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DEVELOPMENT OF NATURAL LOZENGE FORMULATION OF GINGER EXTRACT

MASTER OF SCIENCE THESIS

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APPROVAL

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

07/07/2021

Merve Küçüközkan Savaşçı

My beautiful family and lovely husband who supported me in everything…

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LIST OF SYMBOLS AND ABBREVIATIONS

GI	Gastrointestinal
OTC	Over the counter
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
R	Reference
E	Extract
C	Mobil phase
PVP	Polyvinyl pyrrolidone
PEG	Polyethylene glycol
MC	Methyl cellulose
w/w	Weight per weight
POE	Poly (ethylene oxide)
POP	Poly (propylene oxide)
FDA	Food and Drug Administration (FDA)
GC/MS	Gas chromatography/mass spectrometry analysis
EUCAST	European Committee on Antimicrobial Susceptibility Testing

ABSTRACT

Küçüközkan Savaşçı, M. (2021). Development of Natural Lozenge Formulation of Ginger Extract. Yeditepe University, Institute of Health Science, Department of Drug and Cosmetic Production Technologies. MSc Thesis, Istanbul.

This study was designed with the idea that the antibacterial property of ginger, especially for oral health, is an important stage of proactive treatment. In developing the soft lozenge in question, various formulas were tried, and the optimal formulation was determined. The GL7 soft lozenge formulation was found to be the most suitable formulation. The formulation contains 25% Ginger *(Zingiber officinale)*, 20% Cinnamon (Cinnamomum verum), 1% Acacia, 0.5% Beeswax, 0.5% Lemon Essential Oil, 1% D-Limonen, 1% Stevia, 1% Lemon Juice, 2% Glycerin and, 10% Honey and 10%Strawbery (freeze dried), 20% Pluronic F 127 (Poloxamer 407). The GL7 formula developed does not contain preservatives, so cinnamon is used in the formula, both for their antibacterial properties and taste. On the other hand, the formula was developed using Pluronic F (Poloxamer 407), which is also present in mouthwashes, often recommended by dentists for oral hygiene, with antibacterial properties alone. For the ginger *(Zingiber officinale)* contained as the active ingredient with its antibacterial property, the presence of gingerols and shogaols were detected in the developed formula, which was qualitatively determined by thin layer chromatography (TLC). The microbial properties of GL7 formulation were determined and *C. albicans, E. coli, P. aureus and P. aeruginosa* were found to be effective against bacteria by using disc diffusion method. According to the European Pharmacopoeia for the developed GL7 formulation, dispersion test was carried out and it was found that the dispersion time was 25-30 minutes. With the results obtained, an antibacterial, preservative-free, sugar-free and natural soft lozenge containing ginger extract has been proven to be an alternative dosage form that can be used for oral health.

Key Words: Ginger extract, soft lozenges.

ÖZET

Küçüközkan Savaşçı, M. (2021). Zencefil Ekstraktı ile Doğal Pastil Formülasyonunun Geliştirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, İlaç ve Kozmetik Üretim Teknolojileri Anabilim Dalı. Yüksek Lisans Tezi, İstanbul.

Bu çalışmada, antibakteriyel özelliği bilinen zencefil ekstraktı ile koruyucu ve şeker içermeyen doğal bir pastil formülasyonunun geliştirilmesi amaçlanmıştır. Bu çalışma, özellikle ağız sağlığı için zencefilin antibakteriyel özelliğinin proaktif tedavinin önemli bir safhasını oluşturucağı düşünülerek tasarlandı. Söz konusu yumuşak pastilin geliştirilmesinde farklı formüller denenerek, optimum formülasyon belirlendi. GL7 formülasyonu yumuşak pastil olarak en uygun formülasyon olarak bulundu ve bu formülasyon aktif madde olarak %25 Zencefil *(Zingiber officinale)* ekstraktı, %20 Tarçın, %20 Pluronic F (Poloxamer 407), %1 Akasya zamkı, %0.5 Balmumu, %0.5 Limon Esensiyel yağı, %1 D-Limonen, %1 Limon suyu, %2 Gliserin, %10 kurutulmuş çilek, %10 bal ve %1 Stevia içeriyordu. Geliştirilen GL7 formülü, koruyucu içermemektedir, bu nedenle formülde hem antibakteriyel özelliği hem de tat vermesi sebebiyle tarçın kullanılmıştır. Öte yandan söz konusu formül ağız hijyeni için diş hekimlerin sıklıkla önerdiği gargaralarda da yer alan ve tek başına antibakteriyel özellik gösteren Pluronic F (Poloxamer 407) kullanılarak tasarlanmıştır. Geliştirilen formülde antibakteriyel özelliği ile etken madde olarak yer alan Zencefil *(Zingiber officinale)* için ince tabaka kromatografisi (TLC) ile kalitatif tayin yapılmış gingerols ve shogaols varlığı kanıtlanmıştır. GL7 formülasyonun mikrobiyel özellikleri disc difüzyon metodu ile tayin edilmiş, *C. albicans, E. coli, S. aureus ve P. aeruginosa* bakteri üzerinde etkili olduğu disc difüzyon yöntemi ile belirlenmiştir. Geliştirilen GL7 formülasyonu için Avrupa Farmakopesine göre, dağılma testi yapılmış, dağılma zamanının 25-30 dakika olduğu bulunmuştur. Elde edilen sonuçlar ile zencefil ekstraktı içeren antibakteriyel, koruyucusuz, şekersiz ve doğal bir yumuşak pastilin, ağız sağlığı için kullanılabilecek alternatif bir dozaj formu olduğu kanıtlanmış oldu.

Anahtar Kelimeler: Zencefil ekstraktı, yumuşak pastil

1. INTRODUCTION AND PURPOSE

Since ancient times, plants and plant extract have been used to protect against diseases and treat existing diseases instead of commercially available in synthetic drugs. Although many drugs are commercially available in synthetic form, these drugs can bring significant side effects along with the benefit.

Ginger *(Zingiber officinale),* which has been used as a spice for over two millennia [22, 23], is used for many diseases with its antibacterial properties. According to the Indian system (Ayurveda), ginger is thought to be useful in suppressing dyspepsia, anorexia and inflammation, is also a degassing and digester. Pharmacological studies have shown that ginger is useful in reducing nausea and vomiting associated with chemotherapy, maternity and seasickness [34].

Problems that may arise when oral health is not given importance are one of the most serious problems that directly affect people's quality of life. On the other hand, oral health has become even more important, especially with the pandemic, which shows its negative effects all over the world. The entry point of bacteria into our body is the mouth and throat. This part is the most important part of our body's defense point. Soft lozenges, on the other hand, can be easily used by patients, together with their antibacterial properties, can form an important part of proactive treatment for oral health, and can act as a mouth and throat barrier against diseases.

Therefore, the goal of the study was designed to develop a natural lozenges formulation with ginger extract, which is known for its antibacterial properties, and which does not contain preservatives and sugar for oral health.

On the other hand, alternative prevention and treatment options and reliable products for oral diseases have become an important need in today's global world. Especially in developed countries, bacteria that develop resistance to antibiotics make people think about different treatment options.

The developed natural and antibacterial soft lozenge formula are thought to be an alternative dosage form that can be used for oral health.

Nowadays, antibacterial lozenges are highly preferred by consumers. Therefore, in our study, we thought that we could develop an effective, personalized lozenge form that can be prepared in a community pharmacy.

2. LITERATURE REVIEW

2.1. Mucoadhesive Systems

Oral mucosal drug administration is a systemic drug delivery method that has a variety of benefits over injectable and enteral routes of administration. Due to the oral mucosa's highly vascularized nature, medicines taken through it enter the systemic circulation immediately, bypassing the gastrointestinal tract and liver's first-pass metabolism [1].

Transmucosal Drug Delivery Pathways (i.e., the mucosal linings of the vaginal, rectal, ocular, nose and oral cavity) have distinct advantages over peroral administration for systemic drug delivery. These benefits include the possibility of bypassing the first transition effect, avoiding systemic pre-elimination within the GI tract, and a more favorable enzymatic flora for drug absorption, depending on the specific drug [2].

Mucoadhesive drug delivery methods increase the duration of time a dosage form is in the field of administration or absorption. As a result, they allow for intimate contact in between dosage form and the absorption surface underneath it, therefore enhancing the therapeutic effectiveness of the medication. Numerous mucoadhesive drug delivery methods have been developed in recent years for oral, buccal, nasal, rectal, and vaginal routes for systemic and local effects [3,4].

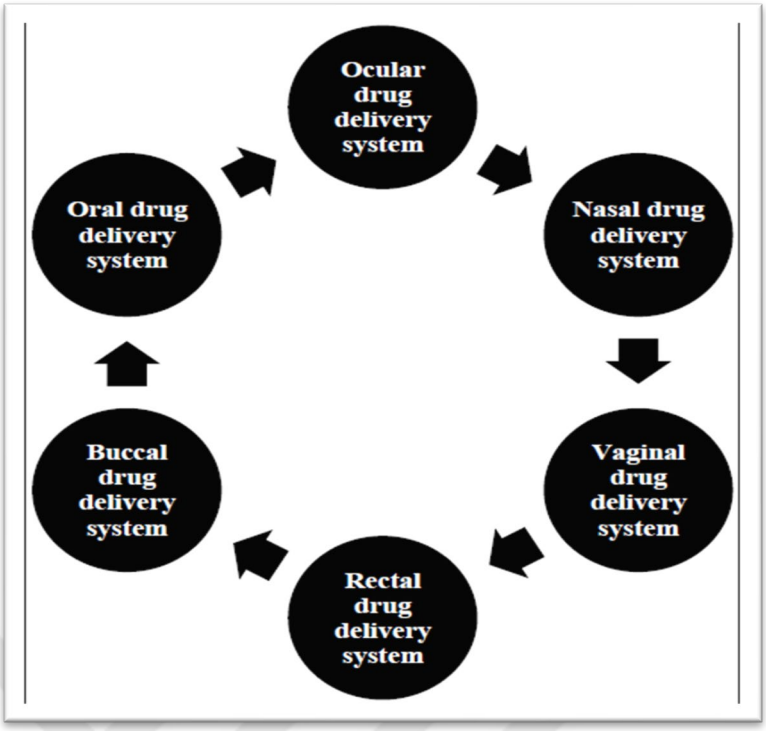


Figure 2.1 Mucoadhesive systems [3].

