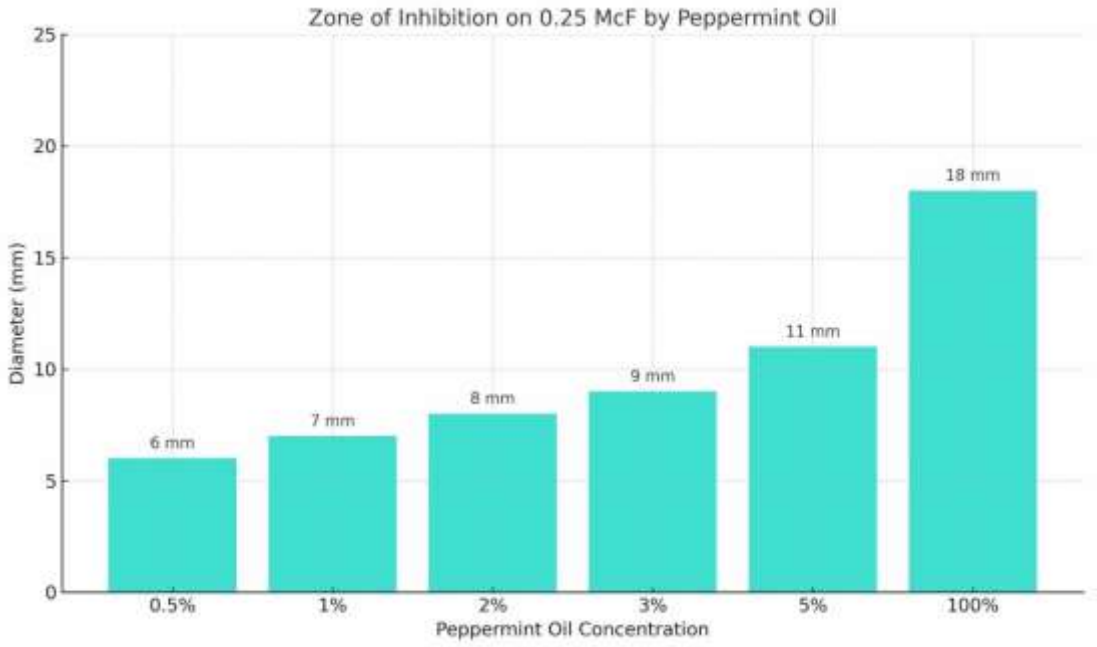

Figure27: Graphical representation of zone of inhibition on 0.25 McF by peppermint oil

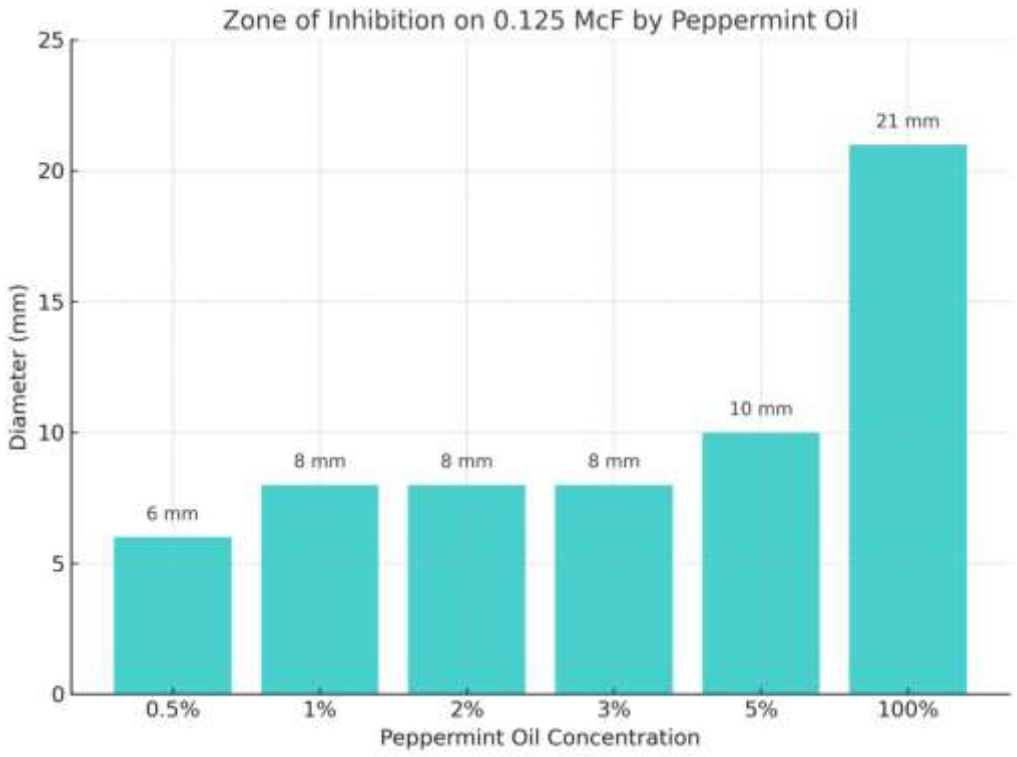

Figure28: Graphical representation of zone of inhibition on 0.125 McF by peppermint oil

