Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 15(2), 448-458, 2025
Journal of the Institute of Science and Technology, 15(2), 448-458, 2025

ISSN: 2146-0574, eISSN: 2536-4618

Biyoloji / *Biology* **DOI: 10.21597/jist.1595057**

Araştırma Makalesi / *Research Article*

Geliş tarihi / *Received*: 03.12.2024 **Kabul tarihi / *Accepted*: 11.02.2025**

Atıf İçin: Hoş, A., İnci, A., Çalış, A., Oktay, E., Demirel, D., İmrak, G., Eyupoglu, O.E. (2025). Antibakteriyel Çiğneme Barlarının Hazırlanması, Biyoaktivitesi ve HPLC-UV İçerik Analizi. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 15(2), 448-458.

To Cite: Hoş, A., İnci, A., Çalış, A., Oktay, E., Demirel, D., İmrak, G., Eyupoglu, O.E. (2025). The Preparation, Bioactivity and, HPLC-UV Contents Analysis of Antibacterial Chewable Bars. *Journal of the Institute of Science and Technology*, 15(2), 448-458.

Antibakteriyel Çiğneme Barlarının Hazırlanması, Biyoaktivitesi ve HPLC-UV İçerik Analizi

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Öne Çıkanlar:
- Antibakteriyel çiğneme barı,
- Balmumu,
- %100 antibakteriyel aktivite

Anahtar Kelimeler:
- Ağız bakımı
- antibakteriyel aktivite
- disk difüzyon yöntemi
- plaka sayımı
- biyobozunur malzeme

ÖZET:

Bu çalışmada, hijyen yöntemlerinin uygulanmasının zor olduğu veya uygulama için yeterli zaman ve malzemenin bulunmadığı durumlarda kullanıcıya kolaylık sağlamak amacıyla antibakteriyel, çevre dostu, tek kullanımlık çiğneme barının geliştirilmesi anlatılmaktadır. İlk olarak karanfil, nar meyve kabuğu ve lavanta uçucu yağları ve metanolik ekstraktları elde edilmiştir. Her bir ekstrakt ve uçucu yağın *Staphylococcus aureus, Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis* ve *Staphylococcus epidermidis*'e karşı antibakteriyel aktivitesini belirlemek için disk difüzyon yöntemi kullanılmıştır. HPLC-UV analizleri HPLC Agilent-1100 modular compact system kullanılarak gerçekleştirilmiştir. Tüm metanolik ekstraktlarda Cinnamic acid, Caffeic acid, Punicalagin, Kaempferol-3-O-rutinoside, Catechin ve Quercetin-3-O-hexoside tespit edilmiştir. Daha sonra, lavanta, karanfil, nar meyvesi kabuğu ekstraktları ve uçucu yağları, balmumu ve damla sakızından oluşan antibakteriyel çiğnenebilir barlar hazırlanmıştır. Ardından antibakteriyel çiğneme barlarının hem insan tükürüğü üzerindeki hem de *S. mitis, S. aureus* ve *S. epidermidis* üzerindeki antibakteriyel aktivitesi belirlenmiştir. Balmumu ve damla sakızı içeren çiğneme barı 1, *S. aureus, S. mitis* ve *S. epidermidis*'e karşı %71-90 antibakteriyel aktivite göstermiştir. Balmumu, damla sakızı, ekstraktlar ve uçucu yağlar içeren çiğneme barı 2, *S. aureus, S. mitis* ve *S. epidermidis*'e karşı %100 antibakteriyel aktivite göstermiştir. Ayrıca, çiğneme barı 2'nin, insan tükürüğüne karşı %99 antibakteriyel aktiviteye sahip olduğu belirlenmiştir. Bu çalışma, antibakteriyel çiğneme barlarının hazırlanması ve antibakteriyel aktivitesinin belirlenmesi ile ilgili ilk araştırmadır. Bu antibakteriyel çiğneme barının insanların kullanımına sunulmadan önce yan etkilerinin belirlenmesi için daha fazla araştırmaya ihtiyaç vardır.