2.2. Buccal Drug Delivery System

2.2.1. The general information of the oral mucosa

The oral immune system's primary function is to protect the teeth, jaws, gingivae, and oral mucosa from infection [5]. The oral cavity is comprised of the lips, tongue, the mouth's base, hard and soft palates, and cheeks. [1] The oral cavity's lining mucosa is protected by a stratified epithelium, and three distinct types of oral mucosa are recognized. [5]. The oral mucosa is composed of the outermost squamous epithelial layer (Figure 2.2). Below this is a basal membrane, followed by a lamina propria and, finally, submucosa [2].

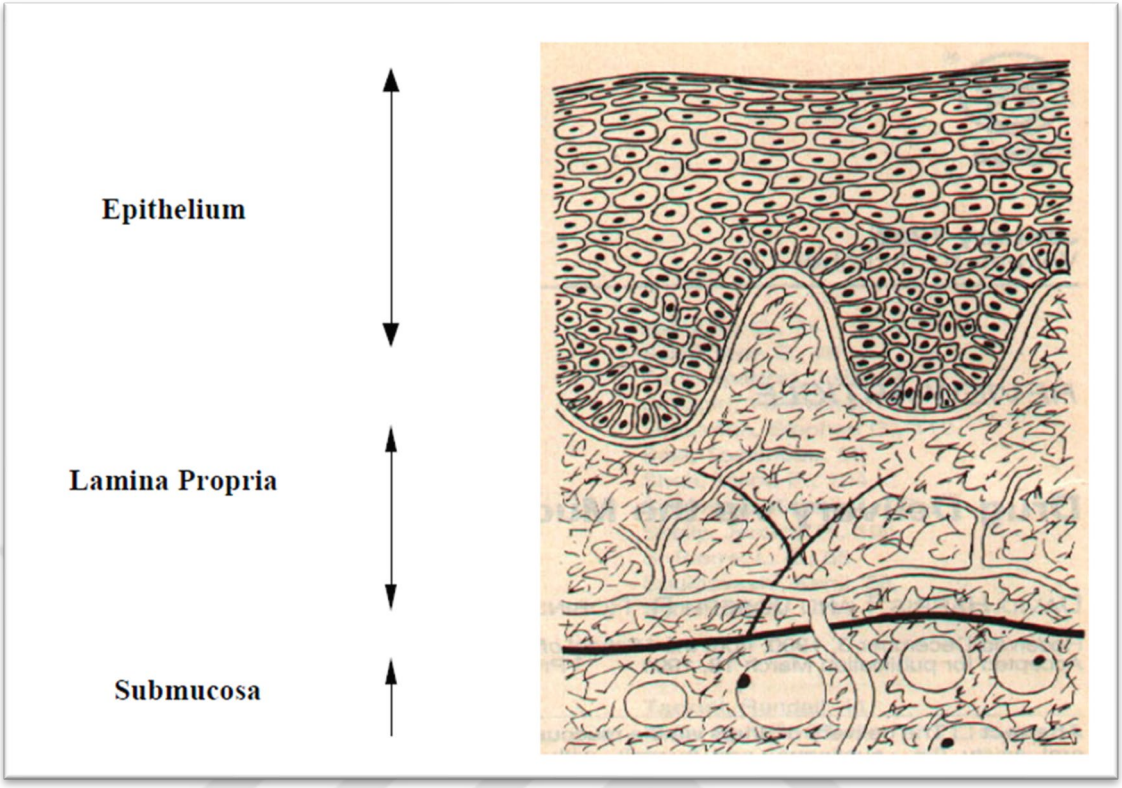


Figure 2.2. Layer of epithelium [7].

The epithelium is similar to the stratified squamous epithelia found in the entire body, which serves as a protective laying for the tissues underneath, as it starts with a mitotically active basal cell layer and progresses through a number of differentiating intermediate layers to superficial layers, where cells are shed from the surface of the epithelium[6]. The buccal mucosa epithelium is approximately 40-50 cell layers thick, whereas the sublingual epithelium is rouughly thinner [7].

Mucus, an intercellular substrate primarily composed of protein and carbohydrate complexes, surrounds the cells of the oral epithelium [8]. Mucus is a viscoelastic hydrogel that serves as a barrier between the cells below. It is primarily composed of 1–5% insoluble glycoproteins in water, 95–99% water, and trace amounts of proteins, enzymes, electrolytes, and nucleic acids. Mucus has a variable composition depending on the source of secretion in the body [6]. The mucous layer possesses protective properties, acting as a barrier and lubricant [8]. The presence of salivary generated by the saliva glands is another feature of the environment of the oral cavity. Saliva is the safe fluid around the cavity tissues in the oral environment. It protects delicate tissues against abrasion by

ground and chemical substances. It enhances the mineralization of the tooth enamel during the early phases of dental caries after the burst and helps remineralize the enamel [8].

2.2.2. Dosage forms for oral mucosa

The Dosage forms for Oral mucosa are presented in Table 2.1.

Table.2.1. Dosage forms for oral mucosa.

Liquid dosage forms	**Semisolid dosage forms**	**Solid Dosage Forms**
They are pharmaceutical solutions or suspensions in suitable aqueous vehicles. These dosage forms are typically used to deliver antibacterial agents directly into the oral cavity, and several antibacterial mouthwashes and breath fresheners are commercially available for this purpose [9].	Gels, creams, and ointments are typically used as semisolid dosage forms and are applied topically to the mucosal surface for either local or systemic effects [10]. Gels, Creams, and Ointments	These are solid dosage forms made by compressing powder mixtures that can be placed in contact with the oral mucosa and allowed to dissolve or adhere, depending on the type of excipients used. They are capable of delivering drugs in multiple directions into the oral cavity or mucosal surface [11]. For instance, tablets and lozanges.

2.3. Lozenges

2.3.1. History of lozenges

The name "Lozenge" is derived from the French "Losenge," which refers to a diamond-shaped geometric object with four equal sides[8].

Lozenges are solid, usually flavored preparations that contain one or more medicines and are designed to dissolve gradually in the mouth or throat [29]. The lozenges are designed to disintegrate on the back of the tongue, limiting drug absorption and maximizing medication action [12].

Lozenges are primarily intended to prolong the duration of the dosage form's retention time, thereby increasing bioavailability. It has a number of advantages, including a reduction in gastric irritation due to the bypass of first-pass metabolism. Because the active ingredients in lozenges are so varied, they can be used for both local and systemic therapy [13]. Lozenges can be formed using either gelatin and/or a sorbitol base and fused sucrose or by compressing sugar-based tablets [31].

Lozenges are utilized in patients who have trouble swallowing solid oral dosage forms, as well as for medications that require a gradual release of medication in the oral cavity or to coat the throat tissues with a drug solution [14].

Lozenges were invented in the twentieth century and are still manufactured commercially. Lozenges are currently available in four forms: medicated lozenges made of caramel, soft lozenges, hard candy lozenges, and compressed tablet lozenges. The majority of lozenge preparations are available over-the-counter (OTC) [12].

Antimicrobials and local anesthetics for throat pain; aromatics, herbals, zinc salts, decongestants, antihistamines, and cough suppressants for cold, allergy, cough, and congestion; and nicotine-like substances for smoking cessation [15].

Antimicrobials and local anesthetics are used to treat throat discomfort; zinc salts, aromatics, decongestants, herbals, antihistamines,cough suppressants and , zinc salts are used to treat colds, coughs, allergies, and congestion; and nicotine-like compounds are used to help smokers quit [15].

2.3.2. Advantages and disadvantages of lozenges

Lozenges have a number of advantages, including the ability to administer them to patients who have difficulty swallowing, the ease with which they can be applied to the geriatric and pediatric populations, the minimal equipment required, and the ease with which they can be prepared in a short time period. On the other hand, lozenges have some disadvantages listed in Table 2.2 [15].

Table.2.2. Advantages and disadvantages of lozenges [14].

Advantages	Disadvantages
•Ease of administration to paediatric and geriatric •Patients •Local and systemic effect through oral cavity •Increased contact time of the drug •Prolonged drug action •Avoid first pass metabolism of drugs •Do not require water for intake •Suitable for patients having difficulty swallowing •Lozenge can be withdrawn if dose is not needed •Modification of formula as per the patient's need •Less production time •Cost of production is less •Provides flavour and pleasant taste to the mouth •Better patient compliance	•Non-ubiquitous distribution of drug in the saliva for •local therapy •Possible draining of drug into the stomach •Accidental swallowing of entire dosage form

2.3.3. Classification of lozenges

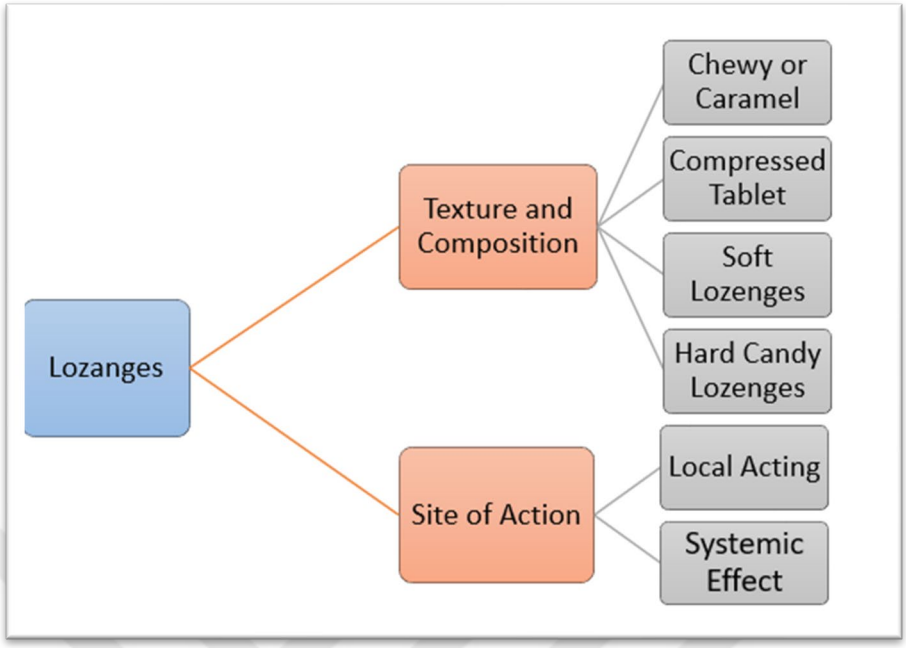


Figure 2.3. Classification of lozenges [15].