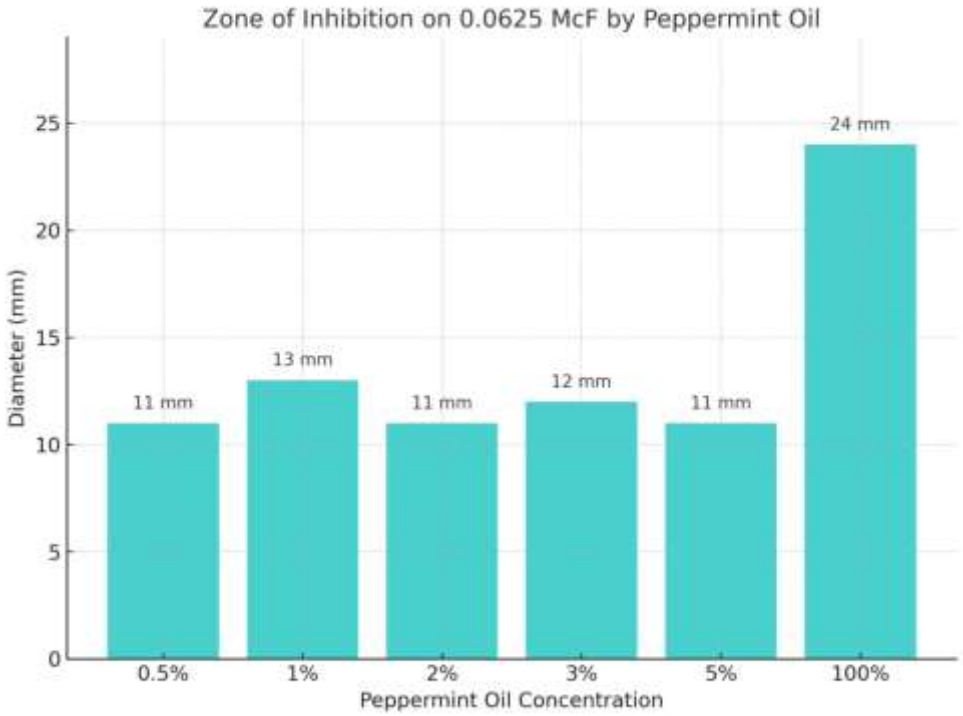

Figure29: Graphical representation of zone of inhibition on 0.0625 McF by peppermint oil

Table 11 summarizes the effectiveness of peppermint oil against bacteria. The oil's concentration affected the inhibitory zones' diameter. At 100% concentration, the greatest inhibition was seen, whereas at 0.5%, the least effect was noted. The discs impregnated with 0.5%, 1%, 2%, 3%, 5%, and 100% (v/v) oil, respectively, had inhibition zones of 7, 8, 9, 10, 11, 13, 17, 18, 21, and 24 mm. The zone of inhibition's diameter grows as the bacterial concentration falls. The zone of inhibition's diameter grows as peppermint oil concentration rises. In **Figure25**, the zone of inhibition by peppermint oil in different concentrations (0.5%, 1%, 2%, 3%, 5%, and 100%) is graphically represented by the zone of inhibition at 0.5, 0.25, 0.125, and 0.0625 McF by peppermint oil.

In **Figures 30-31**, the combination of three essential oils together has proven effective in its antibacterial and antifungal activity against tooth plaque; each of the three essential oils—clove oil, peppermint oil, and sage oil—was incorporated at a concentration of 5%, resulting in a total of 15% essential oils in the final formulation. To achieve this, 50 microliters of each oil are dissolved with 98% ethyl alcohol at a volume of 950 microliters of 98%. Oils have the ability to kill bacteria and fungi, but according to the results, their ability to kill fungi is higher than their ability to kill bacteria. In **Table 12**, the efficacy of the three oils was demonstrated when they were combined, but when other ingredients were added and the finished product was produced, the preparation's efficacy rose, demonstrating the product's success. The inhibition zone diameter against *Staphylococcus aureus* bacteria was 12 mm, and against *Candida* yeast it was 16 mm. In **Table 13**, the inhibition zone diameters caused by the tooth spray are presented. The diameter of the inhibition zone against *Staphylococcus aureus* bacteria was 16 mm, while the inhibition zone against *Candida* yeast measured 22 mm. These results indicate that the spray exhibited stronger antifungal activity compared to its antibacterial effect.

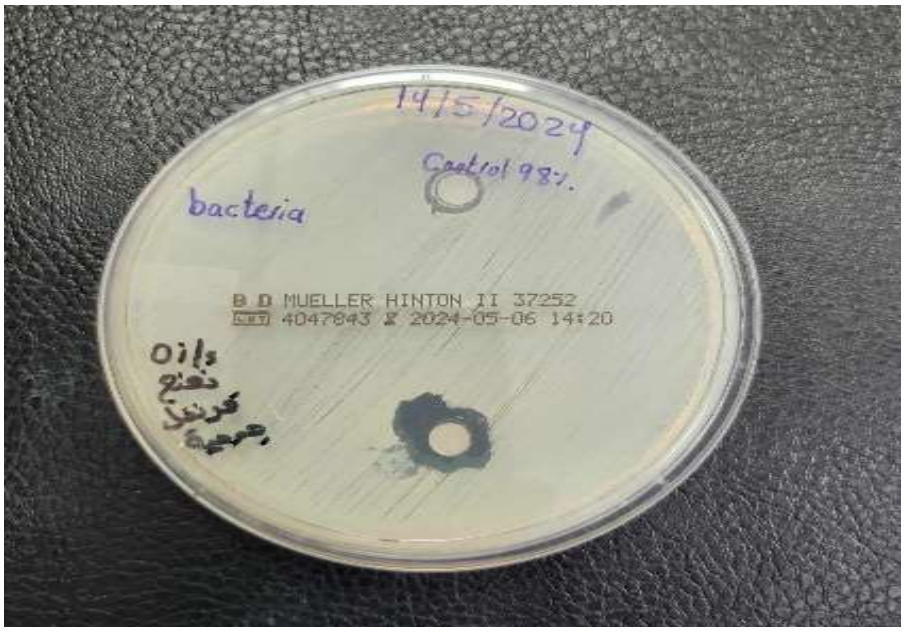

Figure30: Zone of inhibition by clove, sage, and peppermint oil on ***S.A***. bacteria