The Preparation, Bioactivity and HPLC-UV Contents Analysis of Antibacterial Chewable Bars

Highlights:
- Antibacterial chewable bar
- Beeswax
- 100% antibacterial activity

Keywords:
- Oral care
- antibacterial activity
- disc diffusion method
- plate counting
- biodegradable material

ABSTRACT:

This study describes the development of antibacterial eco-friendly disposable chewable bar in order to provide convenience to the user in cases where hygiene methods are difficult to apply or there is not enough time and materials for application. Firstly, essential oils and methanolic extracts of clove, lavender and pomegranate fruit peel were obtained. Disc diffusion method was used to find out the antibacterial activity of each extracts and essential oils against *Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis, Staphylococcus aureus, Staphylococcus epidermidis.* Analyses of HPLC-UV were carried out by using the HPLC Agilent-1100 modular compact system. Cinnamic acid, Caffeic acid, Punicalagin, Kaempferol-3-O-rutinoside, Quercetin-3-O-hexoside, and Catechin were detected in all methanolic extracts. Next, antibacterial chewable bars, which were made of beeswax, mastic gum, extracts and essential oils of lavender, clove, pomegranate fruit peel, were prepared. Then, the antibacterial activity of antibacterial chewable bars was determined against human saliva, *S. mitis, S. aureus, S. epidermidis.* The chewable bar 1, which contains beeswax and mastic gum, showed 71-90% antibacterial activity against *S. aureus, S. mitis* and *S. epidermidis.* The chewable bar 2, which includes beeswax, mastic gum, extracts and essential oils, showed 100% antibacterial activity against *S. aureus, S. mitis* and *S. epidermidis.* Also, chewable bar 2 has 99% antibacterial activity against human saliva.The current research is the first research about the preparation of antibacterial chewable bar and the determination of the antibacterial activity of it. It is needed further research to discover the side effects of this antibacterial chewable bar before using for human.

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Makale 23-25 Şubat 2024 tarihlerinde Mersin'de düzenlenen "Akdeniz 11th International Conference on Applied Sciences" sözlü olarak sunulmuştur.
Etik Kurul Onayı / *Ethics Committee Approval*: Ethical approval for the study was taken from İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee. (Decision Number: 451, Date: 25.05.2022).

INTRODUCTION

Because of the increasing incidence of oral diseases, treatment methods and alternative prevention are necessary. Additionally, effective, safe, and economical products are global need (Halawany, 2012). The natural products isolated from plants include very rich biologically active components which have antibacterial activity (Bairwa et al., 2012). Nowadays, using natural antibacterial products in toothpastes and mouth rinses as extracts of miswak, peppermint, tea tree oil, manuka, and green tea is trending (Hegazy and Awad, 2012). Using mouth wash, chewing sticks, gum, toothpaste, tooth brushes, and dental floss are currently used to maintance oral hygeine (Abhary and Al-Hazmi, 2016).

Good oral health has a great affect on the quality of life and well-being. Poor oral health can be related with various diseases (Halawany, 2012). Oral hygiene means keeping the teeth clean to prevent dental problems such as bad breath, dental cavities, gingivitis, and oral pathologic conditions (periodontitis, gingivitis, and dental trauma such as subluxation, oral cysts and following wisdom tooth extraction) (Bairwa et al., 2012). There is a correlation between the oral microbiota and systemic diseases (Hegazy and Awad, 2012). Dental diseases cause both financial and social problems. For example, the treatment of dental diseases is expensive and a dental pain can lead to waste of time (Bairwa et al., 2012).

Chewing gum, brushing teeth regularly, cleaning interdental with dental floss, cleansing the tongue, rinsing with antiseptic solution, and avoiding fermented carbohydrates are used for reducing the accumulation of plaque in the oral cavity (Waty and Suryanto, 2018). Gum exudates of plants like Myroxylon balsamum, Croton xalapensis and Ficus platyphylla are used in form of chewing gums to keep the cleanliness of teeth. They have much more advantage than commercial gums in reducing the dental caries and masking bad mouth odour. Also, for cleaning the buccal cavity a lot of plants are used as chewing sticks and a component of plant based tooth pastes and gels thanks to their antimicrobial activity (Bairwa et al., 2012). Using extracts of natural origin that supplies usable and safe alternative to antibiotics and other synthetic products to prevent and treat oral and dental problems. That’s why thousands of plants have been utilized for dental and oral problems (Gupta and Shetty, 2018). The essential oils of plants are important constituent of toothpastes and gels (Bairwa et al., 2012).