2.3.3.1. According to site of action

Lozenges are classified in a variety of ways, including their mode of action, which may be local or systemic [15]. Local effects are exemplified by antiseptics and decongestants, whereas systemic effects are exemplified by vitamins and nicotine [16].

2.3.3.2. According to texture and composition

2.3.3.2.1. Chewy or caramel based medicated lozenges

Figure 2.4. Chewy lozenges

Chewy or caramel-based medicated lozenges are a type of lozenge in which the medication is combined with a caramel base and chewed rather than swallowed.

Typically, the formulations are based on a glycerinated gelatin suppository with a strong fruit flavor that contains glycerin, gelatin, and water [17].

2.3.3.2.2. Compressed tablet lozenges

Compression can be used to form a lozenge if the active ingredient is heat-resistant. Granulation is prepared in the same manner as any compressed tablet. Lozenge tablets are distinguished from conventional tablets by their organoleptic properties, non-breaking characteristics, and slower dissolution profiles [15].

Figure 2.5. Compressed tablet lozenges

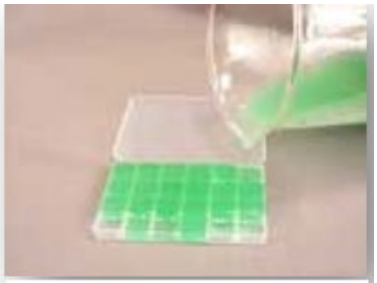


Figure 2.6. Soft lozenges

2.3.3.2.3. Soft lozenges

Soft lozenges have gained popularity due to their ease of preparation and adaptability to a wide variety of medications. Typically, soft lozenges are composed of a blend of polyethylene, glycols, acacia, or a similar material [18].

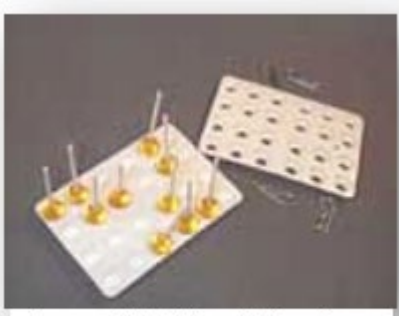


Figure 2.7. Hard Candy Lozenges

2.3.3.2.4. Hard candy lozenges

Hard candy lozenges are amorphous (non-crystalline) or glassy form mixtures of sugar and other carbohydrates. They can also be considered as solid sugar syrup [20].

2.3.4. Preparation Methods

2.3.4.1. Chewy or caramel based medicated lozenges

The candy base is heated between 95 and 125 degrees Celsius and then put to a planetary or sigma paddle mixer. Allow for cooling to a temperature of 120oC for the mass. After that, the whipping agent is applied at a temperature of less than 105°C. The medicaments are then added at temperatures ranging from 95 to 105 degrees Celsius. At a temperature more than 90oC, distribute the color in humectant and add it to the aforementioned mass. Below 85oC, seed crystals and flavor are added; beyond 80oC, lubrication is applied. Rope forming is then used to create the candies [17].

2.3.4.2. Compressed tablet lozenges

Direct compression or wet granulation can be used to make compressed tablet lozenges. Direct compression involves fully mixing the components and then compressing them. Before the process of compression begins, the taste and lubrication are applied. Wet granulation is a process in which the sugar content is mechanically crushed into a fine powder (40-80 net size). The medication is added and well blended. The combined material is granulated with corn syrup or sugar and scanned through a sieve with a mesh size of 2-8. This is followed by drying and milling to a particle size of 10-30 mesh [20].

2.3.4.3. Hard candy lozenges

The candy base is made by dissolving the required amount of sugar in a third of water in a sugar-based stove. Repeat this procedure until the temperature reaches 110 °C. Corn syrup is added and the mixture is heated to a temperature of between 145 and 156°C. The candy mass is removed from the furnace and placed in an oiled transfer container that is balanced on a weight control scale. Following that, color is added using solutions, pastes, or cubes of various colors. After mixing, the mass is transferred to a stainless steel cooling table equipped with a water jacket for the addition of flavoring, medicine, and ground recovery. The material is either poured into the mold or drawn into a strip and trimmed to the desired length as it cools. Following that, lozenges are packaged [31, 33].

2.3.4.4. Soft lozenges

The soft lozenges are formulated with polyethylene glycol 1000 or 1450, chocolate or sugar acacia basis. These ingredients contribute to the lozenges' soft feel. Acacia and silica gel are both included in certain soft lozenges [30].

Hand-rolled soft lozenges are then cut into appropriate size and thickness [15, 20]. Another technique includes heating all components, including the medicine, to around 50 °C and then pouring the mixture into a plastic mold. If polyethylene glycol is used, the mould cavity should be overfilled, as polyethylene glycol shrinks as it cools. This is not necessary with chocolate because it does not shrink [39].

The drawback of this approach is that it requires a high temperature for manufacturing, which precludes the synthesis of heat-labile materials [40,41].

2.3.5. Medical Application

The following types of drug candidates are amenable to incorporation into lozenges:

- Antiseptics
- Local anesthetics
- Antibiotics
- Antihistaminics
- Antitusives
- Analgesics
- Decongestant
- Antifungal
- Demulcents [17, 20].

Table.2.3. Marketed products of lozenges [14,17,20].

Type	Ingredients	Effects	Uses
Amylmetacresol 2,4 diclorobenzylalcohol lozenges	Corn syrup mixed with mucoadhesive polymers	Rapid release of the drug in the mouth	Acute sore throat and as analgesics
Salbutmol sulphate lozenges	Isomalt a tooth friendly sugar substitute mixed with corn syrup	Extended drug release profile for 60 min	Asthma
Ketoconazole lozenges	Sucrose, citric acid, HPMC, HEC	Reduces gatric irritation by passing first pass metabolism	Fungal infections in pediatrics and geriatrics
Paracetamol lozenges	Paracetamol,sucrose, sodium CMC, methyl cellulose	Slow release of medicaments	Fever and pain
Clotrimazole lozenges	Sugar base, acacia/ guar gum/ methyl cellulose, citric acid, artificial flavours and colours	Prolonged oral retension time	Pediatric and geriatric dysphagia
Artesunate oral retentive lozenges	Mucoadhesive polymer like sodium hydroxyl ethyl cellulose is used	Prolonged retension of the lozenges	Malaria in pediatric patients
Montelukast sodium lozenges	Montelukast sodium glucose HPMC	Prolonged retention in the mouth	Asthma
Marshmallow root extract lozenges	Xanthum gum as a gummy base	Increased the disintegration time over 30 min and retened in vitro drug release rate 40 % for 30 min of the lozenges	Irritated oropharyngeal mucosa and associated dry cough
Garlic and ginger lozenges	Sucrose, sodium chloride, pvp, SCMC	Taste masking with good release matrix type lozenges	Inhibitory activity against non resistant c. albicans infections non resistant oral trush
Itroconazole topical delivery lozenges	Rolled into lozenges using PEG base	90% drug release by the end of 60 min and remain stable	Topical application
OndansetronHcl lozenges	Sucrose as a base and eudragit E100 SCMC, HPMC K 4M, methyl cellulose as a binder used	Increased in bioavailability reduction in gastric irritation by passing of first pass metabolism and increased in onset of action	Chemotherapy induced nausea and vomiting
Fluconazole tablet Lozenges	Maize starch acacia, HPMC, E50, Sucrose as base and gelatin as a binder	Increased bioavailability. Reduction in gastric irritation by passing first pass.	Oral thrush

2.4. Ginger (*Zingiber officinale*)

Figure 2.8. *Zingiber officinale.*

Ginger (*Zingiber officinale Roscoe, Zingiberacae*) has been used in cooking for nearly 2000 years [22, 23]. For millennia, ginger has been used as a blood thinner, antiplatelet, antibacterial, antifungal, antiviral, antiworm, anti-inflammatory, and anti-oxidative agent. Although it is classified as a food additive by the US Food and Drug Administration, it has been shown to have beneficial effects on the gastrointestinal and cardiovascular systems, antilipidemic and antihyperglycemic properties, anti-tumor properties, and is effective as an immunomodulatory agent in humans and animals [24, 31].

2.4.1. Botenical information of ginger

Ginger is a member of the Zingiberaceae family. This family contains 24 genera and approximately 300 species. Additionally, the genus Zingiber contains approximately twenty species. Ginger is a tuberculate or rhizomatous perennial plant. The plant grows into an erect annual stalk (pseudo-stem) that reaches a height of 60–90 cm and is covered in dark green leaves. The stems are covered in flat coverings that detach from the handle; the stalk contains between eight and twelve distinct leaves. Leaves with long blades or blades that are straight and stemless; alternating (alternate), lance to linear lance, specula, growing to a height of 10 to 21 cm and a width of 2 to 2.5 cm. On a short stalk, the glomerules ascend sequentially. The glomerulus is formed in the shape of a head surrounded by knives and rises 12–30 cm above the ground. Slowly, the final blade is released. The size of the glomerulus is comparable to that of a thumbnail. The flowers are small and golden in color. The anthers are double, coronate in shape, with a narrow sandy channel and a horn-like shape. Under the right apex, there is an oval ovary and three cells, each of which contains a significant number of eggs, as well as filament, stigma funnel-shaped, horn-shaped, and peripheral hairy anthers. The plant is widely grown in India,

Bangladesh, Taiwan, Jamaica, and Nigeria. It is a perennial herbaceous plant that grows well in warm climates [25].

Medicinal species: *Zingiber officinale.*

Common names: Ginger is referred to as Black ginger, Cochin ginger, African ginger, Jamaican ginger, Gan jiang, Race ginger, Gegibre and Ingwer.

Botanical Family: Zingiberaceae. Ginger shares a close relationship with two more culinary spices: turmeric and cardamom.

Plant description: Ginger is a perennial herb that grows to a height of 2–4 feet and has grass-like leaves up to a foot in length. The underground root, or rhizome, is used for culinary and medicinal purposes..

Where it's grown: Ginger is a tropical plant native to Asia, Africa, India, Jamaica, Mexico, and Hawaii [26].

