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Thermosensitive gel of pomegranate peel extract as alternative for the treatment of local infections

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ABSTRACT

Studies on the use of herbal extracts in the treatment of local diseases are increasing day by day. To maintain activity of extract and provide convenient usage, ideal formulation must be developed for extracts. In this study, we aimed to develop thermosensitive gel of pomegranate peel extract and investigate its antimicrobial activity. The total phenolic content and antioxidant capacity of extract was found 397.00 ± 9.36 mg GAE/100 g, and 10750.00 ± 132.29 mg TE/100 g respectively. The gelation temperature of thermosensitive was measured as 25.3 ± 1.5˚C. The developed gel formulation showed antimicrobial activity against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans* as similar with extract. As a result, the developed thermosensitive gel formulation of pomegranate peel extract could be an alternative for the treatment of local infections by its ease of usage and efficacy in future clinical trials after detailed characterization.

Keywords: pomegranate peel, extract, thermosensitive gel, antimicrobial activity

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INTRODUCTION

Pomegranate peel is an important resource with its bioactive compounds such as tannins, phenolic acids, and flavonoids. Thanks to its content, pomegranate peel provides antioxidant, anti-inflammatory, anticancer, and antimicrobial effects. However, its usage has been limited and it is mostly disposed of as agricultural waste. To turn it into a medicine, studies carried out are increasing day by day[1-6].

On the other hand, these studies are limited to the application of the extract as a solution form. In the drug development process, formulation development study is one of the critical steps to maintain activity of drug candidate and provide convenient usage for patient. Some of the development studies cover the application of pomegranate peel extract with the developed gel formulations. Mittal et al. evaluated the efficacy of the gel they prepared gel using carboxy-methyl cellulose. At the end of the study, they observed that only the gel containing pomegranate peel extract from the three gels they compared was effective enough to reduce the number of *Enterococcus faecalis*[7]. In another study, the extract obtained from peel of pomegranate was formulated as a carboxymethylcellulose-based gel[8]. Gel formulation for pomegranate peel extract, developed by Vasconcelos et al., consisting of carbopol, water, and triethanolamine, and efficacy was demonstrated on various microorganisms[8]. Similarly, carbopol gel formulation was also used to evaluate the effectiveness of pomegranate peel extract in the healing of diabetic wounds, and the results of the study showed that wound healing improved[9]. Additionally, chitosan/gelatin gels containing pomegranate peel extract were prepared by Bertolo et al.[10].

Unlike the gels that are generally formed with carbopol, carboxy methyl cellulose and chitosan, thermosensitive gels offer an important opportunity for local administration of pomegranate peel extract, because formulation is liquid at room temperature and gel at body temperature. With these feature, thermosensitive gel formulation is an attention-grabbing alternative to provide long-term retention and controlled drug release of extract in the application area with easy usage[11-12]. In this study, a thermosensitive gel formulation containing pomegranate peel extract was developed to provide a new alternative to the use of pomegranate peel extract in the treatment of local infections. For this purpose, ethanol extract of pomegranate peel was obtained, total phenolic content and antioxidant activity of the obtained extract were evaluated to standardize the properties of the extract. Then poloxamer 407 based gel formulation of pomegranate peel extract was prepared, gelation temperature was determined, and antimicrobial activity tests were performed against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans*.

METHODOLOGY

Materials

Fruit of *Punica granatum* L. was obtained a local manufacturer from Bilecik, Turkey. Poloxamer 407 and Polyether sulfone (PES) syringe filter were purchased from BASF and Merck, respectively. All chemicals and microbial growth mediums were also obtained from Merck. pH meter (MW801, Milwaukee, Portable Meter), precision balance (TW423L model, Shimadzu Corp.), incubator (En120) was used in the experiments.