Extracts and essential oils from medicinal plants form the basis of many applications, including the preservation of raw or unprocessed foods and pharmaceutical, alternative medicine and natural therapies (Dulger and Gonuz, 2004).

Punica granatum L. (Punicaceae) which is a shrub or a small tree (Fleck et al., 2016). Pomegranate is native to Iran and it has been spread through Asia, North Africa and Mediterranean Europe, including Turkey (Duman et al., 2009). Most of the significant pharmacological activity of the pomegranate fruit peel is due to the presence of polyphenol compounds. These compounds include tannins and flavonoids which are natural preservatives and powerful antioxidants (Newman et al., 2007). Thanks to phytochemicals like delphinidin, cyanidin, pelargonidin, ellagic acid, punicalin, punicalagin, pedunculagin, and different glucosides, which involve anthocyanins, pomegranate has antioxidant, antimicrobial, and antifungal properties (Akarca and Başpınar, 2019).

The earlier production of clove (*Syzygium aromaticum*) was in China and was widely cultivated in Spice Islands, Indonesia, Pemba and Zanzibar. Thanks to eugenol, oleic acids and lipids found in its essential oils clove has antimicrobial and antifungal properties (Nzeako et al., 2006).

Lavender is an indigenous in the Mediterranean sea, Spain, France, Andorra and Italy, but is also cultivated in many other countries in the world. Lavender includes essential oil, anthocyanins, herniarin, phytosterols, coumarin, sugars, coumaric acid, minerals, glycolic acid, valeric acid and its esters, ursolic acid, and tannins (Smigielski et al., 2018). According to the literature, lavender essential oil has different pharmacological effect like antibacterial, antifungal, antioxidant, anxiolytic, anticonvulsant, and anticholinesterase properties hence lavender essential oil has a wide area of applications in pharmaceutical products (Kwiatkowski et al., 2019). Food and Drugs Administration (FDA) classified lavender essential oil as Generally Recognized as Safe (GRAS) (Malcolm and Tallian, 2017).

Mastic, which is obtained from the stem and main leaves of Pistacia lentiscus, is a folk medicine and is used for a food ingredient in Greece and other parts of the Mediterranean region for many centuries. Mastic has antibacterial, anti-inflammatory, and antiulcer properties. Mastic gum had important antiplaque and antigingivitis effects that's why, it may be useful for oral health (Takahashi et al., 2003).

There are *S. mutans, viridans streptococci, S. pneumoniae, S. epidermidis,* and *S. aureus* as pathogenic bacteria in the oral cavity (Waty and Suryanto, 2018). *Streptococcus mutans* can lead to plaque which is one of the main causes of dental infection (Bairwa et al., 2012). *S. mutans* is highly acidogenic and aciduric (Wang and Ren, 2017). *Streptococcus mutans* is a key pathogen in the initiation and progression of caries (Dinis et al., 2022). Since *Streptococcus mutans* metabolize sucrose to organic acids which dissolve the calcium phosphate in teeth. Dissolving the calcium phosphate in teeth cause decalcification and eventual decay (Bairwa et al., 2012). Previous studies have indicated that *Staphylococcus aureus* has a strong connection to dental implant infections (Wang and Ren, 2017). *Streptococcus mitis,* which is one of the earliest commensal colonizers, is found in the human oral cavity from early infancy and throughout life (Engen et al., 2018). *Staphylococcus epidermidis* is a kind of bacteria which is most prevalent in dental caries or dental pulp (Divakar et al., 2017).

It is aimed to produce a practical eco-friendly prototype in chewable form and disposable in order to provide convenience to the user in cases where hygiene methods are difficult to apply or there is not enough time and materials for application.