2.4.2. Composition of ginger

2.4.2.1. Nutrient composition

Fresh ginger includes a moisture content of 80.9 percent, a protein content of 2.3 percent, a fat content of 0.9 percent, a mineral content of 1.2 percent, a fiber content of 2.4 percent, and a carbohydrate content of 12.3 percent. Iron, calcium, and phosphorus are minerals found in ginger. Additionally, it includes thiamine, riboflavin, niacin, and vitamin C. changes significantly depending to content, kind, variety, farming circumstances, drying, curing processes and storage conditions [24].

2.4.2.2. Chemical composition

The most active constituents of the fresh rhizome are gingerols [27]. The volatile oil is mostly composed of sesquiterpene hydrocarbons, such as zingeberene (35%), curcumen (18%), and farnesene (10%) [28]. Gingerols, shogaols, paradols, and zingerone are all non-volatile pungent chemicals. Paradol is comparable to gingerol and is produced when shogoal (phenylalkanones) is hydrogenated [23]. Ginger is a source of fatty acids, waxes, carbs, vitamins, and minerals. Additionally, ginger rhizomes contain a powerful

proteolytic enzyme known as zingibain. Ginger's strong flavor is generated from non-volatile phenylpropanoid derivatives, most notably ginger and shogaols. Ginger supplementation in fish diets has been shown to improve disease resistance by enhancing the host's innate immunological activities, which are critical for disease resistance. Ginger may be involved in a variety of biological processes, including anti-oxidative, cardiovascular, anti-inflammatory, anticarcinogenic, hypolipidemic, anti-nausea, antithrombotic, and antibacterial ones [24].

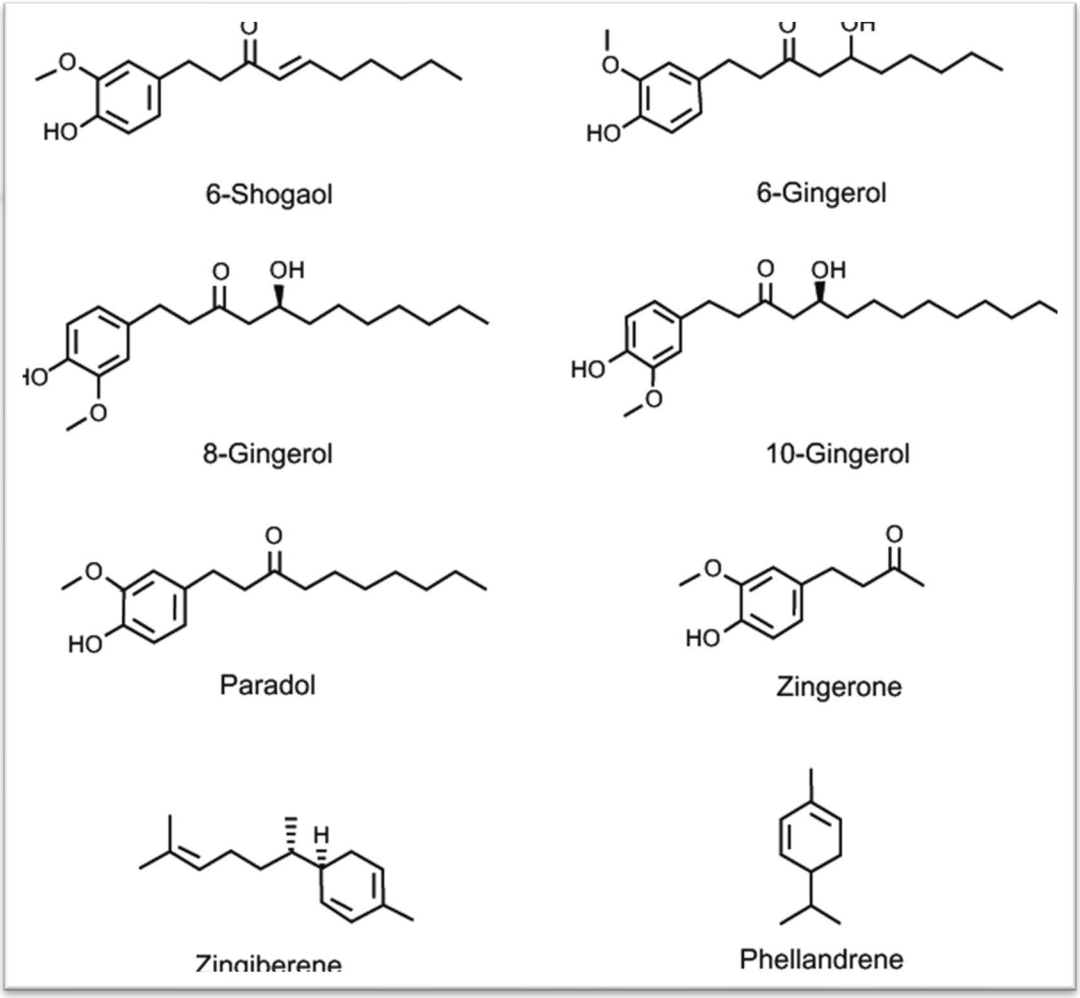


Figure 2.9. Chemical composition of ginger

2.4.3. Antimicrobial activity of ginger

Ginger is a powerful antibacterial agent and, to a lesser extent, an antifungal agent. Ginger's active ingredients have been shown in vitro to inhibit the growth of colon bacteria such as Es*cherichia coli, Proteus spp., Staphylococci, Streptococci,* and *Salmonella,* as well as to possess antibacterial properties against *Helicobacter pylori* [37].

Using the agar diffusion method, it was discovered that fresh ginger juice inhibited the growth of *Aspergillus niger, Saccharomyces cerevisiae, Mycoderma spp*., and *Lactobacillus acidophilus* at ambient temperatures (4, 10, 12 and 14 percent, respectively) [37].

Additionally, some in vitro studies examining ginger's antibacterial effect on fungi have revealed that ginger has no effect on certain fungi (*Aspergillus niger and Aspergillus flavus*) [23]. However, another study discovered that ginger extract possesses the broadest spectrum of antifungal activity of 29 plant extracts. [38].

2.4.4. Antioxidant properties of ginger

Antioxidants are compounds that bind active components to their active sites, thereby reducing the risk of various health complications. Antioxidants are abundant in plant-based foods. Among vegetables, ginger has a variety of therapeutic properties that help to resolve health inconsistencies [35]. Numerous in vitro studies on ginger roots and extracts have been conducted to determine the presence of polyphenol compounds with high antioxidant activity (6-gingerol and its derivatives) [36].

Ginger enhances the body's defense system. Numerous in vitro studies have established that numerous chronic diseases are associated with oxidative stress, and that these conditions have protective effects due to ginger's active ingredients' high antioxidant activity. If oxidative stress is sustained for an extended period of time, it can cause DNA damage. Gingeroller is considered an inhibitor of enzymes. It acts as an inhibitor of the activity of a variety of enzymes [35].

2.4.5. Pharmaceutical application

Ginger has a long history of use in Indian, Chinese, and Japanese systems of medicine. Ginger, which is believed to be beneficial in the Indian system (Ayurveda) for suppressing anorexia, dyspepsia, and inflammation, is also a degasser and digester.

Ginger has been shown to be beneficial in preventing nausea and vomiting associated with chemotherapy, pregnancy, travel, and seasickness in pharmacological studies. Additionally, dry ginger drops can be used to treat otalgia, cephagia, asthma, cough, colic, diarrhea, bloating, nausea, and vomiting [34].

Additionally, ginger is used as an anti-inflammatory and pain reliever, as well as for arthritis treatment, due to its protective effect against diabetes, migraine, and asthma, as well as peptic ulcer disease and gastrointestinal (GI) disorders such as stomach and colic [35].

Antioxidant and cancer-protective properties of ginger compounds are a result of their free radical scavenging activity. Ginger has been shown to be beneficial in the treatment of duodenal ulcers and mucosal damage caused by inflammatory bowel disease and colitis. Additionally, ginger is used to treat colds and influenza-like symptoms, as well as anxiety and stress, dyslipidemia, and cardiovascular function [35].

2.4.6. Extraction

6-gingerol, *Zingiber officinale's p*rimary bioactive phenolic compound, is well-known for its ability to scavenge free radicals in the treatment of a variety of ailments. Due to its simplicity, efficiency, and broad applicability, solvent extraction is the most frequently used method for extracting this primary component. The solvents most frequently used in the extraction procedure are hot or cold aqueous mixtures of acetone, ethanol, methanol, and ethyl acetate, and the extraction methods most frequently used are reflux and maceration.

Maceration yielded better results than reflux in extraction studies with ginger using the solvents mentioned above, while ethanol was significantly superior to acetone and methanol among the solvents [21].

The maceration method involves storing the coarse or powdered plant at room temperature for at least three days while adding a solvent. After three days, the mixture is pressed or filtered. Heat is transferred via convection and conduction in this traditional method [19].

3. MATERIALS & METHODS

3.1. Materials

The materials used in this study are given in Table 3.1.

Table 3.1. List of materials

Ingredient	Company
Ginger (*Zingiber officinale*)	Turkish market
Cinnamon (*Cinnamomum verum*)	Turkish market
Acacia	Sigma aldrich, Germany
Beeswax	Sigma aldrich, Germany
Lemon Essential Oil	Sigma aldrich, Germany
D-Limonen	Sigma aldrich, Germany
Stevia	Sigma aldrich, Germany
Lemon Juice	Turkish market
Glycerin	Honeywell, Germany
Gelatin	Sigma aldrich, Germany
Pluronic F 127 (Poloxamer 407)	Sigma aldrich, Germany
Honey	Turkish market
Strawbery	Turkish market
Carbomer	Sigma aldrich, Germany
Triethanolamine	Sigma aldrich, Germany
PVP (Polyvinylpyrrolidone)	Sigma aldrich, Germany
Methyl cellulose(MC)	Sigma aldrich, Germany
PEG 1000	Sigma aldrich, Germany
Distilled Water	Polifarma, Turkey

3.2. Equipment

The equipments used in this study are listed in Table 3.2.

Table 3.2. List of equipments

Equipment name	Company
Evaporator	Heidolph
Lyophilizatior alpha 1-4 LSC	Christ, Germany
Eletronic Balance	Mettler Toledo
Sonicator	Meditech
Thin Layer Chromatography	MZ-Analysentechnik GmbH, Germany

3.3. Methods

3.3.1. Ginger extraction

For the preparation of the lozenge, it is first necessary to extract ginger, which is the active ingredient of the lozenge. Extraction steps are in Table 2.3.