Preparation pomegranate peel extract

150 grams of fresh pomegranate peel was ground with a knife grinder, and 1000 ml of ethanol/water (70/30) mixture cooled to +4 °C was added to the peel[13]. The resulting suspension was shaken at 150 rpm for 2 hours at room temperature in a dark room. The resulting supernatant was filtered and concentrated under vacuum at +40 °C. The obtained extract was filtered through a 0.22 μm PES syringe filter and stored at -20°C. The solids content of the obtained extract was determined after all water had been removed.

Determination of total phenolic component and antioxidant activity

Total phenolic component and antioxidant activity were determined to standardize the properties of the extract. The Folin–Ciocalteu method was used to quantitatively determine the phenolic substances contained in the pomegranate peel. This method, which is widely used to measure plant-derived phenols, was carried out by Singleton and Rossi's method[14,15]. Briefly, 0.2 N folin reagent was added to the samples and then incubated by adding sodium carbonate solution and calculated by absorbance measured at 765 nm. The result is expressed as mg GAE (Gallic Acid Equivalent)/100 grams of sample. The 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging is a frequently used method to determine antioxidant activity. The free radical scavenging activity of the samples was determined according to DPPH method. In this method, 4 mL of 0.004% (w/v) methanolic DPPH solution and extract solutions were mixed, and the absorbance of the samples was measured at 517 nm after they were incubated for 30 minutes at room temperature in the dark[16,17]. Using Equation 1, the DPPH scavenging activity (%) of the samples was calculated. The DPPH radical scavenging activities of the extracts were calculated as trolox equivalents (mg TEs/g).

Equation 1:

$$\textit{The DPPH Scavenging activity (\%)} = \frac{\textit{Absorbance of Control - Absorbance of Sample}}{\textit{Absorbance of Control}} \text{ x100}$$

Preparation of extract containing thermosensitive gel and determination of gelation temperature

Thermosensitive gel formulation containing pomegranate peel extract was prepared with cold method[18,19]. Briefly after pomegranate peel extract concentrate, cooled down to 4˚C and poloxamer 407 (18% w/v) was added slowly and mixed. The mixture was stored overnight at 4˚C to obtain a clear solution. For the determination of the solid content originating from the extract, the extract was completely dried, and its weight was weighed. Based on this weight, the gel concentration was prepared to be 39,6 mg/mL. For the determination of gelation temperature, 10 ml of gel was added into a 30 R vial and cooled to 4˚C. Then, while the vial was stirred continuously at 150 rpm, the temperature was increased to 1˚C per minute and the point at which magnetic stirrer bar stopped turning was determined as the gelation temperature.

Antimicrobial activity of extract containing gel

In antimicrobial activity tests, 2 Gram (+) bacterial cells, 1 Gram (-) bacterial cell and 1 yeast cell obtained from the American Type Culture Collection were used. Information on the microorganisms used is given in Table 1.

Table 1. Microorganisms and media used in antimicrobial activity tests (MHA: Mueller Hinton Agar, SDA: Sabouraud Dextrose Agar)

Microorganism		Medium
Enterococcus faecalis ATCC 2942	Gram (+) bacteria	MHA
Staphylococcus aureus ATCC 29213	Gram (+) bacteria	MHA
Pseudomonas aeroginosa ATCC 27853	Gram (-) bacteria	MHA
Candida albicans ATCC 24433	Yeast	SDA

In order to test the antimicrobial activities of the obtained formulation, the agar well diffusion method[20], which is similar to the Disk Diffusion, NCCLS, 2003 method, was used. Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for yeasts were used. Bacterial strains used in the studies were adjusted according to Mc Farland 0.5 (1.5×10^8) and yeast strains

were adjusted according to Mc Farland 2 (6 x 10^8) and inoculated into media autoclaved at 121°C for 15 minutes. Only ethanol-impregnated discs were used as negative control and commercial antibiotics (Azithromycin (0.97 µg/L) and Voriconazole (3.90 µg/L)) were used as positive control. After adding test samples, bacteria were incubated at 37°C for 24 hours and yeasts at 30°C for 48 hours. At the end of the incubation period, it was observed whether inhibition zones were formed around the discs and the inhibition zones formed around the discs were measured using a millimetric ruler. Trials were carried out under aseptic conditions and in 3 parallels, and the tests were repeated twice to determine their accuracy[21]. ANOVA followed by Fisher's LSD post hoc test was used to compare more than two groups with Minitab®16 (Minitab Inc.; State College, PA, USA). When p-value was <0.05 (*), the difference between groups was considered statistically significant.