MATERIALS AND METHODS

Materials

Plant materials used in this study were purchased in Turkey. Test microorganisms used in experiments were *Staphylococcus aureus* (ATCC 29213), *Streptococcus mutans* (CNCTC 8/77), *Streptococcus salivarius* (CNCTC 64/59), *Streptococcus mitis* (clinical isolate), *Staphylococcus epidermidis* (ATCC 12228). They were obtained from Medipol Mega Hospitals Complex Microbiology Laboratory and İstanbul Medipol University, School of Pharmacy, Pharmaceutical Microbiology Laboratory.

Preparation of Extracts

Plant materials (lavender, clove, pomegranate fruit peel) were dried in shade for 7 days and dried materials were ground by using a mill. The powders of plant materials were kept in methanol for 3 days in a dark place at a rate of 1:10 (w/v). The solvent in the extracts were removed by using rotary evaporator (BUCHI Rotavapor R3 and BUCHI Heathing Bath B-100) under vacuum at 45°C for 15 minutes (Tunç et al., 2013).

Preparation of Essential Oils

20 g of the powdered plant materials (lavender, clove, pomegranate fruit peel) were transfered into a glass balloon then 200 mL of ultra distilled water and 2 mL of HCl were added. After the mixture began to boil inside the Clevenger apparatus at 135 °C for 4 h. Finally, the obtained essential oil was transferred into sterile containers (Seidi et al., 2021).

HPLC-UV Analysis of Phenolic Acid Content of Extracts

HPLC Agilent-1100 modular compact system (USA) with autosampler unit, UV-DAD, thermostable column cabinet with three trays utilized for the gradient analysis included with reagent programmable channel injection.

Experimental determinations were performed using a closed Purospher star, C18 column with guard column (5 µm, 4.6 × 250 mm) (Merck, Germany). The injection volume of all samples was 20 µL. The analysis time was 30 min. Data analysis (retention time, peak area and detection limits) was performed using Chem-station of Agilent Program. The mobile phase consisted of quaternary solvents in a harmony: solvent A was methanol, solvent B was formic acid/acetonitrile/aqua (3.5/48.25/48.25/48.25, v/v/v) mixture, solvent C was 1.5% formic acid: aqua (v/v) mixture and solvent D was HPLC grade acetonitrile. The executive pump mobile phase flow rate was set to 0.7 mL/min. Detection signals were respectively set at max. 280 nm for UV phenolic peaks. The column oven was set to room temperature of 23-25°C. The reaction coil made of polytetrafluoroethylene tubing (0.22 mm i.d.) was set to 1.5 m length. All samples were passed through a 0.20 micron filter before HPLC injection (Sinan et al., 2021). For this study, the validation parameters for LOD and LOQ were set at 3 times and 10 times the average standard deviation of noise, respectively, according to the International Conference on Harmonization guidelines (Armbruster and Pry, 2008).

Preparation of Antibacterial Chewable Bars

0.4 g of each extracts, 50 µL of each essential oils were added into the melted beeswax (10.17 g) and mastic gum (0.485 g). To mix the essential oils and extracts homogeneously, beeswax was melted and it was added essential oils and extracts. The melted mixture was poured into the template and allowed to cool. After that the cooled thin layer was cut to a certain size (2 cm × 2 cm) and used in experimental studies (Figure 1) (Hoş et al., 2020). The chewable bar that contains only beeswax was used as a control. The chewable bar that contains beeswax and mastic gum was named as chewable bar 1. The chewable bar which includes beeswax, mastic gum, extracts and essential oils was named as chewable bar 2.

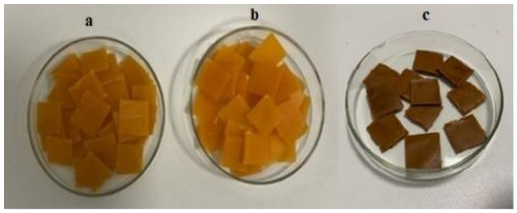

Figure 1. **a)** Beeswax (Control), **b)** Chewable bar 1, **c)** Chewable bar 2

Determination of Antibacterial Activity of Extracts and Essential Oils

Disc diffusion method was used to find out the antibacterial properties of each extracts and essential oils against to *Staphylococcus aureus, Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis, Staphylococcus epidermidis* (Semerci et al., 2020). The extract concentrations were obtained as 200 mg/mL by adding methanol to each extract. Sterile discs (6 mm in diameter) were saturated by 20 µL of extracts or 10 µL of essential oils and were allowed to dry. Methanol saturated discs were used as negative control.