Figure31: Zone of inhibition by clove, sage, and peppermint oil on ***candida*** yeast

Table12: Inhibition zone diameter in (mm) by clove, sage, and peppermint oil on bacteria and yeast

Essential oil	**Inhibition zone diameter in (mm)**
Clove, sage, and peppermint oil on *S.A.* bacteria	12 mm
Clove, sage, and peppermint oil on *candida yeast*	16 mm

Table13: Inhibition zone diameter (mm) by tooth spray on bacteria and yeast

Tooth spray	**Inhibition zone diameter in(mm)**
Tooth spray on *S.A.* bacteria	16 mm
Tooth spray on *candida* yeast	22 mm

Based on the previous results, the antimicrobial activity of clove, peppermint, and sage essential oils was confirmed against both bacteria and yeast. The data showed that as the bacterial concentration decreased (e.g., at 0.0625 McFarland), the diameter of the inhibition zone increased, indicating enhanced effectiveness of the oils. Increasing the concentration of each oil led to a corresponding increase in the diameter of the inhibition zone, which can be attributed to the higher presence of active antimicrobial compounds such as eugenol in clove oil, menthol and menthone in peppermint oil, and thujone in sage oil. At a bacterial concentration of 0.0625 McFarland, peppermint oil at 100% concentration exhibited the

largest inhibition zone (24 mm), followed by clove oil (21 mm) and sage oil (17 mm). These differences highlight the varying chemical compositions and potencies of the oils' active ingredients. This inverse relationship can be explained by the reduced bacterial load, which allows the active components in the oils to act more efficiently and diffuse more freely in the medium, resulting in a larger inhibition zone. Despite their different phytochemical compositions, the three oils share several antimicrobial constituents, such as phenolic compounds, terpenes, and flavonoids. These shared properties provide a degree of compatibility and potential synergy, which justifies their blending in formulations. However, the combination of the three oils alone resulted in a bacterial inhibition zone of 12 mm and an antifungal zone of 16 mm. In comparison, the formulated tooth spray, which contains these essential oils along with pharmaceutical excipients, demonstrated enhanced activity—producing a 16 mm zone against bacteria and a 22 mm zone against yeast. This increase in efficacy could be due to the presence of excipients that may improve oil solubility, stabilize active compounds, or enhance their diffusion, thereby amplifying the antimicrobial effects.

In **Figure 32**, the zone of inhibition caused by the tooth spray on both *Staphylococcus aureus* and *Candida* are illustrated .The spray produced a 22 mm inhibition zone against Candida. And a 16mm zone against Staphylococcus aureus, indicating greater antifungal than antibacterial activity.

Figure 32: Zone of inhibition by tooth spray on bacteria and yeast

In **Figure 33**, the absence of ZOI in the Mueller Hinton agar suggests that the organism is resistant to the tested antimicrobial and that the antimicrobial agent has no antimicrobial effect on it. In the case of the control, no inhibition zone was seen. The discs containing 70% and 98% ethyl alcohol, methylparaben, and $NaHCO_3$ did not exhibit an inhibitory zone. This suggests that the growth of bacteria was unaffected by these components. The success of the

tooth spray depends on the oils' ability to eradicate germs and fungi; the addition of materials does not negate the oils' ability to do so.

Figure33: zone of inhibition by 98%, 70% alcohol, 0. 1% methyl paraben, and 0.8% NaHCO3 on yeast and bacteria

3.3.1 Table 14: properties of materials used in tooth spray

The properties of materials used in tooth spray are summarized in Table 12

	Clove oil	**Peppermint oil**	**Sage oil**	**Vegetable glycerin**	**Methylparaben**	**NaHCO3**	**Stevia**
Odor	Clove bud essential oil	Sharp odor that's cool and refreshing	Warm camphoraceous odor	No odor	Odorless	Odorless	Odorless
Color	Colorless to pale yellow	Pale yellow	Colorless to yellow liquid	Transparent	White powder	White crystalline powder	White
Smell	Like cloves with a sweet aspect.	Fresh, sharp, menthol smell	Herbaceous scent with notes of camphor and eucalyptus	No smell	No smell	No smell	No smell
Taste	Warm and spicy with faint touches of cinnamon	Similar to odor	Bitter taste	Sweet	Slight burning taste	Slightly alkaline (bitter) taste	More intense than sugar
Component	Eugenol	Menthol and menthone	Camphor, thujone, and pinene.	$C_3H_8O_3$	$C_8H_8O_3$	Sodium cation and a bicarbonate anion	Stevioside and rebaudioside A
Functions	Active ingredient	Active ingredient	Active ingredient	Humectant	Preservative	Neutralize acid	Sweet agent

4.3.2. The primary formulations

The trial formulations chosen from the STFs containing essential oil are the main formulations. We selected various components that were thought to be effective in removing dental decay. Step 1 described the primary formulas. Based on the parameters and outcomes, it was determined that the tooth spray containing natural substances is highly effective, as shown in **Table 2**.