RESULTS and DISCUSSION

Determination of total phenolic component and antioxidant activity of pomegranate peel extract

The total phenolic content and antioxidant capacity of the pomegranate peel extract were determined as 397.00 ± 9.36 mg GAE/100 g and 10750.00 ± 132.29 mg TE/100 g, respectively. When this result was compared with previous literature results, Gozlekci et al. analyzed 4 pomegranate cultivars grown in Turkey ("Lefan," "Katirbasi," "Cekirdeksiz-IV," and "Asinar"). In their examination, they determined that the highest phenolic content was in the peel extract for all cultivars. They determined that this value varies between 1775.4-3547.8 mg GAE/L, depending on the pomegranate cultivars[22]. The type of pomegranate grown in Inhisar, Bilecik is the "Devedisi" cultivar, which is different from these four. On the other hand, the reason for the slightly higher value we obtained could be related to the step of preparing the pomegranate peel for extraction. In our study, pomegranate peel was not dried in the sun or in the oven, so higher activity could be preserved. In order to show the effect of drying, Marchi et al. examined the differences between the pomegranate peel by drying them in an oven and a lyophiliser[23]. At the end of the study, they showed that drying with temperature reduces the antioxidant capacity. While 595.7 µmol Trolox/g activity was determined in lyophilized samples, this value decreased to 351.3 µmol Trolox/g.[23]. Also, the evaluation of the solvent used, which is another critical point in the extraction method, was carried out by Malviya et al. In the extraction study performed with methanol, ethanol, water and their combinations, it was determined that the extracts obtained with 70 ethanol: 30 water or 100% water had the highest activity and phenolic con-

tent[24]. This finding also explains the high activity obtained with the 70 ethanol: 30 water ratio used in our study. Within the scope of our study, thanks to the fact that the products were used without drying and the extraction process was carried out at a temperature not exceeding +40°C, relatively higher total phenolic content and antioxidant capacity were obtained.

Preparation of extract containing thermosensitive gel and determination of gelation temperature

As the temperature increases, poloxamer 407 copolymer molecules assembly as spherical micelles. Its structure includes a dehydrated polypropylene oxide core and an outer core composed of hydrated polyethylene oxide chains. In cases where the concentration is sufficient, these micelles form the gel structure (Figure 1)[25].

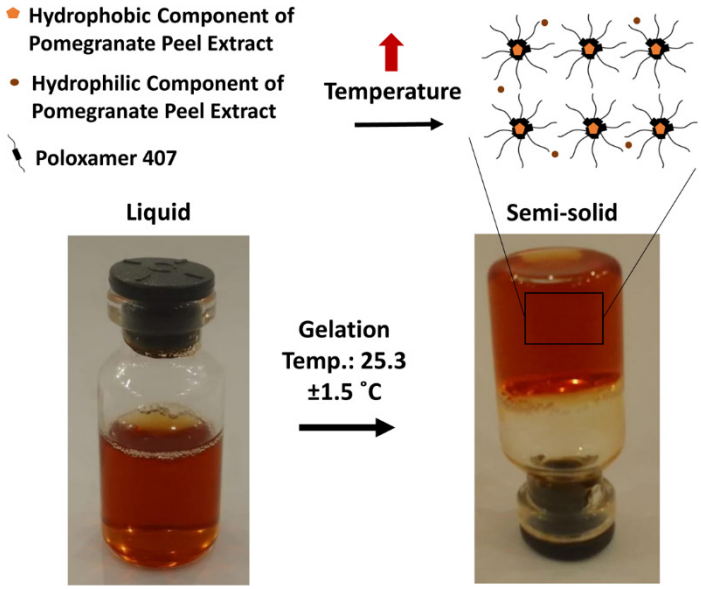

Figure 1. Schematic representation of extract containing thermosensitive gel and determination of gelation temperature