Bacterial suspension was prepared from 24h culture and the bacterial density was adjusted to 0.5 McFarland by using a densitometer. The bacterial suspension which was adjusted to 0.5 McFarland was inoculated to 5% Sheep Blood Mueller Hinton Agar for viridans streptococci and Mueller Hinton Agar for another test microorganisms by using sterile swabs. After that, the discs were gently pressed onto the Agars. Incubation period was performed under 5-10 % CO_2 condition at 37°C for 24 hours for viridans streptococci and was carried out at 37°C for 24 hours for other test microorganisms. Finally, the diameters of the inhibition zone were measured by using an electronic digital caliper after the incubation period.

The determination of antibacterial effects of extracts and essential oils was performed three times under aseptic conditions.

Determination of Antibacterial Effects of Chewable Bars

The antibacterial effects of chewable bars were determined by the plate counting method against *Streptococcus mitis, Staphylococcus aureus,* and *Staphylococcus epidermidis* (Hoş et al., 2020). Also, the antibacterial effects of chewable bars were determined by the plate counting method against human saliva. Saliva samples were taken from 20 humans (10 females + 10 males) to determine the antibacterial effect of the chewable bars. A mixture of females' saliva sample was obtained by mixing the females' saliva samples. Males' saliva sample mixture was obtained by mixing males' saliva samples. The human saliva was collected from people whose ages are between 18 and 60, healthy (without gingival bleeding, not using dental braces, not using antibiotics, not having canker sores, herpes and thrush, not having any infection, not allergic to the herbal materials used in the chewable bars). Ethical approval for the study was confirmed by İstanbul Medipol University Non-Interventional Clinical Research Ethics Committee. (Decision Number: 451, Date: 25.05.2022).

Each surface of the chewable bar was sterilized under UV light treatment for 5 min. The 50 µL of 24 h bacterial culture (3×10^5 CFU/mL) was inoculated onto the chewable bar surface. Also, the 50 µL of the mixture of males' saliva and the mixture of females' saliva were inoculated onto the chewable bar surface separately. Then, all of the surfaces were covered by polyethylene film (1.5 cm×1.5 cm). Finally, the inoculated chewable bars were incubated for 24 h at 37 °C and a relative humidity (RH) of higher than 90%. After 24 h, the chewable bars were then washed with 20 mL of 0.87% NaCl solution. 100 µL of this solution was inoculated to the 5% Sheep Blood Agar for viridans streptococci and Nutrient Agar for another test microorganisms by the spread plating method at the end of the washing process. The incubation step was performed at 37°C for 24 h. Especially for viridans streptococci, the incubation step was carried out under 5-10 % CO_2 condition at 37°C for 24 h. After the incubation period, the colonies were counted. The antibacterial effect was calculated by using the following relationship:

$$R = [(B-C)/B] \times 100$$

Where R is the antibacterial effect (%) of the chewable bar samples, B is the mean number of the bacteria on the control sample (CFU) and C is the mean number of the bacteria on the modified test sample (CFU).

RESULTS AND DISCUSSION

Antibacterial analysis have two main parts. Firstly, it was determinated the antibacterial effect of extracts and essential oils of lavender, clove, pomegranate fruit peel by disc diffusion method against *Staphylococcus aureus, Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis,* and *Staphylococcus epidermidis.* Table 1 showed the antibacterial activity of the extracts (lavender, clove,

pomegranate fruit peel) against the test microorganisms. Otherwise, the antibacterial activity of essential oils (lavender, clove, pomegranate fruit peel) against test microorganisms was given in Table 2. As it was shown both Table 1 and Table 2, extract and essential oil of clove has antibacterial activity against more bacteria than others.