4.4. Stability studies

The stability of the successful formulation was studied under two storage conditions at zero time and after incubation for 2 months **(Table15**). The sample was taken from the chamber, and tests were done, such as pH, viscosity, and microbial tests. Protected from light for each period again for each storage conditions. The results of the stability are shown in **Table15.**

Table15-a: The stability study of the spray formulation at 0 time and after incubation for 2months under **25°C ± 2°C/60% RH ± 5%**.

Time (months)	**pH**	**Viscosity**	**Diameter zone of inhibition (Bacteria)**	**Diameter zone of inhibition (Yeast)**
0	8.53	0.223	16 mm	22 mm
2	8.59	0.227	11 mm	23 mm

Table15-b: The stability study of the spray formulation at 0 time and after incubation for 2months under **40°C ± 2°C/75% RH ± 5% RH**

Time (months)	**pH**	**Viscosity**	**Diameter zone of inhibition (Bacteria)**	**Diameter zone of inhibition (Yeast)**
0	8.53	0.223	16 mm	22 mm
2	8.9	0.224	15 mm	20 mm

In **Figure 34:** the diameter of the inhibition zone at zero time is shown for both *Staphylococcus aureus* and *Candida albicans* .The inhibition zone measured 16mm against bacteria and 22 mm against yeast .indicating a stronger antifungal activity of the tooth spray. In **Figure 35** and **36**, the diameter of the inhibition zone against *Staphylococcus aureus* was measured after incubation of the tooth spray for two months under different storage conditions. At **25°C ± 2°C/60% RH ± 5%,**the inhibition zone measured 11mm,while at **40°C ± 2°C/75% RH ± 5% ,**it increased to 15mm.

The decrease in the inhibition zone against *Staphylococcus aureus* under **25°C ± 2°C/60% RH ± 5 %,(** from 16 mm to 11 mm) suggests that, over time, some loss of antibacterial activity occurred under moderate storage conditions. This could be due to slow oxidative degradation of key active components in the essential oil, such as phenolic compounds, even at lower temperatures, especially if the packaging allowed slight air exposure.

On the other hand, under **40°C ± 2°C/75% RH ± 5%,** the inhibition zone only decreased slightly (from 16 mm to 15 mm). This relatively stable performance at a higher temperature may be explained by two possibilities:

1. Some active components may have become more bioavailable due to increased fluidity of the spray at higher temperature.
2. There may have been a compensating effect where minor degradation occurred, but reaction by –products or enhanced diffusion preserved most of the antibacterial effect. This highlights how temperature and humidity affect not only degradation but also the bioavailability and diffusion of active ingredients, which can influence antimicrobial outcomes in complex ways.

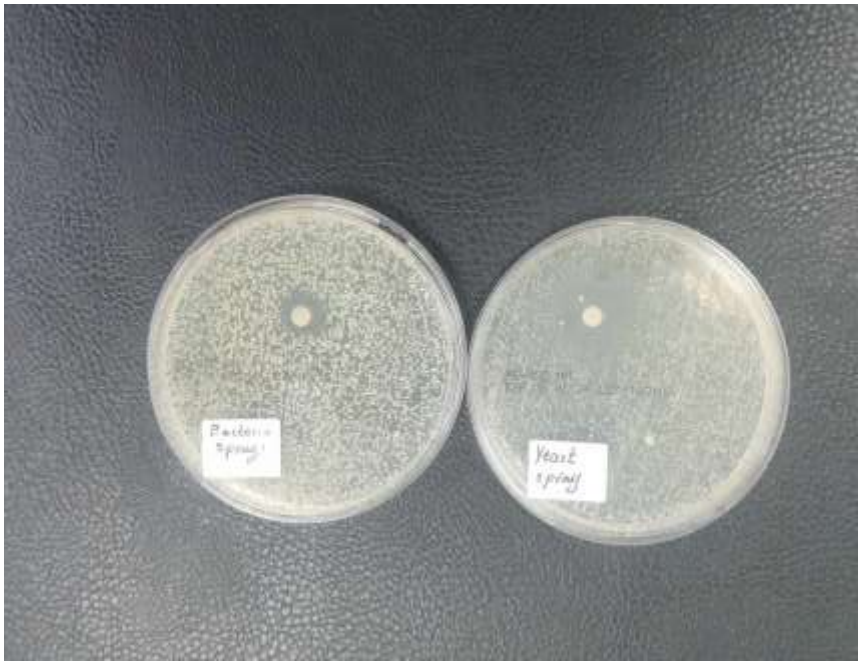

Figure 34: Diameter zone of inhibition at zero time on bacteria and yeast

Figure35: Diameter zone of inhibition on bacteria after incubation for 2months under **25°C ± 2°C/60% RH ± 5%.**

Figure 36: Diameter zone of inhibition on bacteria after incubation for 2months under **40°C ± 2°C/75% RH ± 5% RH**

In **Figure 37**, the diameter of the inhibition zone against *Candida albicans* was evaluated after two months of storage under two different conditions. At **25°C ± 2°C/60% RH ± 5%,** the inhibition zone measured 23 mm**,** while at **40°C ± 2°C/75% RH ± 5%, 5%,**it decreased to 20 mm. This decline in antifungal activity at elevated temperature and humidity could be explained by the thermal degradation and increased volatility of essential oil components.