By this way, it can become gel form solution at body temperature. Especially thanks to its hydrophobic core, it offers an important opportunity to transport therapeutics, limited solubility in water. Although the ethanol is removed and the extract is filtered after ethanol extraction, the nano-sized precipitated substances can exist in extract. Another advantage of this gel prepared with Poloxamer 407 is that it can dissolve these precipitates and form a homogeneous solution. In this way, effectiveness of these substances could be increase[26]. In our study, a simple and industrially applicable formulation was developed by adding polymer

directly to the extract, which was concentrated and cooled. Since the gelation temperature of the obtained gel was measured, this value was found to be 25.3 ± 1.5˚C and it was determined that the gel obtained was reversible. This value obtained shows that the purpose of instant gelation at body temperature, which is in solution in cold, is achieved. When other studies conducted were examined, the gelation temperature of the gel developed by Cetin et al. for wound healing was found to be 28˚C[27]. There are studies showing that the gelation temperature prepared with poloxamer 407 can affect by other substances in the medium[25, 28,29]. So, the finding we obtained in our study is compatible with the literature. On the other hand, it is clearly seen in the literature that the gels prepared using 18% w/v poloxamer 407 have a fluid liquid consistency below the determined gelation temperature. This has been clearly shown in our previous studies. It has also been shown in our previous studies that the viscosity of the gel prepared with poloxamer 407 at this concentration has the expected spreadable properties[18,27,28]. In this study, based on our previous experiences, we primarily focused on the gelation temperature and activity, which are the main variable properties of the gel. A similar approach has previously been used by other groups developing gel formulations for pomegranate peel extract. Mittal et al. evaluated the efficacy of the gel; they prepared gel using carboxy-methyl cellulose[7]. In this study, gel was prepared, and antimicrobial assessment was performed directly as methodology. In another study, gel formulation for pomegranate peel extract, developed by Vasconcelos et al., consisting of carbopol, water, and triethanolamine. Similar methodology was used in this study[8]. Additionally, chitosan/gelatin gels containing pomegranate peel extract were prepared by Bertolo et al.[10]. In this study, rheological evaluation, total phenolics content, and antioxidant activity were measured as major parameters without antimicrobial activity tests. All these studies showed that each study can determine its methodology based on its own focused target without doing all the analysis. Since it has been clearly demonstrated in the literature that poloxamer thermosensitive gels can be easily applied to the application site, have long-term retention, and provide controlled release properties. So, *in vitro* antimicrobial activity tests were carried out to show that the obtained gel's efficacy rather than performing *in vivo* studies.

Antimicrobial activity of extract containing gel

Agar well diffusion method was used to determine antimicrobial activity of 4 different test microorganisms. The formed zone diameters after 24 and 48 hours of incubation were measured for bacteria and yeast, respectively. Inhibition zone diameters were found as 25, 21 ± 1.41, 23.5 ± 0.7, 15.5 ± 0.7 against *Enterococcus faecalis* (Figure 2), *Staphylococcus aureus* (Figure 3), *Pseudomonas aeruginosa* (Figure 4) *and Candida albicans* (Figure 5), respectively.