Table 1. Inhibition zone diameters of methanolic extracts (lavender, clove, pomegranate fruit peel)

	Inhibition Zone Diameters (mm)		
Test Microorganisms	Pomegranate Fruit Peel	Clove	Lavender
S. aureus	14.80±0.36	11.07±1.42	0
S. mutans	0	12.10±0.70	0
S. mitis	7.50±0.36	11.57±1.82	0
S. salivarius	0	0	0
S. epidermidis	16.80±0.56	13.77±0.61	7.70±0.26

±standard deviation.

Table 2. Inhibition zone diameters of essential oils (lavender, clove, pomegranate fruit peel)

	Inhibition Zone Diameters (mm)		
Test Microorganisms	Pomegranate Fruit Peel	Clove	Lavender
S. aureus	0	13.83±0.47	7.53±0.61
S. mutans	0	0	0
S. mitis	0	10.97±1.44	0
S. salivarius	0	0	0
S. epidermidis	0	15.90±0.46	0

±standard deviation.

Secondly, it was analyzed the antibacterial activity of chewable bar 1 and chewable bar 2 by plate counting method against human saliva and test microorganisms (*S. mitis*, *S. aureus*, *S. epidermidis*). The antibacterial activity of chewable bar against human saliva was carried out three times under aseptic conditions. Table 3 showed the number of colonies for control (beeswax) and chewable bar 2. It was determined that chewable bar 2 has highly antibacterial effect on both females' and males' saliva.

Table 3. Number of colonies for control, and chewable bar 2

	Number of Colonies (CFU/mL)					
Chewable Bar	Females' Saliva			Males' Saliva		
	1st Experiment	2nd Experiment	3rd Experiment	1st Experiment	2nd Experiment	3rd Experiment
Beeswax (Control)	134×10^3	154×10^3	135×10^3	26×10^3	5×10^3	26×10^3
Chewable Bar 2	0	40	0	40	70	40

The antibacterial effect of chewable bar 2 against bacteria in human saliva was determined by plate counting method. As it shown in Figure 2, colony forming units significantly decreased compared to control.

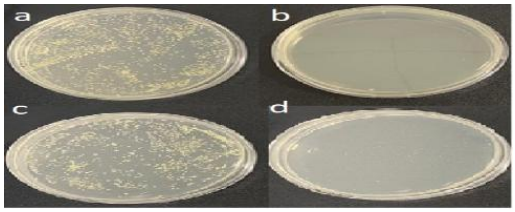

Figure 2. Colonies after incubation period, **a)** control against females' saliva, **b)** chewable bar 2 against females' saliva, **c)** control against males' saliva, **d)** chewable bar 2 against males' saliva

Table 4 showed the number of colonies for control (beeswax) chewable bar 1 and chewable bar 2. It was determined that chewable bar 1 and chewable bar 2 have highly antibacterial effect on *S. aureus, S. mitis* and *S. epidermidis.*

Table 4. Number of colonies for control, chewable bar 1, and chewable bar 2

Number of Colonies (CFU/mL)			
Chewable Bar	*S. aureus*	*S. mitis*	*S. epidermidis*
Beeswax (Control)	301×10^3	402×10^3	62×10^3
Chewable Bar 1	31×10^3	116×10^3	7×10^3
Chewable Bar 2	0	0	0

The results of the calculated antibacterial effects (R%) of the chewable bars against *S. aureus, S. mitis* and *S. epidermidis* are given in Table 5. According to the test results shown in Table 5, chewable bar 2 exhibited 100% antibacterial effect against *S. aureus, S. mitis* and *S. epidermidis.* Also, chewable bar 2 has 99% antibacterial activity against human saliva. Additionally chewable bar 1, which contains only beeswax and mastic gum, showed 71-90% antibacterial activity against *S. aureus, S. mitis* and *S. epidermidis.*

Table 5. Antibacterial activity (R%) of chewable bars

Antibacterial Activity (R%) of Chewable Bar					
Chewable Bar	*S. aureus*	*S. mitis*	*S. epidermidis*	Females' Saliva	Males' Saliva
Beeswax (Control)	0	0	0	0	0
Chewable Bar 1	90	71	89	nd	nd
Chewable Bar 2	100	100	100	99.9	99.7

nd: non detected.