Table 3.3. Extraction steps of ginger

 **Figure 3.1.**Weighed process	**Step.1** Fresh ginger is peeled and cut into small pieces, taken into a beaker, weighed.

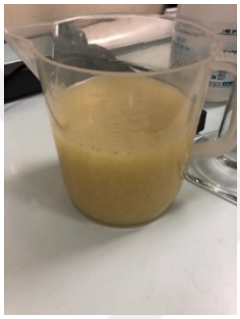
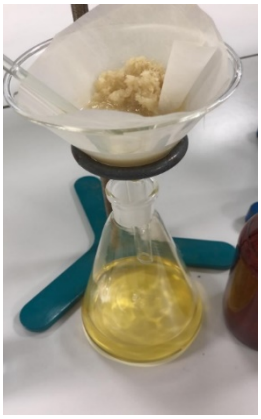
 **Figure 3.2.** Homogenizing process	**Step.2** The fresh ginger cut into pieces is homogenized.
 **Figure 3.3.** Maceration &soaking process with EtoH	**Step.3:** Maceration &Soaking Process with EtoH
 **Figure 3.4.** Filtration process	**Step.4:** The mixture kept in ethanol is filtered.

 **Figure 3.5.** Evaporation process	**Step.5:** Alcohol is blown in rotary evaporator device.
 **Figure 3.6:** Freeze- dried (Lyophilization) process	**Step.6:** Freeze- dried (Lyophilization) Proces

After the fresh ginger was lyophilized and dried, different lozenge formulas were prepared. A detailed table of prepared lozenge formulas is given in Section 3.3.2.

3.3.2. Ginger lozenge formulation

After ginger extract and freeze-dried cinnamon was prepared, the different lozenge bases are formulated. The preparation of soft lozenge components and their amounts are listed in Table 3.4.

Table.3.4. Composition of soft lozenge formulations (w/w %) and their codes

		Formulation codes						
Components amount (%)	**Function**	**GL1**	**GL2**	**GL3**	**GL4**	**GL5**	**GL 6**	**GL7**
Ginger (*Zingiber officinale*)	Active Ingredient	25	25	25	25	25	25	25
Cinnamon (*Cinnamomum verum*)	Active Ingredient + Flavour	20	20	20	20	20	20	20
Acacia	Binder	1	1	1	1	1	1	1
Beeswax	Lubricant	-	-	-	-	-	-	0.5
Lemon Essential Oil	Flavouring Agents	-	-	-	-	-	-	0.5
D-Limonen	Flavouring Agents	-	-	-	-	-	-	1
Stevia	Flavouring Agents	-	-	-	-	-	-	1
Lemon Juice	Flavouring Agents	1	1	1	1	1	1	1
Glycerin	Humectants	2	2	2	2	2	40	2
Gelatin	Binder	-	-	-	-	-	15	-
Pluronic F 127 (Poloxamer 407)	Surfactant	-	-	-	-	-	-	20
Honey	Flavouring Agents	10	10	10	10	10	10	10
Strawbery (freeze dried)	Flavouring Agents	10	10	10	10	10	10	10
Carbomer	Binder	-	-	-	2	-	-	-
Triethanolamine	Surfactant	-	-	-	1	-	-	-
PVP (Polyvinylpyrrolidone)	Binder		-	10	3	-	-	-
Methyl cellulose(MC)	Binder	-	-	-	-	10	-	-
PEG 1000	Binder	5	1	5			-	-
Distilled Water	Excipient	q.s.	q.s.	q.s.		q.s.	q.s.	q.s.

All lozenge formulations contain freeze-dried cinnamon and ginger extracts, as well as acacia, glycerin, lemon juice, honey, and freeze-dried strawberry, as well as polymers. Acacia is used to give the lozenge its texture and smoothness. The humectant is glycerin, and the flavors are lemon juice, honey, and freeze-dried strawberry. Polymers are frequently used as binders in formulations.

To prepare the active ingredient, combine the active ingredients and acacia in a sonicator for 5 minutes (microemilation is performed).

Strawberries are used as a sweetener; 1 kg of strawberries are blended into small pieces and combined with 1 liter of water. Then it is homogenized. It is stored for one day. The mixture is then blended until smooth and chilled overnight in the refrigerator. It is refrigerated.

Another step is the polymerization. After 100 ml, all polymers are prepared and the active ingredient is added to the formulation over the polymer. The procedure for preparing polymers is detailed in Table 3.5.

Table 3.5. Preparation of polymers

Polymers	**Preparation method**
PEG 1000	PEG is water soluble (approximately 630 mg/ml at 20°C). By dissolving PEG in water, a PEG solution was prepared.
PVP (Polyvinylpyrrolidone)	0.5 g PVP was dissolved in 10 mL deionized water for 2 hours at room temperature while stirring, and the solution was then used to make the PVP solution.
Triethanolamine	Triethylamine is soluble in water at 20 degrees Celsius to a concentration of 112.4 grams per liter. The solution was prepared by dissolving the ingredients in water.

Carbomer	1 g Carbopole powder is weighed and dissolved in 50 ml water.
Methylcellulose	In hot water, methylcellulose MC swells. At temperatures ranging from 80 to 90 degrees Celsius, the powder was dispersed with high shear in approximately 1/3 of the required amount of water. Once evenly distributed, the remaining water (cold or ice water) was added with moderate stirring. When the base is cooled to between 0 and 10 degrees Celsius, the maximum clarity, hydration, and viscosity are achieved.
Gelatin-glycerin base	The glycerogelatins are polymerized polymers. They were initially prepared by softening the Heat the gelatine and glycerine in the water for approximately 10 minutes on a steam bath set to 80 degrees Celsius until dissolved.
Pluronic F 127 (Poloxamer 407)	18 g Pluronic F 127 is weighed in and then chilled to -20 degrees for one night. The mixture is then brought to 4 degrees and allowed to left for one night before being mixed in a homogenizer.

The following flow charts depict the steps involved in the preparation of the formulations. Each formulation resulted in the preparation of 20 lozenges weighing 1 g.

GL1

*GL1 formulation was prepared by using 25% Ginger (*Zingiber officinale*), 20% Cinnamon (*Cinnamomum verum*) as active ingredients, 1% Acacia, 2% Glycerin and 5% PEG 1000, %1 Lemon Juice, 10% Honey and 10 %Strawbery (freeze dried).

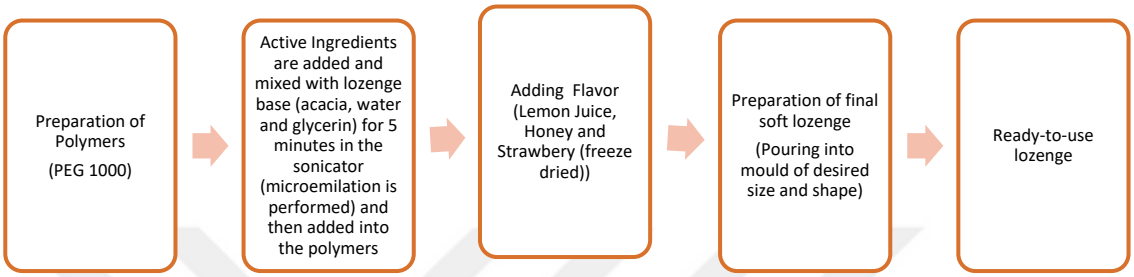


Figure 3.7. Flowchart of the GL1 formulation.

GL2

GL2 formulation was prepared in the same ingredints with GL1 formulation, however 1% PEG 1000 was used instead of 5% PEG 1000.

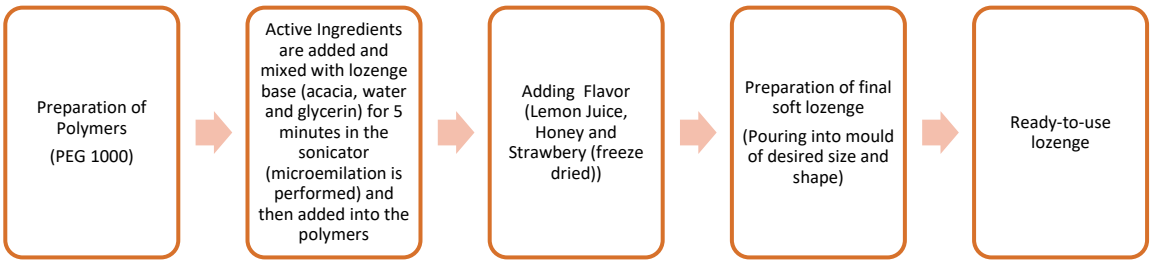


Figure 3.8. Flowchart of the GL2 formulation.

GL3

GL3 formulation was prepared by using the same ingredient in GL1 formulation. The difference from GL1 formulation, 10% PVP (Polyvinylpyrrolidone) is added.

Figure 3.9. Flowchart of the GL3 formulation.

GL4

GL4 formulation was prepared by using %25 Ginger (*Zingiber officinale*), %20 Cinnamon (*Cinnamomum verum*) as active ingredients, %1 Acacia, %2 Glycerin, 1% Carbomer, 1% Triethanolamine, 3% PVP (Polyvinylpyrrolidone), 1 % Lemon Juice, 10 % Honey and 10% Strawbery (freeze dried).

Figure 3.10. Flowchart of the GL4 formulation.

GL5

*GL5 formulation was prepared by using methyl cellulose MC (10%).

Figure 3.11. Flowchart of the GL5 formulation.

GL6

The composition of gelatin-glycerin bases which were used into GL6 formulation respectively containing 40% glycerin, 15% gelatin, 25% Ginger *(Zingiber officinale)*, 20% Cinnamon (Cinnamomum verum) as active ingredients, 1% Acacia, and 5% PEG 1000, %1 Lemon Juice, 10% Honey and 10 %Strawbery (freeze dried).