The variation in antifungal activity of the tooth spray under different storage conditions suggests that temperature plays a critical role in the stability and performance of essential oil components. At **25°C ± 2°C/60% RH ± 5%,** the inhibition zone against *Candida albicans* increased after two months(from 22 mm to 23 mm), indicating that the formulation remained chemically stable and that the active compounds may have undergone beneficial diffusion.

In contrast, at **40°C ± 2°C/75% RH ± 5% RH,** the inhibition zone decreased (from 22 mm to 20 mm), likely due to thermal degradation or evaporation of volatile antifungal constituents, such as eugenol. These compounds are known to be heat-sensitive, and prolonged exposure to elevated temperature and humidity (75%) can lead to oxidation or breakdown of functional groups, reducing their antifungal effectiveness.

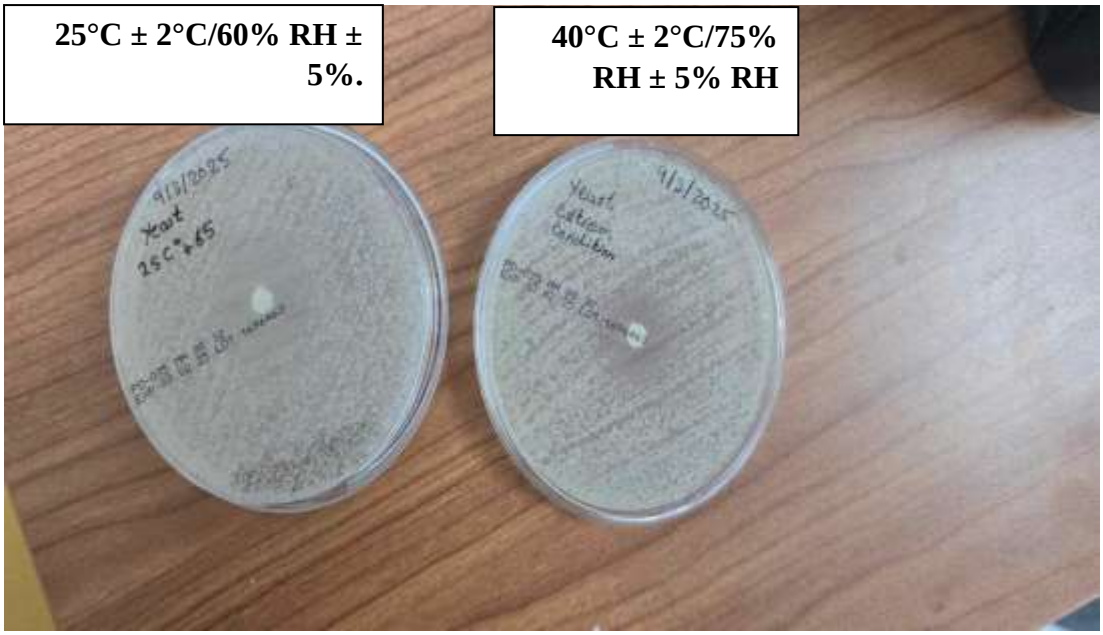

Figure 37: Diameter zone of inhibition on yeast after incubation for 2months under**25°C ± 2°C/60% RH ± 5%and under 40°C ± 2°C/75% RH ± 5%**

Chapter Four:

Conclusion:

Clove, sage, peppermint, and other essential oil-based tooth sprays offer antibacterial, antifungal, and anti-plaque qualities without causing cytotoxicity. As a result, a blend of these essential oils may be created as natural remedies to stop frequent mouth illnesses. Other types of oral products, such as toothpaste or mouthwash, might be created in addition to the spray formulation. We anticipate obtaining stable formulations with suitable characteristics. Since the spray itself may vary greatly, it is challenging to relate dental spray manufacturing study results to a wide range of clinical settings. The speed, direction, size, and quantity of droplets that emerge from the oral cavity vary for each handset, depending on the type, location, orientation, and specific operation of the dental instrument as well as how the instrument and generated spray interact with the hard and soft tissues of the these products; many are said to have chosen them since they are risk-free and have no adverse effects. Tooth offers antimicrobial and antibacterial properties, helps cure tooth decay, and provides customers with fresh breath which gives them confidence in a convenient pump.

The combination of clove, sage, and peppermint essential oils, each used at a concentration of 5% as active ingredients, demonstrated good compatibility when blended together. Based on the diameter of the zone of inhibition and stability tests, the results were promising and met the required specifications. When the three oils were mixed, their antimicrobial effectiveness was enhanced compared to each oil used individually.