HPLC-UV analysis were performed with HPLC Agilent-1100 modular compact system. Punicalagin, Cinnamic acid, Caffeic acid contents were found significantly higher than other phenolics in HPLC-UV analysis of all extracts (Table 6 and Figure 3). Linearity of the methodology was tested in the range of 37-86 ppm for detected 6 phenolic acids (Table 6). All statistical Agilent-Chem-station detection limit data and concentrations were reported significantly with standard deviations (p<0.05).

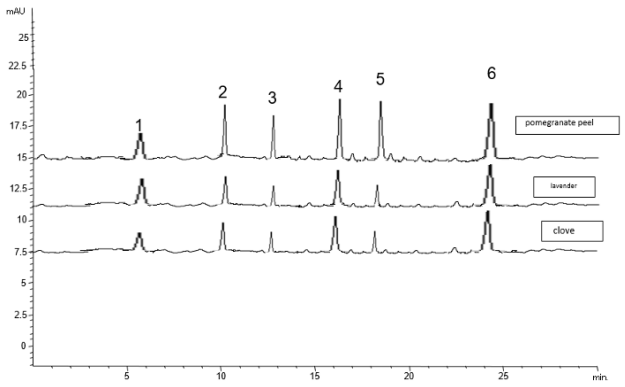

Figure 3. Chromatogram profile of defined peaks at 280 nm for all extracts (Pomegranate peel, lavender and clove methanolic extracts) **1)** Quercetin-3-O-hexoside, **2)** Kaempferol-3-O-rutinoside, **3**) Catechin, **4)** Punicalagin, **5)** Cinnamic acid, **6)** Caffeic acid)

Table 6. The highest detection and amount limits of the peaks defined at 280 nm for all extracts of samples

Peak Numbers	Component Name	Retention Time (RT) (min.)	Concentrations (for 280 nm, ppm)	Limit of Detection (LOD, ppm, 280 nm)	Limit of Quantitation (LOQ, ppm, 280 nm)	Recovery (%)
1	Quercetin-3-O-hexoside	5.5	23.5±0.02	36.1±0.03	108.3±0.01	99.6
2	Kaempferol-3-O-rutinoside	10	35.1±0.04	39.1±0.03	117.3±0.03	89.7
3	Catechin	12.5	28.2±0.04	37.1±0.03	111.3±0.04	105.6
4	Punicalagin	16	72.5±0.03	55.1±0.03	165.3±0.03	103.2
5	Cinnamic acid	18.5	140.1±0.04	74.1±0.03	222.3±0.03	102.4
6	Caffeic acid	24.5	128.2±0.04	72.3±0.03	216.9±0.04	98.5

±SD: Average Standard Deviation, 95 % confidence interval, critical ratio: $p<0.05$, ppm: parts per million.

We developed antibacterial chewable bar which has 100% antibacterial activity against to *S. aureus*, *S. mitis* and *S. epidermidis* in addition to these, it has 99% antibacterial activity on human saliva in this study. This study is the first one that's why this study can lead to development of similar product.

The use of medicinal plants has been widespread since ancient times. Before the 19th century, the main ingredients used in medicines were plants in nature. Because of the high capacity of medicinal plants, they can be used against to pathogenic agents (Mirpour et al., 2015).

Up to now, researches indicated that the parts of plants were used for oral care uses. Especially, leaves of plants were the dominant parts in oral care uses with the percentage of 25.44%. It is followed by root with the percentage of 20.17%, seed/nut/fruit with the percentage of 18.42%, bark with the percentage of 14.03%, young stem/stem/rachis with the percentage of 12.28%, whole plant with the percentage of 9.65%, and gum/latex with the percentage of 8.77%. Focusing on the areas of usage, plants were used for relieve from toothache (29.82%), as dentifrice/toothbrush (25.43%) and mouthwash/gargle (16.66%), against common dental diseases (14.03%), against mouth-related stomatitis/ulcer/gingivitis (12.28%), and gum bleeding/disorders (10.53%). Plants can be used as a gargle form of decoction of plant parts, toothbrush or powder of dried material for dental care (Anitha and Praveen, 2015).