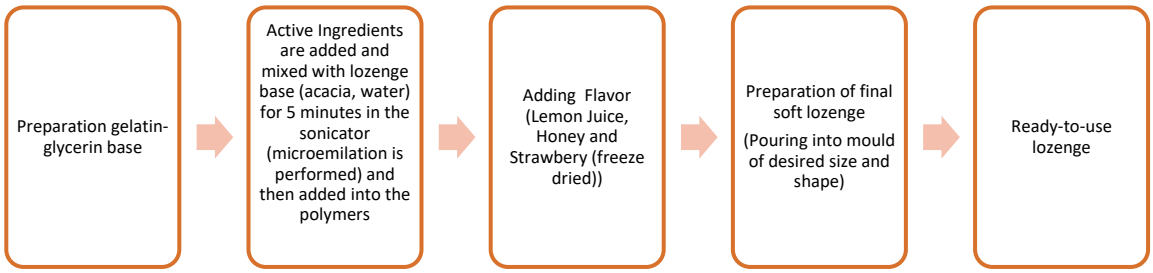


Figure 3.12. Flowchart of the GL6 formulation.

GL7

GL7 formulation was prepared by using 25% Ginger (*Zingiber officinale*), 20% Cinnamon (*Cinnamomum verum*) as active ingredients, 1% Acacia, 0.5% Beeswax, 0.5% Lemon Essential Oil, 1% D-Limonen, 1% Stevia, 1% Lemon Juice, 2% Glycerin and 10% Honey and 10 %Strawbery (freeze dried), 20% Pluronic F 127 (Poloxamer 407).

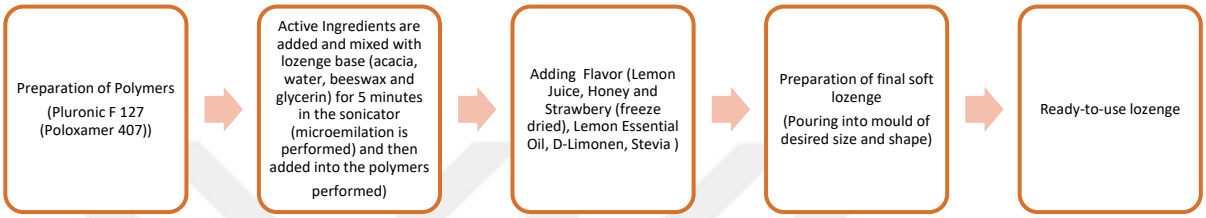


Figure 3.13. Flowchart of the GL7 formulation.

3.4. Quality Controls

3.4.1. Physically appearance

All formulations are evaluated based on how they physically appear to the observer. To be considered optimal, the lozenge produced with the optimal formula should have a homogeneous appearance that is not easily broken, as well as being sticky. Quality control analyses were carried out on the most optimal formula to ensure that it was as accurate as possible.

3.4.2. Disintegration time test

Using the method described in the European Pharmacopoeia 7.0 monograph, the disintegration time test was carried out in a buffer solution with the pH set at 7.2. We used the Pharma Test Disintegration Apparatus [53].

3.4.3. Thin layer chromotografy

A sensitive and accurate high-performance thin-layer chromatography (HPTLC) method was developed to determine the amount of 6-gingerol present in the rhizomes of Zingiber officinale (family: Zingiberaceae), commonly referred to as ginger. The stationary phase for HPTLC was methanol extracts of rhizomes from three different sources, with the mobile phase being n-hexane and diethyl ether (40:60 v/v). The test solution, reference solution, and mobile phase are prepared in accordance with the monograph of the European Pharmacopoeia 8.0 [54].

Figure 3.14. R:reference, E:extract, C:mobil phase

Test solution (E): Combine 5 mL methanol R and 1.0 g powdered herbal drug in a shaker and shake for 15 minutes before filtering the solution.

Reference solution (R): 10 mL of methanol R is added to 10 µL of citral R, and 10 mg of resorcinol R is added to 10 mL of citral R. Prepare the solution as soon as possible before using it.

Mobile phase (C) : hexane R, ether R (40:60 V/V).

Plate : TLC silica gel plate R.

Application: 20 µL as bands.

Development: in an unsaturated tank, over a path of 15 cm.

Drying : in air.

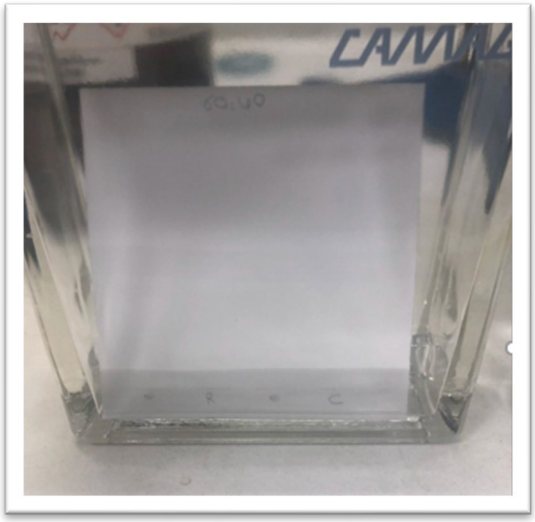


Figure 3.15. Thin layer chromatografy method

Detection: Applying an a 10 g/L solution of vanillin R in sulfuric acid R and observing in daylight while heating at 100-105 °C for 10 minutes will yield the following results:

An intense red zone (resorcinol) can be seen in the lower half of the chromatogram obtained with the reference solution, while two violet zones (citral) can be seen in the upper half.

Thin Layer Chromatographic Identification are shown in the Figure 3.16.

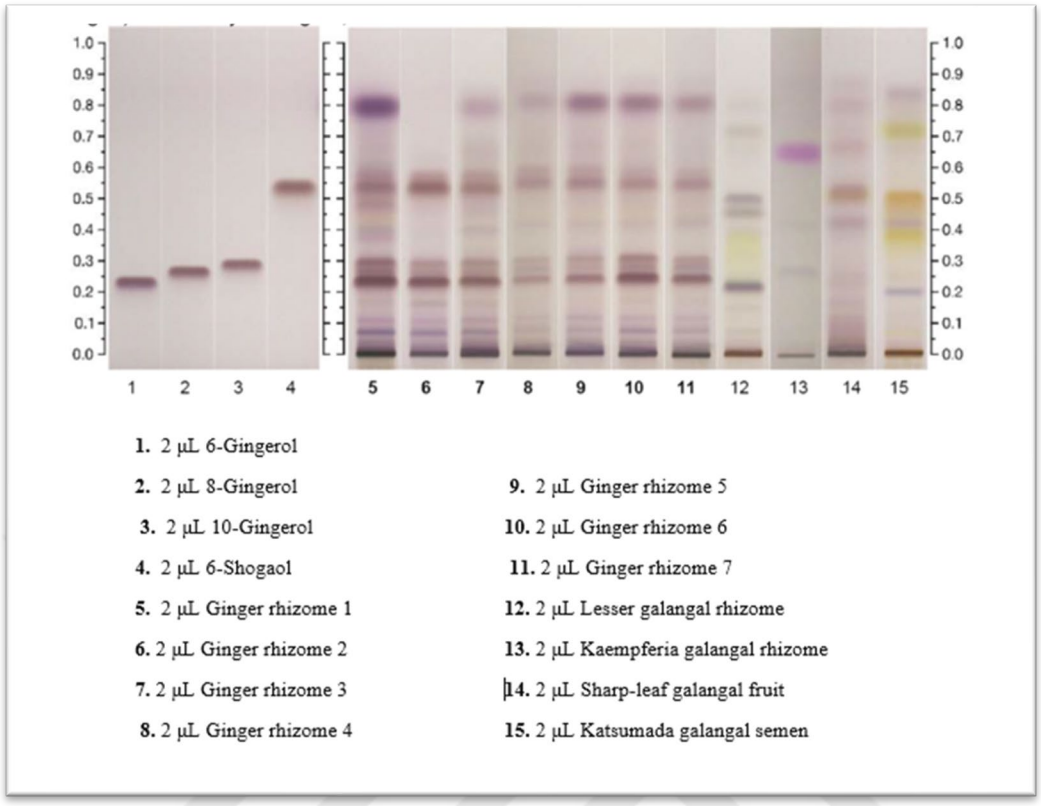


Figure 3.16. Thin layer chromatographic identification [55].

3.4.4. Microbiology test

Ginger *(Zingiber officinale)* has been used as a spice for over 2000 years and, according to the World Health Organization, has antimicrobial properties against a variety of human pathogens. Among the polyphenolic compounds found in its roots and extracts are 6-gingerol and its derivatives, which exhibit significant antimicrobial activity. [36].

The most frequently used method for determining the antimicrobial activity of Z. officinale is the Disc Diffusion (Agar-Based) method. As a result, the disc diffusion method was used to determine the antibacterial activity of two complexes against *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 6538).

All compounds were dissolved in H2O. Standard discs containing ofloxacin 5g (Antibacterial Agent) and nystatin (Antifungal Agent) 100 units were used as positive controls and references for the agar-based disc diffusion method, respectively.

After reaching the turbidity of 0.5 McFarland standards, sterile Ecuvion sticks were used to inoculate agar plates containing Mueller Hinton Agar (for bacteria) and Sabouraud percent 2 Dextrose Agar (for fungal suspensions) (fungal). Blank paper disks with a diameter of 6.0 mm were impregnated with stock solutions at the tested concentrations and then dried on an agar plate. After 18 to 24 hours of incubation at 37 degrees Celsius for microorganisms and 25 degrees Celsius for bacteria, growth inhibition zones around disks were measured.

The sizes of the inhibition zones on the agar surface surrounding the discs were used to determine the microbial species' sensitivities to the various compounds. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) determined the diameters of the zones in the disc diffusion experiment using a sliding caliper [56].

4. RESULTS AND DISCUSSION

4.1. Quality Controls Results

4.1.1. Physically appearance

All formulations are evaluated according to physically appearance as seen in Table 4.1.

Table 4.1. Evalution of soft lozenge formulations

Formulation codes	Physical evaluation of formulation
GL1	Heterogenous
GL 2	Heterogenous
GL 3	Heterogenous
GL 4	Lozenge was deteroted(fragile)
GL 5	Lozenge did not form (very soft)
GL 6	Heterogenous
GL 7	Homogenous

The all formulations were evaluated as an physical appearence and content uniformity.

GL1 formulation could not be able to homogenous a lozenge form. GL1 formulation was prepared by using 25% Ginger *(Zingiber officinale)*, 20% Cinnamon (Cinnamomum verum) as active ingredients, 1% Acacia, 2% Glycerin and 5% PEG 1000, %1 Lemon Juice, 10% Honey and 10 %Strawbery (freeze dried).

GL2 formulation was prepared in the same ingredints with GL1 formulation, however 1% PEG 1000 was used instead of 5% PEG 1000. It is aimed to obtain a homogeneous lozenge by reducing the PEG 1000 ratio, but the product is not homogeneous.

