Investigation of Propolis and Boron Containing Disinfectants and Comparison with WHO-Recommended Formulation against COVID-19

Propolis ve Bor İçeren Dezenfektanların İncelenmesi ve DSÖ Tarafından COVID-19'a Karşı Kullanılması Önerilen Formülasyon ile Karşılaştırılması

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ABSTRACT

Objective: The need for disinfectants has increased worldwide due to the developing pandemic COVID-19. If access to water is limited, the use of alcohol-based disinfectant formulations recommended by the WHO. In this study, disinfectant gel formulations containing propolis and boric acid with lower alcohol levels than the WHO-recommended formulation were developed.
Materials and Methods: Four different gels formulations containing propolis and boric acid were developed. A gelling agent was added to the developed formulations to make it remain longer on the skin than liquid solution formulations. Texture profile analyses, spreadability, and bioadhesion tests of the formulations were evaluated. After the characterization studies, bactericidal and fungicidal activity assays were performed with final and the WHO-recommended formulations.
Results: The results showed that increasing of gelling agent increased the viscosity, bioadhesion and textural values. The final formulation containing propolis and boric acid had acceptable gel properties. Propolis and boric acid containing disinfectant gel formulation included lower alcohol level than the WHO-recommended formulation showed a similar bactericidal and fungicidal activity with WHO-recommended formulation.
Conclusions: Propolis and boric acid-containing disinfectant gel formulations were successfully produced. Final formulation with a lower alcohol level might be an alternative for the WHO-recommended formulation with similar effectiveness.

Key Words: Disinfectant, propolis, boric acid, COVID-19, hydroalcoholic gel, carbomer

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ÖZET

Amaç: COVID-19 salgını nedeniyle dünya genelinde dezenfektanlara olan ihtiyaç artmıştır. Eğer suya erişim kısıtlı ise, DSÖ tarafından alkol bazlı dezenfektanların kullanılması önerilmektedir. Bu çalışmada, DSÖ tarafından önerilen formülasyondan daha düşük alkol oranına sahip propolis ve borik asit içeren dezenfektan jel formülasyonları geliştirilmiştir.
Yöntemler: Propolis ve borik asit içeren dört farklı jel formülasyonu geliştirilmiştir. Geliştirilen fomülasyonlara, cilt üzerinde sıvı çözelti formülasyonlarından daha uzun süre kalmasını sağlamak için jelleştirici madde ilave edilmiştir. Formülasyonların tekstür profili analizleri, sürülebilirlik ve yapışma testleri değerlendirilmiştir. Karakterizasyon çalışmalarından sonra, bakterisidal ve fungisidal aktivite çalışmaları, son ve DSÖ tarafından önerilen formülasyonlar ile gerçekleştirilmiştir.
Bulgular: Jelleştirici madde oranının artırılmasının viskozite, yapışma ve tekstür değerlerini artırdığı gösterilmiştir. Propolis ve borik asit içeren son formülasyon kabul edilebilir jel özelliklerine sahiptir. Propolis ve borik asit içeren daha düşük alkol oranına sahip dezenfektan jel formülasyonu, DSÖ tarafından önerilen formülasyon ile benzer bakterisidal ve fungisidal aktivite göstermiştir.
Sonuçlar: Propolis ve borik asit içeren dezenfektan jel formülasyonları başarıyla üretilmiştir. Geliştirilen son formülasyon, DSÖ tarafından önerilen formülasyondan daha düşük alkol seviyesine ve benzer etkinliğe sahip bir alternatif olabilir.

Anahtar Sözcükler: Dezenfektan, propolis, borik asit, COVID-19, hidroalkolik jel, karbomer

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INTRODUCTION

The need for disinfectants has increased due to the worldwide COVID-19 pandemic. Disinfectants are one of the vital pharmaceutical products. When access to water and soap is limited, it is recommended to use alcohol-based hand disinfectants which contain at least 60% alcohol to reduce the spread of the new coronavirus (1). There are different types of disinfectants (*e.g.* surface, skin, and device) in terms of their active substance. Unlike cleaning and sanitizing, disinfecting destroys germs (bacteria, virus, and mold) on the surfaces. The effectiveness of a disinfectant is related with the active substance, concentration, contact time, and coverage (2). In addition, humidity, microorganism type, and amount on the applied surface are also important criteria on the efficiency. Both ethyl alcohol and isopropyl alcohol damage the outer cell membrane of germs, which is related with the antimicrobial activity. The World Health Organization (WHO) recommended two different alcohol-based hand rubs (formulation I and formulation II) for hand disinfection (3). Siddharta et al. reported that both formulations were found efficient on inactivating of Zika virus, Ebola virus, and emerging coronaviruses such as Severe Acute Respiratory Syndrome (SARS) coronavirus and Middle East Respiratory Syndrome (MERS) coronaviruses (4). Reducing the alcohol level is possible with the addition of other antiseptic agents to a disinfectant formulation.