References

- Almajed, O. S., Aljouie, A. A., Alharbi, M. S., & Alsulaimi, L. M. (2024). The impact of socioeconomic factors on pediatric oral health: A review. Cureus. https://doi.org/10.7759/cureus.53567
- Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. https://doi.org/10.1021/jf403496k
- Beaulieu, L. Y., Logan, E. R., Gering, K. L., & Dahn, J. R. (2017). An automated system for performing continuous viscosity versus temperature measurements of fluids using an Ostwald viscometer. Review of Scientific Instruments, 88(9). https://doi.org/10.1063/1.4990134
- Beigi, M., Torki-Harchegani, M., & Pirbalouti, A. G. (2018). Quantity and chemical composition of essential oil of peppermint (Mentha × piperita L.) leaves under different drying methods. International Journal of Food Properties, 21(1), 267–276. https://doi.org/10.1080/10942912.2018.1453839
- Ben Hassine, D., et al. (2021). Clove buds essential oil: The impact of grinding on the chemical composition and its biological activities involved in consumer's health security. Biomedical Research International, 2021. https://doi.org/10.1155/2021/9940591
- Bloch-Zupan, A. (2001). Is the fluoride concentration limit of 1,500 ppm in cosmetics (EU guideline) still up-to-date? Community Dental Health, 18(4), 222–225.
- Bowen, W. H. (2013). The Stephan Curve revisited. Odontology, 101(1), 2–8. https://doi.org/10.1007/s10266-012-0092-z
- Browne, D., Whelton, H., & O'Mullane, D. (2005). Fluoride metabolism and fluorosis. Journal of Dentistry, 33(3 Spec. Iss.), 177–186. https://doi.org/10.1016/j.jdent.2004.10.003
- Chen, H., Davidson, P. M., & Zhong, Q. (2014). Impacts of sample preparation methods on solubility and antilisterial characteristics of essential oil components in milk. Applied and Environmental Microbiology, 80(3), 907–916. https://doi.org/10.1128/AEM.03010-13
- Chi, D. L., & Scott, J. A. M. (2019). Added sugar and dental caries in children: A scientific update and future steps. In W.B. Saunders. https://doi.org/10.1016/j.cden.2018.08.003
- de Sousa, D. P., et al. (2023). Essential oils: Chemistry and pharmacological activities. Biomolecules, 13(7), Article 1144. https://doi.org/10.3390/biom13071144
- Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines, 3(4), 25. https://doi.org/10.3390/medicines3040025
- Didehdar, M., Chegini, Z., & Shariati, A. (2022). Eugenol: A novel therapeutic agent for the inhibition of Candida species infection. Frontiers in Pharmacology. https://doi.org/10.3389/fphar.2022.872127
- Đurović, S., et al. (2022). The effect of various extraction techniques on the quality of sage (Salvia officinalis L.) essential oil, expressed by chemical composition, thermal properties, and biological activity. Food Chemistry: X, 13. https://doi.org/10.1016/j.fochx.2022.100213

- Dye, B. A. (2017). The global burden of oral disease: Research and public health significance. Journal of Dental Research, 96(4), 361–363. https://doi.org/10.1177/0022034517693567
- Dye, B. A., Thornton-Evans, G., Li, X., & Iafolla, T. J. (2011). Dental caries and sealant prevalence in children and adolescents in the United States, 2011-2012 key findings data from the National Health and Nutrition Examination Survey, 2011-2012. Centers for Disease Control and Prevention. http://www.cdc.gov/nchs/data/databriefs/db191_table.pdf#1
- Edit, G., de Andrade, C. G., Negrini, T. de C., & Arthur, R. A. (2019). Role of Candida albicans on enamel demineralization and on acidogenic potential of Streptococcus mutans in vitro biofilms. Journal of Applied Oral Science, 27. https://doi.org/10.1590/1678-7757-2018-0593
- Fatmawati, F., Wachyuni, A. P., & Azhary, D. P. (n.d.). Review of Menthol in Oral Care Formulation as Freshner and Preventing Halitosis. International Journal of Pharmaceutical Research and Applications, 7, 1085. https://doi.org/10.35629/7781-070210851091
- Featherstone, J. D. B. (2008). Dental caries: A dynamic disease process. Australian Dental Journal, 53(3), 286–291. https://doi.org/10.1111/j.1834-7819.2008.00064.x
- Ferhat, M. A., Meklati, B. Y., Smadja, J., & Chemat, F. (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. Journal of Chromatography A, 1112(1–2), 121–126. https://doi.org/10.1016/j.chroma.2005.12.030
- Gawish, A. S., ElMofty, M. S., Jambi, S., Felemban, D., Ragheb, Y. S. E., & Elsayed, S. A. (2024). Phytotherapy in periodontics as an effective and sustainable supplemental treatment: A narrative review. Journal of Periodontal & Implant Science, 54(4), 209–223. https://doi.org/10.5051/jpis.2301420071
- Hagel, J. M., Chen, X., & Facchini, P. J. (2019). Production of methylparaben in Escherichia coli. Journal of Industrial Microbiology & Biotechnology, 46(1), 91–99. https://doi.org/10.1007/s10295-018-2102-9
- Haro-González, J. N., Castillo-Herrera, G. A., Martínez-Velázquez, M., & Espinosa-Andrews, H. (2021). Clove essential oil (Syzygium aromaticum L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules, 26(21). https://doi.org/10.3390/molecules26216387
- Heng, C. (2016). Tooth decay is the most prevalent disease. Federal Practitioner, 33(10), 31–33. PMID: 30766141; PMCID: PMC6373711.
- Izyan, N., et al. (2019). Glycerol in Food, Cosmetics and Pharmaceutical Industries: Basics and New Applications. International Journal of Scientific & Technology Research, 8(10), 212–219. Retrieved from www.ijstr.org
- Kassebaum, N. J., Bernabé, E., Dahiya, M., Bhandari, B., Murray, C. J. L., & Marcenes, W. (2015). Global burden of untreated caries: A systematic review and metaregression. Journal of Dental Research, 94(5), 650–658. https://doi.org/10.1177/0022034515573272 Fejerskov, O. (1997). Community dentistry and oral epidemiology concepts of dental caries and their consequences for understanding the disease. Community Dentistry and Oral Epidemiology, 25, 5–12.
- Kim, M.-H., Lee, M. K., & Hwang, Y. S. (2019). Effect of oral spray on dental plaque bacteria and oral epithelial cells. Journal of Dental Hygiene Science, 19(2), 107–112. https://doi.org/10.17135/jdhs.2019.19.2.107