GL3 formulation was prepared by using the same ingredient in GL1 formulation. The difference from GL1 formulation, 10% PVP (Polyvinylpyrrolidone) is added. The lozenge was harder than the expecting one because of PVP ingredient but the GL3 formulation also could not be able to homogenous a lozenge form.

GL4 formulation was prepared by using %25 Ginger (*Zingiber officinale*), %20 Cinnamon (*Cinnamomum verum*) as active ingredients, %1 Acacia, %2 Glycerin, 1% Carbomer, 1% Triethanolamine, 3% PVP (Polyvinylpyrrolidone), 1 % Lemon Juice, 10 % Honey and 10% Strawbery (freeze dried). GL4 formulation base was lead to deterioration of the structure. GL4 formulation base was fragile.

GL5 lozenge formulation was composed of synthetic polymers (methyl cellulose MC (10%)).This formulation could not be evaluated as a lozenge, it did not form and also it was very soft.

The composition of gelatin-glycerin bases which were used into GL6 formulation respectively containing 40% glycerin, 15% gelatin, 25% Ginger *(Zingiber officinale)*, 20% Cinnamon (Cinnamomum verum) as active ingredients, 1% Acacia, and 5% PEG 1000, %1 Lemon Juice, 10% Honey and 10 %Strawbery (freeze dried). GL6 formulation could not be able to homogenous a lozenge form. The gelatin amount was not sufficient may be affected the texture of this formulation.

Consequently, all trial formulations were evaluated by physical appearence. The GL7 formulation was found convenient as a homogenous lozange. GL7 formulation was prepared by using 25% Ginger (*Zingiber officinale*), 20% Cinnamon (*Cinnamomum verum*) as active ingredients, 1% Acacia, 0.5% Beeswax, 0.5% Lemon Essential Oil, 1% D-Limonen, 1% Stevia, 1% Lemon Juice, 2% Glycerin and, 10% Honey and 10 %Strawbery (freeze dried), 20% Pluronic F 127 (Poloxamer 407).

According to the physically appearance results the optimum lozenge formulation was obtained as GL7 formulation as seen in table 4.2.

Table 4.2. Optimum formulation composition of GL7 soft lozenge and its components

Components	**Amounts (w/w %)**	**Each Lozenge (1g)**
Ginger *(Zingiber officinale)*	25%	250 mg
Cinnamon *(Cinnamomum verum)*	20%	200 mg
Pluronic F 127 (Poloxamer 407)	20%	200 mg
Honey	10%	100 mg
Strawbery (freeze dried)	10%	100 mg
Glycerin	2%	20 mg
Acacia	1%	10 mg
D-Limonen	1%	10 mg

Stevia	1%	10 mg
Lemon Juice	1%	10 mg
Beeswax	0.5%	5 mg
Lemon Essential Oil	0.5%	5 mg
Distilled Water	q.s.	80 mg

Each lozenge was 1g and containing 200 mg Ginger *(Zingiber officinale)*. Disintegration time test, microbiology test, and thin-layer chromatography were carried out with the optimal formulation (GL7).

4.1.2. Disintegration time test result

It is the intent of lozenges that they dissolve slowly in the mouth. The disintegration time of the optimal lozange formulation (GL7) was found to be 20-25 minutes.

4.1.3. Thin layer chromatografy result

In the chromatogram obtained with the test solution, two intense violet zones (gingerols) are visible below (Figure 4.1.) the zone due to resorcinol in the chromatogram obtained with the reference solution, two other less intense violet zones (shogaols) are visible in the middle, between the zones due to resorcinol and citral in the chromatogram obtained with the reference solution, and another zone is visible above the zone due to resorcinol

Figure 4.1. The result of thin layer chromatografy

4.1.4. Microbiology test results

At a concentration of 10 mg/ml, the disc diffusion method was used to test the antibacterial activity of ginger extract and the optimum formula GL7 against *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 10536), *Pseudomonia aeruginosa* (ATCC 15442), and *Staphylococcus aureus* (ATCC 6538).

When evaluating only ethanol solution ginger extract by disc agar method, ethanol ginger extract showed zone of inhibition of 12 mm and 8 mm againts *Escherichia coli* and *Staphylococcus aureus* (Table 4.3.).

Table 4.3. Antibacterial and antifungal activities of ginger extract (inhibition zone)

Compound	Conc. Of test Compound	Zone of Inhibition *C. albicans* ATCC 10231	Zone of Inhibition *E. coli* ATCC 10536	Zone of Inhibition *S. aureus* ATCC 6538	Zone of Inhibition P. aeruginosa ATCC 15442
Ofloxacin (Antibacterial Agent)	5 µg	-	32 mm	40 mm	35 mm
Nystatin (Antifungal Agent)	100 units	12 mm	-	-	-
Ginger Extract		-	12 mm	8 mm	-
Placebo		-	-	-	-

On the other hand, when evaluating the optimum formula GL7 by disc agar method, GL7 formulation showed zone of inhibition of 12 mm, 14 mm, 12 mm, 13 mm againts *Candida albicans, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* (Table 4.4.).

Table 4.4. Antibacterial and antifungal activities of GL7 formulatin (inhibition zone)

Compound	Conc. Of test Compound	Zone of Inhibition *C. albicans* ATCC 10231	Zone of Inhibition *E. coli* ATCC 10536	Zone of Inhibition *S. aureus* ATCC 6538	Zone of Inhibition *P. aeruginosa* ATCC 15442
Ofloxacin (Antibacterial Agent)	5 µg	-	30 mm	40 mm	35 mm
Nystatin (Antifungal Agent)	100 units	34 mm	-	-	-
GL7 Formulation	10 mg/ml	12 mm	14 mm	12 mm	13 mm
Placebo	10 mg/ml	-	-	-	-

Our result showed that GL7 formulation were able to inhibit growth of both gram-negative *(E. coli, & P. aeruginosa)* and gram-positive bacteria *(S. Aureus & C. albicans).*

4.2. Discussion

The study was designed with the idea that the antibacterial property of ginger, especially for oral health, is an important stage of proactive treatment. In developing the soft lozenge in question, various formulas were tried, and the optimal formulation was determined.

The GL7 soft lozenge formulation was found to be the most suitable formulation. The formulation contains 25% Ginger (*Zingiber officinale*), 20% Cinnamon (*Cinnamomum verum*), 1% Acacia, 0.5% Beeswax, 0.5% Lemon Essential Oil, 1% D-Limonen, 1% Stevia, 1% Lemon Juice, 2% Glycerin and, 10% Honey and 10%Strawbery (freeze dried), 20% Pluronic F 127 (Poloxamer 407).

According to the European Pharmacopoeia for the developed GL7 formulation, dispersion test was carried out and it was found that the dispersion time was 25-30 minutes.

For the ginger (*Zingiber officinale*) contained as the active ingredient with its antibacterial property, the presence of gingerols and shogaols were detected in the developed formula, which was qualitatively determined by thin layer chromatography (TLC).

Gingerols are the primary constituents of ginger rhizomes. Due to their thermal instability, they readily undergo dehydration reactions, resulting in the formation of shogaols, which impart dried ginger's distinctive pungent flavor. The following table details the chemical structures of gingerol and shagaol.

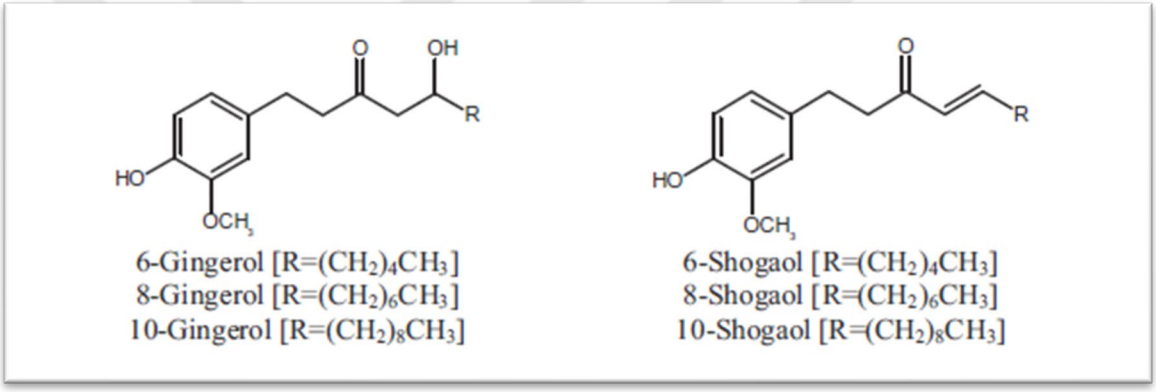


Figure 4.2. Chemical structures of gingerols and shogaols.

Both gingerols and shogaols exhibit a wide range of biological activities, including anti-cancer, antioxidant, antimicrobial, anti-inflammatory, and anti-allergic properties, as well as various activities involving the central nervous system (e.g., memory enhancement) [57].

In our natural antibacterial soft lozenge formulations, ginger purchased from the Turkish market is used and the formulations are made by extracting fresh ginger with Etonal. Therefore, the TLC method is very important for the qualitative determination of ginger content. As a result of our analysis, gingerol and shoagols in ginger extract are the main reasons why our formula shows antibacterial properties.

The other studies in ginger determination by thin layer chromatography (TLC) method were examined.

Sujay Rai et al. determined the amount of 6-gingerol present in ginger rhizomes in their investigation using a sensitive and accurate High-Performance TLC method. TLC was performed on methanol extracts of rhizomes obtained from three different sources using n-hexane and diethyl ether (40:60 v/v) as mobile phase and three different methanol extracts. 6-gingerol was detected in an average amount of 60.44 l of ginger extract containing 2.53 mg/g ginger extract. They concluded at the conclusion of their study that this method accurately quantifies 6-gingerol and that the TLC method can be used for routine ginger quality testing [58].

Usama I. Aly and colleagues detected the presence of 6-Gingerol in various parts of ginger using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), as well as qualitative and quantitative analysis, in their study. All samples of ginger 6-gingerol were detected using the TLC method, which was also used to qualitatively evaluate the samples. The quantitative determination was performed using the HLPC method, and the amount of 6-gingerol found in rhizomes (both in vivo and in vitro) and in vitro microrhizomes was found to be greater than that found in calli, shoots, and roots in vitro [59].