Propolis is a mixture of various compounds, such as resins, waxes, essential oils and pollen. Honeybees collect propolis from various plants and process them with their enzymes. Therefore, the phytochemical content of propolis is quite variable from region to region and depends on the time of collection. Honeybees use propolis to protect their hives against bacterial and fungal infections or other animals. Propolis contains many phenolic compounds including flavonoids, phenolic acids and phenolic acid esters as the structural components. Caffeic acid phenethyl ester (CAPE) is one of the major active components (5). Phenolic acids and flavonoids of propolis play the main role on antibacterial activity based on the inhibition of nucleic acid synthesis or the cytoplasmic membrane damage by perforation (5). In some studies, propolis has been demonstrated to possess antifungal, antiviral, antioxidant, astringent, and immunostimulant effects. In addition to these effects, it was reported to have anticancer, nerve regeneration, and neovascularization effects (6). Propolis has unique antimicrobial, antioxidant and anti- inflammatory effects on wound healing based on different mechanisms such as quenching of free radicals, inhibition of lipid peroxidation and nuclear factor NF-κB or treating the wound biofilms. Some reports demonstrated that propolis based wound dressings have an antibacterial activity with appropriate biocompatibility (7). Propolis has also a skin hydration effect, which helps to prevent dry skin from the problem of alcohol (8).

Boron is found in nature as minerals that are bound to sodium, calcium, and magnesium oxides. Boron elements are mainly used in glass, ceramic, and detergent industry and are emerging in medical area due to their anticancer, antiviral, antifungal, and antibacterial activities. In addition, the use of some boron-based compounds against type-2 diabetes mellitus and human African trypanosomiasis treatments was reported (9). Boric acid is a weak acid of boron and exhibits antimicrobial, antifungal, and antiseptic properties (10). Boric acid solutions are in use for the treatment of external and middle ear infections due to its strong antiseptic and acidic properties (11).

In this study, propolis and boric acid-containing disinfectant formulation development was aimed. Although some reports have revealed the disinfection properties of these substances separately, no study using both substances has been found in the literature (12,13). Carbomer was used to make the disinfectant solution to gel formation, and mechanical properties of gel formulations were investigated. Bactericidal and fungicidal activity assays were carried out with a final gel formulation and the WHO-recommended formulation I for comparison of the effectiveness of both formulations.

MATERIALS and METHODS

Materials

Boric acid was purchased from Botafarma, Turkey. Ethanolic (70 % v/v ethanol) propolis extract was kindly provided by Gürle Arıcılık, Turkey. Ethanol was purchased from Merck, Germany. Carbomer 390 was provided from Tinci Materials Technology, China.

Preparation of the disinfectant formulations

For the preparation of the disinfectant formulations, 1% of boric acid was dissolved in ethanol (70%, v/v). After obtaining a clear solution, propolis extract was added to this solution at 1% concentration. Next, a yellowish solution was obtained with gentle mixing. Then, Carbomer was added at different concentrations (Table 1). The pH of solutions was adjusted with triethanolamine to 6.0 under mechanical stirring. The hydroalcoholic gels were allowed to stay for 24 h at room temperature for cleaning the air bubbles prior to the characterization. The WHO-recommended disinfectant formulation I was prepared as WHO directions (3). First, hydrogen peroxide (3%) was added to ethanol (96%, v/v). Then, glycerol was added to this solution. Finally, the bottle was completed with required amount of distilled water. Final concentration of the alcohol and hydrogen peroxide was 80% and 0.125%, respectively.

Table 1. The contents of proposed disinfectant formulations

	Propolis extract (%, v/v)	Boric acid (%, w/v)	Carbomer 390 (%, w/v)
F1	1	1	0.00
F2	1	1	0.50
F3	1	1	0.75
F4	1	1	1.00

Viscosity studies

Viscosity values of the solutions were measured using a cone-plate rheometer (Brookfield DV-III, UK). A small quantity of formulation (~0.5 mL) was placed on a plate and the viscosity value was measured at 20 rpm using the spindle CP-52. The measurements were triplicated at room temperature.

Texture profile analyses

Texture profile analyses (TPA) of the disinfectant formulations were characterized with a TA-XT Plus Texture Analyzer (Stable Micro Systems, UK). For this purpose, mechanical properties (hardness, adhesiveness, cohesiveness, and elasticity) and spreadability (firmness) values were evaluated. TPA of the formulations was determined using a flat probe. The probe was immersed into the formulation double time. The delay period was 15 s between the cycles. Different mechanical parameters of the gel formulations were calculated by Exponent software using the force-time plots.