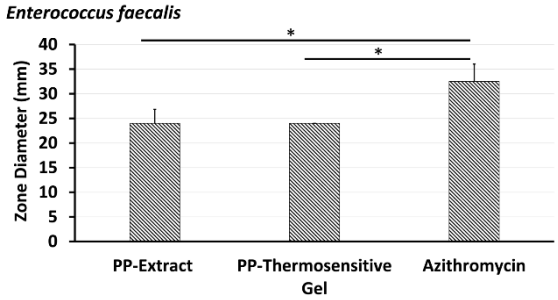

Figure 2. Inhibition zone diameters of PP-Extract (pomegranate peel extract), PP-Thermosensitive Gel (pomegranate peel extract containing thermosensitive gel) and Azithromycin (0.97 µg/L) against *Enterococcus faecalis,* *p<0.05

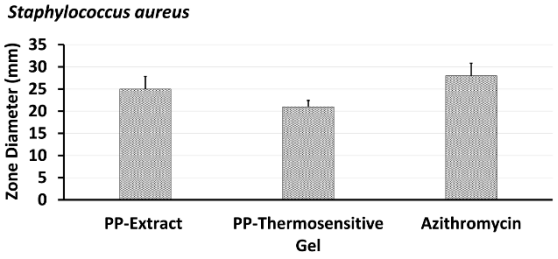

Figure 3. Inhibition zone diameters of PP-Extract (pomegranate peel extract), PP-Thermosensitive Gel (pomegranate peel extract containing thermosensitive gel) and Azithromycin (0.97 µg/L) against *Staphylococcus aureus* *p<0.05.

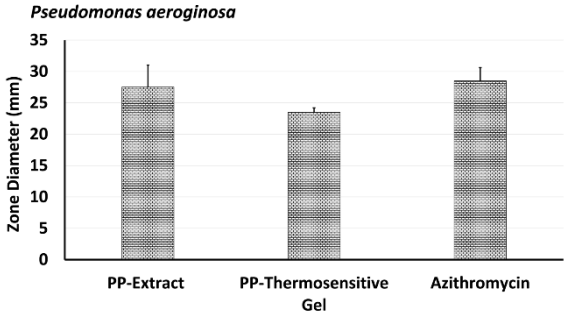

Figure 4. Inhibition zone diameters of PP-Extract (pomegranate peel extract), PP-Thermosensitive Gel (pomegranate peel extract containing thermosensitive gel) and Azithromycin (0.97 µg/L) against *Pseudomonas aeruginosa,* *p<0.05

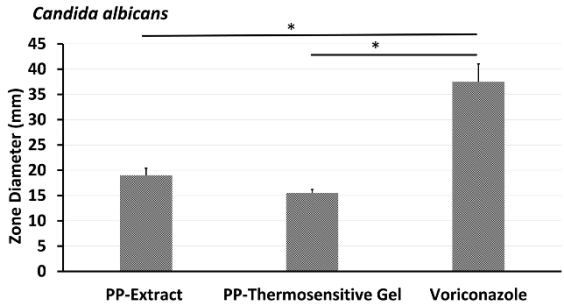

Figure 5. Inhibition zone diameters of PP-Extract (pomegranate peel extract), PP-Thermosensitive Gel (pomegranate peel extract containing thermosensitive gel) and Voriconazole (3.90 µg/L) against *Candida albicans*, *p<0.05

In addition, solid Nutrient Agar medium was prepared, and *Staphylococcus aureus* was inoculated into the whole petri dish with a smear. Then, a line dividing the petri dish in half was drawn and the gel obtained from pomegranate extract was applied to half of it as a thin layer and left to incubate in an oven at 37 ºC for 24 hours. At the end of the incubation, bacterial growth was observed in the non-gel-applied part of the petri dish, while very intense bacterial growth was not observed in the gel-applied part (Figure 6).