G. S. El-Baroty et al. used analytical TLC and gas chromatography/mass spectrometry (GC/MS) to characterize essential oils extracted from cinnamon and ginger rhizomes, as well as TLC-bio-autography experiments to determine the antimicrobial and antioxidant compounds contained in these components. Cinnamaldehyde (CA) and eugenol were identified as the most active antibacterial components in cinnamon bark oil and -sesquiphellandrene, caryophyllene, and zingiberene as the most active antibacterial components in ginger rhizome oil, respectively, using bioautography on TLC and GC-MS. According to G. S. El-Baroty et al., both oils contain compounds with significant antimicrobial and antioxidant activity, as well as protective properties [60].

Since our formula includes ginger as an active ingredient and cinnamon, which functions both as an active ingredient and as a flavoring agent, G. S. El-Baroty et al. study, which determines the antibacterial properties of these ingredients, is valuable for our study.

The disc diffusion method, on the other hand, was used to determine the microbiological properties of ginger extract as well as the optimal formula.

Previous research has demonstrated that ethanol extracts of Zingiber officinale were capable of inhibiting the growth of both gram-negative and gram-positive bacteria [42], with the inhibitory effect being more pronounced for gram-positive bacteria [43].

Ashraf A. Mostafa et al. investigated the antimicrobial activity of five plant extracts (*Punica granatum, Syzygium aromaticum, Zingiber officinales,* and *Thymus vulgaris)* against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi*. As was the case in our study, ethanolic extracts of ginger (*Zingiber officinales*) were used, as was the agar disc diffusion technique. The study examined the antibacterial activity of ginger (*Zingiber officinales*) and other plants against food poisoning bacteria, including two gram positive strains (*Bacillus cereus and Staphylococcus aureus*) and three gram negative strains (*E. coli, S. typhi & P. aeruginosa*). The results indicated that each of the plant extracts tested was potentially effective at inhibiting the growth of food poisoning bacteria, albeit to varying degrees [49]. However, our study demonstrated that ginger extract is effective against both gram positive and gram negative bacteria (*S. aureus and two strains of bacteria*) (*E. coli, & P. aeruginosa*).

Anjan Giriraju and GY Yunus conducted another study to determine the effectiveness of 10% ethanolic ginger extract against microorganisms that threaten dental health. The three oral microorganisms most frequently implicated in the causation of oral infections are *Streptococcus mutans, Candida albicans,* and *Enterococcus faecalis.* According to the study's findings, 10% ethanolic ginger extract inhibited *S. mutans, Candida albicans,* and *E. faecalis* at maximum zones of inhibition of 8 mm, 14 mm, and 11 mm, respectively [50].

Since our lozenge formulation was also designed to protect oral health, its microbial effect on *Candida albicans* was investigated. According to our study, the optimum formula GL7 showed zone of inhibition of 12 mm against *Candida albicans.*

In their study, U. Santo Grace et al. examined the antimicrobial activity of an ethanolic extract of *Zingiber officinale* (ginger) against gram-positive *Staphylococcus aureus* and gram-negative *Enterococcus aureus.* The ethanolic extract of ginger tested in the study demonstrated significant antibacterial activity against *Staphylococcus aureus* and *Enterococcus feacalis*. In comparison to *Enterococcus feacalis,* the ginger extract exhibited the strongest inhibitory activity against *Staphylococcus aureus* (23 mm zone)

at dilutions up to 15 µl. Although the zone of inhibition between *Staphylococcus aureus* and *Enterococcus faecalis i*s similar, it is clear that the ethanolic extract of ginger is more effective against gram-positive organisms [51].

While we used ethanol solution for the microbiological test in our study, S. P. Malu et al. discovered that the microbial activity of ginger was investigated using a variety of solvents in their study. Ginger extracts were prepared in the study using the solvents n-hexane, ethyl acetate, ethanolic soxhlet, and water. Antibacterial activity and inhibition of bacterial growth were determined for the extracts. The results indicated that all extracts except water extract possessed antibacterial activity, with dose-dependent inhibition of bacterial growth. Additionally, the results indicated that ginger extracts possess antibacterial properties and may be used to treat bacterial infections (*Coliform Bacillus, Staphylococcus Epidermidis,* and *Streptococcus viridans)* [52].

In our microbiology test, we used the disc diffusion method to determine the antibacterial activity of ginger extract and the optimum formula GL7 against *Candida albicans (ATCC 10231), Escherichia coli (ATCC 10536), Pseudomonas aeruginosa (ATCC 15442)*, and *Staphylococcus aureus (ATCC 6538)*. When ethanol solution ginger extract was evaluated using the disc agar method, it demonstrated a zone of inhibition of 12 mm and 8 mm against Escherichia coli and Staphylococcus aureus, respectively.

On the other hand, when evaluating the optimum formula GL7 by disc agar method, GL7 formulation showed zone of inhibition of 12 mm, 14 mm, 12 mm, 13 mm againts *Candida albicans, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa.* Our result showed that GL7 formulation were able to inhibit growth of both gram-negative *(E. coli, & P. aeruginosa)* and gram-positive bacteria *(S. Aureus & C. albicans).*

According to the results, the optimal formula GL7 appears to have an effect on more zones and more bacteria than ginger extract. When GL7 formula is examined, it is seen that cinnamon is known for its antibacterial properties in the formula content.

Cinnamon has been shown to be effective against pathogenic bacteria in model systems using *Escherichia coli and Staphylococcus* [44].

Yassine El Atki et al. assessed the antibacterial activity of cinnamon essential oil alone and in combination with several classical antibiotics against three multidrug-resistant bacteria, *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa,* to determine if synergy was possible. Cinnamon appears to have an antibacterial effect on bacteria, according to the study's findings [45].

The GL7 formula developed does not contain preservatives, so cinnamon is used in the formula, both for their antibacterial properties and also taste.

On the other hand, the formula was developed using Pluronic F (Poloxamer 407), which is also present in mouthwashes, often recommended by dentists for oral hygiene, with antibacterial properties alone.

Pluronics®, based on the polymer's structure, are a significant class of biomedical polymers currently being developed (Figure 4.3.). They are one-of-a-kind materials composed of poly(ethylene oxide) (PEO), poly(propylene oxide) (PPO), and poly(ethylene oxide) (PEO) triblock copolymers (PPO). Unlike the pluronic PEO block, which is hydrophilic and thus soluble in water, the PPO block is hydrophobic and thus insoluble in water.

They self-assemble into micelles in aqueous environments due to the presence of a hydrophobic PPO core and a hydrophilic PEO outer shell that forms an interface with the liquid. Additionally, because these micelles are amphiphilic, they can contain lipophilic molecules in their central hydrophobic core region.

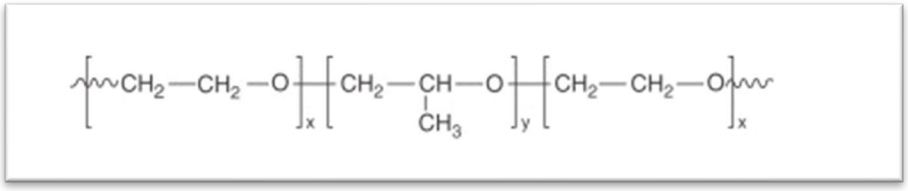


Figure 4.3. Chemical structures of Pluronics [$(PEO)_x$–$(PPO)_y$–$(PEO)_x$].

Thus, pluronic micelles are effective drug carriers because their assemblies act as passive drug reservoirs, allowing them to be combined with other drugs. These assemblies slowly release hydrophilic-hydrophobic encapsulated excipients into physiological fluids, allowing drugs to be delivered into subcellular compartments over time [48].

Pluronic F (Poloxamer 407)-hydrogels exhibit thermosensitive properties, making them attractive candidates for pharmaceutical formulations; additionally, they have been approved for human use by the Food and Drug Administration (FDA) and are widely used in the biomedical field due to their low toxicity and compatibility with a wide variety of excipients and biomolecules [46].

A recent study assessed that Pluronic F (Poloxamer 407) can be used as an adjunct in formulations for application in antibacterial photodynamic therapy to reduce adhesion and destroy adhered bacteria [47].

The use of Pluronic F (Poloxamer 407) and cinnamon (that antimicrobial property is known) is thought to increase the antibacterial property of our optimal formula GL7.

5. CONCLUSION

The most important point of defense of our body is the mouth. The entry point of bacteria into our body is our mouth and throat. Oral health is important in preventing disease and protecting the body's defenses. Oral health has become even more important, especially with the pandemic showing its negative effects around the world. Soft lozenges on the other hand can be easily used by patients, along with their antibacterial properties they can form an important part of prophylactic treatment for oral health and can act as a barrier in the mouth and throat against diseases.

This study was designed with the idea that the antibacterial property of ginger, especially for oral health, is an important stage of proactive treatment. In developing the soft lozenge in question, various formulas were tried, and the optimal formulation was determined.

In this thesis, the formulations were developed by preferring natural ingredients. All formulations contained 25% Ginger *(Zingiber officinale)*, 20% Cinnamon *(Cinnamomum verum)* and does not contain preservatives, so cinnamon is used in the formulas, both for their antibacterial properties and taste.

For the ginger *(Zingiber officinale)* contained as the active ingredient with its antibacterial property, the presence of gingerols and shogaols were detected in the developed formula, which was qualitatively determined by thin layer chromatography (TLC).

On the other hand, the optimum formula GL7 was developed using t Pluronic F (Poloxamer 407) has thermo-sensitive and hydrogels properties which are also present in mouthwashes, often recommended by dentists for oral hygiene, with antibacterial properties alone. Besides, the microbial properties of GL7 formulation were determined *C. albicans, E. coli, P. aureus, and P. aeruginosa* were found to be effective against bacteria by using disc diffusion method.

In the result of study, a natural antibacterial soft lozenge form containing ginger extract has been developed. Developed soft lozenges, can be easily used by patients, together with their antibacterial properties, can form an important part of proactive treatment for oral health, and can act as a mouth and throat barrier against diseases.

Due to emerging antibiotic resistance, alternative prevention and treatment options for oral health have become the need of today, it is believed that the natural and antibacterial soft lozenge formula developed is an alternative dosage form that can be used for oral health.

Besides, On the other hand, we have studied an effective, personalized lozenge form that can be prepared in a community pharmacy and we think that such forms can be developed to be prepared in community pharmacy.

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