In one study, the HPLC chromatogram of pomegranate peel ethanolic extract revealed the presence of ferulic acid, quercetin, benzoic acid, gallic acid, caffeic acid, vanillic acid, syringic acid, cinnamic acid, and sinapic acid at the highest concentration (Kafeel et al., 2023). In our study, Kaempferol-3-O-rutinoside, Catechin, Punicalagin, Cinnamic acid and, were also detected in pomegranate peel extract, in addition to its quercetin and caffeic acid content. In another pomegranate peel and clove nano emulsion study, the quercetin content was found to be high (Omar et al. 2023). Likewise, in the present study, the quercetin content was also found in pomegranate peel, clove and lavender methanolic extract which is in the chewable bar. In the HPLC-MS detection study conducted with lavender alcohol extract, cinnamic acid derivatives were detected (Ablikim et al., 2021), as we found in our study.

Koptaget reported that the antibacterial effect of clove essential oil against to *S. aureus* with the 17.35 mm inhibition zone diameter (Koptaget, 2019). However Babu et al. declared that the antibacterial effect of clove essential oil against to *S. aureus* with the 25.00 ± 0.05 mm inhibition zone

diameter (Babu et al., 2011). In our study we determined that the antibacterial effect of clove essential oil against to *S. aureus* as 13.83±0.47 mm inhibition zone diameter.

Lavandula angustifolia (Lamiaceae), which is used in traditional and folk medicines in different parts in the world, is a powerful aromatic and medicinal herb. Djenane et al. found that the inhibition zone diameter of essential oil from *L. angustifolia* against *S. aureus* was 19.45±1.37 mm (Djenane et al., 2012). However in our study we found the inhibition zone diameter of lavender essential oil against *S. aureus* as 7.53±0.61 mm.

Doğan and Güler reported that the inhibition zone diameter of 1 g/mL ethanol extract of *Punica granatum* L. peel was 14 mm against to *S. aureus* (Güler and Doğan, 2022). To compare with our study, we found that the inhibition zone diameter of 200 mg/mL methanol extract of *Punica granatum* L. peel was 14.80±0.36 against to *S. aureus*. Changes in the concentration of active compounds in pomegranate depend on fruit variety, anatomical part, ripeness time, extraction type and phenolic extraction method (Akarca and Başpınar, 2019).

CONCLUSION

In our literature research, no study was found about the preparation of antibacterial chewable bar. For this reason, the present investigation is the first investigation on antibacterial chewable bar.

The prepared chewable bar 1 containing beeswax and mastic gum has antibacterial activity against *S. aureus, S. mitis* and *S. epidermidis* between 71-90%. The prepared chewable bar 2 including beeswax, mastic gum, extracts and essential oils has 100% antibacterial activity against *S. aureus, S. mitis* and *S. epidermidis.* In addition to these, chewable bar 2 showed 99% antibacterial activity against human saliva.

These antibacterial eco-friendly chewable bars might be a good candidate for dental care especially, in cases where hygiene methods are difficult to apply or there is not enough time and materials for application.

We hope that the results of this study will provide development of new antibacterial dental care products. Further research needs to discover the side effects of this antibacterial chewable bar before utilizing as a dental care product in human.

ACKNOWLEDGEMENTS

This study was supported by TÜBİTAK 2209-A project with the number 1919B012105372.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

Ayşegül Hoş: Conceptualization, Methodology, Formal Analysis, Investigation, Writing-Original Draft, Supervision, Visualization. Ayşe İnci: Investigation, Writing-Original Draft, Visualization. Ebrar Oktay: Project Administration, Investigation. Dilara Demirel: Investigation. Gülpembe İmrak: Investigation. Ayşe Çalış: Investigation, Writing-Original Draft. Ozan Emre Eyupoglu: Investigation, Formal Analysis, Writing-Original Draft.

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