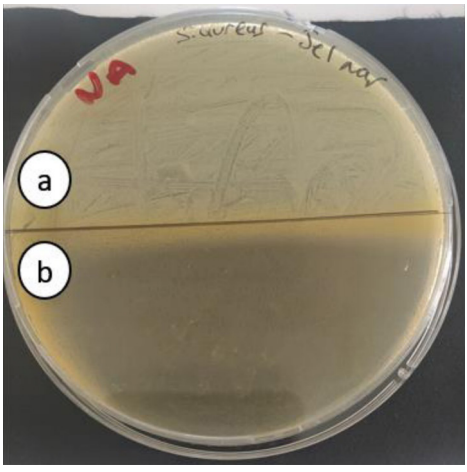

Figure 6. (a) Non-gel-applied part of *Staphylococcus aureus* inoculated petri dish. (b) Pomegranate peel extract containing thermosensitive gel applied part of *Staphylococcus aureus* inoculated petri dish.

When results are examined, it is observed that both the ethanol extract of pomegranate peel and its thermosensitive formulation show antimicrobial activity against to all tested organisms. Difference between these two groups is not statistically significant. On the other hand, slightly decreased activity shown in gel group. After addition of gel formulation to microorganisms, gel form occurs and extract release occurs with controlled release manner. Also, *in vitro* condition, water is limited when compared the vaginal, oral, and wound application sides. In the absence of water, it will take time for the gel to dissolve and the drug release from the gel will be limited. This may be the likely reason for this limited decrease in activity. Since the release in the application area cannot be fully imitated due to the need to provide a sink condition in the release study to be carried out in the *in vitro* environment, the medium in the petri dish, which would have the lowest water content, was tested as a worst-case scenario. Also, azithromycin and voriconazole activity showed, successfully as positive control groups. Although the antimicrobial activity of the extract and the gel obtained with this extract seems to be lower than the control groups, it is clearly understood that this effect will be related to the concentration of the positive controls and extract used[30]. And antimicrobial activity results of empty gel were not included in the results because no activity was observed in the empty gel group.

In another study, pomegranate peel was dried at 33˚C for 7 days and ground, then extracted and formulated as a carbopol-based gel. Here 0.5 ml extract was obtained from 540 mg powder. It has been stated that 1:64 dilution is effective for *Candida albicans*. In this method, due to drying, oxidation and degradation could be occurred. Because of difference in methods, studies are not comparable. On the other hand, antifungal activity was successfully demonstrated in both studies[8]. Conducting antimicrobial activity studies with pomegranate peel extracts, Demir et al. determined that the inhibition zone diameters were 21.00 and 18.50 mm for *Staphylococcus aureus* and *Enterococcus faecalis*, respectively[31]. In our new formulation have equal or greater effect against these organisms. It was examined that the ethanol extracts of pomegranate peel obtained in this study showed antibacterial activity against both gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus)* and gram-negative (*Pseudomonas aeruginosa)* organisms. The results obtained because of antimicrobial activity experiments are compatible with previous studies using pomegranate peel ethanol extracts and these effects were successfully maintained in thermosensitive gel formulation.

Pomegranate peel is an important resource with its antioxidant, anticancer, and antimicrobial effects. However, its usage has been limited and it is mostly disposed of as agricultural waste. The studies carried out to turn it as a drug with its bioactive compounds. As a result, developed thermosensitive gel formulation of pomegranate peel extract could be an alternative for the treatment of oral, vaginal, dermal antifungal and antibacterial infections by its ease of usage, and efficacy.

STATEMENT OF ETHICS

Not applicable.

CONFLICT OF INTEREST STATEMENT

None.

AUTHORS CONTRIBUTIONS

Adem Sahin: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition.

Ülküye Dudu Gül: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. Gizem Bayazıt: Investigation, Formal analysis, Writing - review & editing. Mustafa Sinan Kaynak: Supervision, Writing - review & editing.