- Koch, M., & Graetz, C. (2021). Spray mist reduction by means of a high-volume evacuation system—Results of an experimental study. PLoS One, 16(9), Article e0257137. https://doi.org/10.1371/journal.pone.0257137
- Koo, H., & Bowen, W. H. (2014). Candida albicans and Streptococcus mutans: A potential synergistic alliance to cause virulent tooth decay in children. Future Medicine Ltd. https://doi.org/10.2217/fmb.14.92
- Kramm, K., Orth, M., Teiwes, A., Kammerhofer, J. C., Meunier, V., Pietsch-Braune, S., & Heinrich, S. (2023). Influence of nozzle parameters on spray pattern and droplet characteristics for a two-fluid nozzle. Chemie Ingenieur Technik, 95(1–2), 151–159. https://doi.org/10.1002/cite.202200152
- Lagier, J. C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., & Raoult, D. (2015). Current and past strategies for bacterial culture in clinical microbiology. Clinical Microbiology Reviews, 28(1), 208–236. https://doi.org/10.1128/CMR.00110-14
- Marchese, A., et al. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. International Journal of Molecular Sciences. https://doi.org/10.1080/1040841X.2017.1295225
- Masyita, A., & Sari, R. M. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chemistry: X, 13, 100217. https://doi.org/10.1016/j.fochx.2022.100217
- McCormack, M. G., Smith, A. J., Akram, A. N., Jackson, M., Robertson, D., & Edwards, G. (2015). Staphylococcus aureus and the oral cavity: An overlooked source of carriage and infection. American Journal of Infection Control, 43(1), 35–37. https://doi.org/10.1016/j.ajic.2014.09.015
- Meier, T., Deumelandt, P., Christen, O., Stangl, G. I., Riedel, K., & Langer, M. (2017). Global burden of sugar-related dental diseases in 168 countries and corresponding health care costs. Journal of Dental Research, 96(8), 845–854. https://doi.org/10.1177/0022034517708315
- Mohamed, M. A. E. A., Abdel Hameed, H. E. S., Elshenawy, E. S. A., El-Salmawy, H. A. A., & Shaltout, R. E. (2021). Numerical simulation and experimental study for the impact of in-flow nozzle on spray characteristics. ACS Omega, 6(49), 33498–33510. https://doi.org/10.1021/acsomega.1c04272
- Moon, S. E., Kim, H. Y., & Cha, J. D. (2011). Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Archives of Oral Biology, 56(9), 907–916. https://doi.org/10.1016/j.archoralbio.2011.02.005
- Muchalal, M., & Crouzet, J. (1985). Volatile components of clove essential oil (Eugenia caryophyllus spreng): Neutral fraction. Agricultural and Biological Chemistry, 49(6), 1583–1589. https://doi.org/10.1080/00021369.1985.10866947
- Nair, B. (2001). Final report on the safety assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. International Journal of Toxicology, 20(Suppl 3), 61–73.
- National Institute of Dental and Craniofacial Research. (2021). Oral health in America: Advances and challenges. U.S. Department of Health and Human Services. https://www.nidcr.nih.gov/research/oralhealthinameica
- Nuñez, L., & Aquino. (2012). Microbicide activity of clove essential oil (Eugenia caryophyllata). Brazilian Journal of Microbiology, 1255–1260. https://doi.org/10.1590/S1517-838220120004000003

- Palacios, C., Rivas-Tumanyan, S., Morou-Bermúdez, E., Colon, A. M., Torres, R. Y., & Elías-Boneta, A. R. (2016). Association between type, amount, and pattern of carbohydrate consumption with dental caries in 12-year-olds in Puerto Rico. Caries Research, 50(6), 560–570. https://doi.org/10.1159/000450655
- Pathirana, H. N. K. S., Wimalasena, S. H. M. P., DeSilva, B. C. J., Hossain, S., & Gang-Joon, H. (2019). Antibacterial activity of clove essential oil and eugenol against fish pathogenic bacteria isolated from cultured olive flounder (Paralichthys olivaceus). Slovenian Veterinary Research, 56(1), 31–38. https://doi.org/10.26873/SVR-590-2018
- Pelkonen, O., Abass, K., & Wiesner, J. (2013). Thujone and thujone-containing herbal medicinal and botanical products: Toxicological assessment. Regulatory Toxicology and Pharmacology, 65(1), 100–107. https://doi.org/10.1016/j.yrtph.2012.11.002
- Peres, M. A., et al. (2019). Oral health 1 Oral diseases: A global public health challenge. Retrieved from https://vizhub.healthdata.org/
- Pitts, N. B., et al. (2017). Dental caries. Nature Reviews Disease Primers, 3, Article 17030. https://doi.org/10.1038/nrdp.2017.30
- Polyakova, M., et al. (2024). The effect of oral care foams and a spray on salivary pH changes after exposure to acidic beverages in young adults. Dentistry Journal (Basel), 12(4). https://doi.org/10.3390/dj12040093
- Porte Godoy, A., & Maia-Porte, A. (2013). Chemical composition of sage (Salvia officinalis L.) essential oil from the Rio de Janeiro State (Brazil). Revista Brasileira de Plantas Medicinais, 15(3), 438–441. https://doi.org/10.1590/s1516-05722013000300018
- Prasad, N., Vijay, S., Reddy, A. Y., & Nonitha, S. (2019). Effects of menthol-flavored substances at the cellular level on oral mucosal sites. Retrieved from www.ncbi.nlm.nih.gov/pmc/journals/1480
- Ramsey, J. T., Schuepbach, R. E., Noory, T. M., Chambers, K. D., & Koob, K. K. (2010). Essential oils and health. Yale Journal of Biology and Medicine, 83(2), 291–300. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2893021/
- Reddy, A., Norris, D. F., Momeni, S. S., Waldo, B., & Ruby, J. D. (2016). The pH of beverages in the United States. Journal of the American Dental Association, 147(4), 255–263. https://doi.org/10.1016/j.adaj.2015.10.019
- Roberts, W. E., Mangum, J. E., & Schneider, P. M. (2022). Pathophysiology of demineralization, Part II: Enamel white spots, cavitated caries, and bone infection. Springer. https://doi.org/10.1007/s11914-022-00723-0
- Saharkhiz, M. J., Motamedi, M., Zomorodian, K., Pakshir, K., Miri, R., & Hemyari, K. (2012). Chemical composition, antifungal and antibiofilm activities of the essential oil of Mentha piperita L. ISRN Pharmacology, 2012, 1–6. https://doi.org/10.5402/2012/718645
- Samuel, P., et al. (2018). Stevia Leaf to Stevia Sweetener: Exploring Its Science, Benefits, and Future Potential. Journal of Nutrition, 148(7), 1186S–1205S. https://doi.org/10.1093/in/nxy102
- Sarkhouh, S. M. (2014). Investigating the ultrastructure of enamel white spot lesions (WSL) using Optical Coherence Tomography at different length scales (Clinical Doctorate in Pediatric Dentistry thesis).
- Shelon, S. P. H., Souza Pinto, C., Farago, P. V., Santos, F. A., & Wambier, D. S. (2011). Physical-chemical characteristics of whitening toothpaste and evaluation of its effects on enamel roughness. https://doi.org/10.1016/j.jdent.2011.02.006
- Shreyas, A. J., & Anbalagan, C. (n.d.). Extraction of glycerin oil and production of natural bar soap by using pongamia seeds. Retrieved from http://ymerdigital.com