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REFERENCES

1. Xiang Q, Li M, Wen J, Ren F, Yang Z, Jiang X, et al. The bioactivity and applications of pomegranate peel extract: a review. J Food Biochem, 2022;46(7):e14105. Doi: 10.1111/jfbc.14105

2. Giri NA, Gaikwad NN, Raigond P, Damale R, Marathe RA. Exploring the potential of pomegranate peel extract as a natural food additive: a review. Curr Nutr Rep, 2023 Jun;12(2):270-289. Doi: 10.1007/s13668-023-00466-z

3. Kumar N, Daniloski D, Pratibha Neeraj, D'Cunha, NM, Naumovski N, Petkoska AT. Pomegranate peel extract – a natural bioactive addition to novel active edible packaging. Curr Nutr Rep, 2022;156:111378. Doi: 10.1016/j.foodres.2022.111378

4. Teniente AL, Flores-Gallegos C, Esparza-González SC, Campos-Múzquiz LG, Nery-Flores SD, Rodríguez-Herrera R. Anticancer effect of pomegranate peel polyphenols against cervical cancer. Antioxid, 2023;12(1). Doi: 10.3390/antiox12010127

5. Chen J, Liao C, Ouyang X, Kahramanoğlu I, Gan Y, Li M. Antimicrobial activity of pomegranate peel and its applications on food preservation. J Food Qual, 2020;2020. Doi: 10.1155/2020/8850339

6. Tito A, Colantuono A, Pirone L, Pedone E, Intartaglia D, Giamundo G, et al. Pomegranate peel extract as an inhibitor of Sars-Cov-2 spike binding to human ACE2 receptor (*in vitro*): a promising source of novel antiviral drugs. Front in Chem, 2021;9:638187. Doi: 10.3389/fchem.2021.638187

7. Mittal A, Tejaswi S, Shetty S, Uk A. Comparison of antibacterial activity of calcium hydroxide, *Azadirachta indic*a (neem), *Ocimum tenuiflorum* (tulsi) and *Unica granatum* (pomegranate) gels as intracanal medicaments against *Efaecalis*: an *in-vitro* study. Pharmacogn J, 2021;13:988. Doi: 10.5530/pj.2021.13.127

8. Vasconcelos LC, Sampaio FC, Sampaio MC, Pereira MS, Higino JS, Peixoto MH. Minimum inhibitory concentration of adherence of *Punica granatum* Linn (pomegranate) gel against *S. mutans, S. mitis* and *C. albicans*. Braz Dent J, 2006;17(3):223-227. Doi: 10.1590/s0103-64402006000300009

9. Karim S, Alkreathy HM, Ahmad A, Khan MI. Effects of methanolic extract based-gel from Saudi pomegranate peels with enhanced healing potential on excision wounds in diabetic rats. Front Pharmacol, 2021;12:704503. Doi: 10.3389/fphar.2021.704503.

10. Bertolo MRV, Martins VCA, Horn MM, Brenelli LB, Plepis AMG. Rheological and antioxidant properties of chitosan/gelatin-based materials functionalized by pomegranate peel extract. Carbohydr Polym, 2020;228:115386. Doi: 10.1016/j.carbpol.2019.115386

11. Chen Y, Lee JH, Meng M, Cui N, Dai CY, Jia Q, et al. An overview on thermosensitive oral gel based on poloxamer 407. Mater, 2021;14(16). Doi: 10.3390/ma14164522

12. Fan R, Cheng Y, Wang R, Zhang T, Zhang H, Li J, et al. Thermosensitive hydrogels and advances in their application in disease therapy. Polym, 2022;14(12). Doi: 10.3390/polym14122379

13. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. AJEV, 1965. Doi: 10.5344/ajev.1965.16.3.144

14. Lamuela-Raventós, RM. Measurement of antioxidant activity and capacity: Recent Trends and Applications. Wiley; 2017. 107-115. Doi: 10.1002/9781119135388.ch6

15. Cuendet M, Hostettmann K, Potterat O, Dyatmiko W. Iridoid glucosides with free radical scavenging properties from fagraea blumei. Helv Chim Acta, 1997;80(4):1144-1152. Doi: 10.1002/hlca.19970800411

16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958;181(4617): 1199-1200. Doi: 10.1038/1811199a0

17. Timur SS, Şahin A, Aytekin E, Öztürk N, Polat K H, Tezel N, Gürsoy R N, Çalış S. Design and *in vitro* evaluation of tenofovir-loaded vaginal gels for the prevention of HIV infections. Pharm Dev Techn, 2018;23(3):301-310. Doi: 10.1080/10837450.2017.1329835

18. Machado HA, Abercrombie JJ, You T, Deluca P, Leung KP. Release of a wound-healing agent from PLGA microspheres in a thermosensitive gel. Biomed Res Int, 2013;387863. Doi: 10.1155/2013/387863

19. Gül Ü, İrdem E, Yavuz Ş, Ilhan S. Determination of dye biosorption capacity of lichens and reusability of wastes as antimicrobial agents. JTA, 2020;112:1-9. Doi: 10.1080/00405000.2020.1797263

20. Prakash J W, Antonisamy JM, Jeeva S. Antimicrobial activity of certain fresh water microalgae from Thamirabarani River, Tamil Nadu, South India. Asian Pac J Trop Biomed, 2011;1(2, Supplement):170-173. Doi: 10.1016/S2221-1691(11)60149-4

21. Gözlekçi S, Saraçoğlu O, Onursal E, Ozgen M. Total phenolic distribution of juice, peel and seed extracts of four pomegranate cultivars. Pharmacogn Mag, 2011;7(26):161-164. Doi: 10.4103/0973-1296.80681

22. Marchi L, Monteiro A, Mikcha J, Santos A, Menconi Chinellato M, Marques DR at al. Evaluation of antioxidant and antimicrobial capacity of pomegranate peel extract (P*unica granatum* L.) under different drying temperatures. Chem Eng Trans, 2015;44:121-126. Doi: 10.3303/CET1544021

23. Malviya S, Arvind Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. JFST, 2014;51(12):4132-4137. Doi: 10.1007/s13197-013-0956-4

24. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of Poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm Res, 2006;23(12):2709-2728. Doi: 10.10 07/s11095-006-9104-4

25. Cafaggi S, Russo E, Caviglioli G, Parodi B, Stefani R, Sillo G, et al. Poloxamer 407 as a solubilising agent for tolfenamic acid and as a base for a gel formulation. EUFEPS, 2008;35(1-2):19-29. Doi: 10.1016/j.ejps.2008.05.010

26. Cetin N, Menevse E, Celik ZE, Ceylan C, Rama ST, et al. Evaluation of burn wound healing activity of thermosensitive gel and PLGA nanoparticle formulation of quercetin in Wistar albino rats. J J Drug Deliv Technol, 2022;75:103620. Doi: 10.1016/j.jddst.2022.103620

27. Fakhari A, Corcoran M, Schwarz. Thermogelling properties of purified poloxamer 407. Heliyon, 2017;3(8):e00390. Doi: 10.1016/j.heliyon.2017.e00390

28. Dumortier G, El Kateb N, Sahli M, Kedjar S, Boulliat A. Chaumeil JC. Development of a thermogelling ophthalmic formulation of cysteine. Drug Dev Ind Pharm, 2006;32(1):63-72. Doi: 10.1080/03639040500390934

29. Gormez A, Bozari S, Yanmis D, Gulluce M, Sahin F, Agar G. Chemical composition and antibacterial activity of essential oils of two species of *Lamiaceae* against phytopathogenic bacteria. Pol J Microbiol, 2015;64(2):121-127. Doi: 10.33073/pjm-2015-018

30. Demir T, Akpınar Ö, Kara H, Gungor H. *In vitro* antidiabetic, antiinflammatory, cytotoxic, antioxidant and antimicrobial activities of pomegranate (*Punica granatum* L.) Peel. Akademik Gıda, 2019;61-71. Doi: 10.24323/akademik-gida.544647