- Sinko, P. J. (2013). Martin's physical pharmacy and pharmaceutical sciences (6th ed.). Lippincott Williams & Wilkins.
- Spatafora, G., Li, Y., He, X., Cowan, A., & Tanner, A. C. R. (2024). The evolving microbiome of dental caries. Microorganisms, 12(1), Article 121. https://doi.org/10.3390/microorganisms12010121
- Turek, C., & Stintzing, F. C. (2013). Stability of essential oils: A review. Comprehensive Reviews in Food Science and Food Safety. https://doi.org/10.1111/1541-4337.12006
- Ulanowska, K., & Olas, B. (2021). Biological properties and prospects for the application of eugenol—a review. International Journal of Molecular Sciences, 22(7), 3671. https://doi.org/10.3390/ijms22073671
- Wiener, R. C., Shen, C., Findley, P. A., Sambamoorthi, U., & Tan, X. (2017). The association between diabetes mellitus, sugar-sweetened beverages, and tooth loss in adults: Evidence from 18 states. Journal of the American Dental Association, 148(7), 500–509.e4. https://doi.org/10.1016/j.adaj.2017.03.012
- Xu, J. G., Liu, T., Hu, Q. P., & Cao, X. M. (2016). Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against Staphylococcus aureus. Molecules, 21(9). https://doi.org/10.3390/molecules21091194
- Xu, N., et al. (2021). Enhancing the oxidative stability of algal oil emulsions by adding sweet orange oil: Effect of essential oil concentration. Food Chemistry, 355, Article 129645. https://doi.org/10.1016/j.foodchem.2021.129645
- Zapata, A., & Ramirez-Arcos, S. (2015). A comparative study of McFarland turbidity standards and the Densimat photometer to determine bacterial cell density. Springer New York LLC. https://doi.org/10.1007/s00284-015-0801-2
- Zimmerman, E. (1967). Automatic recording of viscosity changes with the Ostwald capillary viscometer. Analytical Biochemistry, 21(1), 81–85. https://doi.org/10.1016/0003-2697 (67)90085-1

تحضير وتقييم بخاخ الأسنان

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الملخص:

تُستخدم الزيوت العطرية بشكل واسع في الوقاية من أمراض الفم والأسنان، مثل تسوس الأسنان، التهاب اللثة، ورائحة الفم الكريهة، نظرًا لخصائصها المضادة للبكتيريا والفطريات .هدفت هذه الدراسة إلى تطوير وتقييم بخاخ فموي عشبي يحتوي على زيوت عطرية، وذلك من حيث التركيب والجرعة والخصائص المضادة للميكروبات .يتميز البخاخ الناتج بخصائص تجعله خيارًا مناسبًا لصحة الفم، حيث يتكون من زيوت عطرية طبيعية ومكونات أخرى، ويترك رائحة زكية داخل الفم بعد استخدامه .كما أن التركيبة المقترحة تتسم بالثبات والمرونة وعدم الالتصاق، وتخلو من الروائح أو البقع غير المرغوبة، مما يعزز تقبّلها من قبل المستخدمين .وأظهرت النتائج أن المكونات العشبية للبخاخ تمتلك تأثيرات مضادة للبكتيريا والفطريات، خاصة عند استخدامه في بيئة فموية ذات درجة حموضة منخفضة($pH<5.5$) ، حيث تعمل القواعد المضافة على معادلة الحموضة ورفع pH الفم إلى الفم إلى 8.5,مما يساعد على الوقاية من التسوس.