Spreadability, which is an important parameter for gels, is related with the firmness of formulation (14). The ease of spread makes a product more preferable. The spreadability studies were performed using the male cone probe and female perspex cone-shaped sample holder. The sample holder was completely filled with the formulation designed. Then, cone probe was moved down into the sample holder at a defined rate of 1 mm/s and to a defined depth of 23 mm (15). The force-time plot provided the firmness and work of shear values of the gel formulation.

Bioadhesion studies

Bioadhesion values of the formulations were evaluated using the texture analyzer on a rat skin. The rat skin was fixed with cyanoacrylate glue to the lower end of the probe. The diameter of the probe was 12 mm. A small quantity (~0.5 mL) of the formulation was dropped on the heavy duty platform with an aluminum plate. The skin was contacted with the formulation for 120 s with an applied force of 0.2 N and the rate of 1 mm/s. The area under force (N)–distance (mm) curve was calculated using the Exponent software, and the work of bioadhesion value was measured. All the measurements were triplicated at room temperature.

Bactericidal and fungicidal activity assays

Bactericidal and fungicidal activity assays were carried out according to TS EN 1276 and TS EN 1650 standards at Microbiological Analysis Laboratory of Ege University (EGEMİKAL), Izmir, Turkey (16). TS EN 1276 is a quantitative suspension test for the assessment of the bactericidal activity of chemical antiseptics and disinfectants. *Enterococcus hirae* (ATCC 10541), *Escherichia coli* (K12 NCTC 10538), *Pseudomonas aeruginosa* (ATCC 15442), and *Staphylococcus aureus* (ATCC 6538) were used as the test organisms.

TS EN 1650 is a quantitative suspension test for the evaluation of the fungicidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas. *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans* (ATCC 10231) were used as the test organisms. The contact time between disinfectant and microorganism was 1 min.

RESULTS

In this study, four different disinfectant formulations were designed and prepared with various gelling agent concentrations. It was observed that all the formulations colored mild yellowish due to propolis extract (Fig.1).

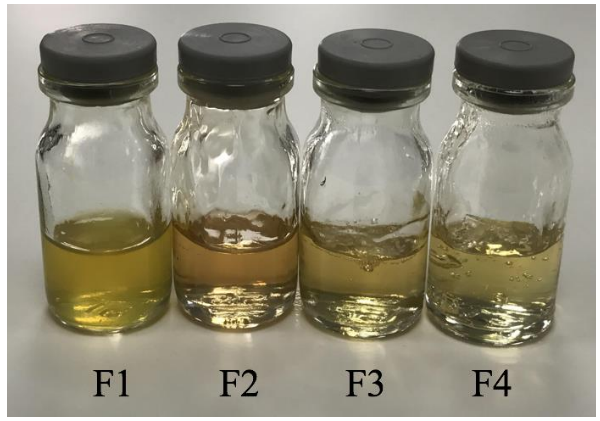

Figure 1. The appearance of formulations

As expected, the increase of Carbomer concentration from 0 to 1 %, changed all the characteristic parameters (Table 2).

Table 2. Different characterization parameters of formulations

	F1	F2	F3	F4
Viscosity (cPs)	1.1 ± 0.1	3767.0 ± 81	6355.0 ± 21	9499.0 ± 37
Firmness (g)	5.19 ± 0.1	107.4 ± 11.2	272.0 ± 2.6	362.4 ± 15.2
Work of Shear (g.sec)	1.57 ± 0.2	83.3 ± 7.7	184.9 ± 25.8	296.4 ± 17.9
Hardness (g)	11.8 ± 0.1	121.8 ± 5.8	276.9 ± 10.0	378.1 ± 7.5
Adhesiveness (g.sec)	-4.6 ± 0.8	-225.6 ± 54.0	-532.9 ± 113.9	-896.7 ± 152.7
Springiness	0.5 ± 0.0	0.6 ± 0.2	0.8 ± 0.1	0.9 ± 0.0
Cohesiveness	1.0 ± 0.0	0.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.0
Work of bioadhesion (mJ/cm²)	0.01 ± 0.00	0.10 ± 0.02	0.19 ± 0.01	0.31 ± 0.03

Our outcomes indicated that F1 formulation, without Carbomer, has very low viscosity, TPA (except cohesiveness), and bioadhesion values. After addition of Carbomer for gelling and the triethanolamine for pH adjustment, the viscosity values were observed to increase. This upsurge caused an increase in spreadability parameters (firmness and work of shear values).

Carbomer displayed good bioadhesive properties and increased Carbomer concentration and bioadhesion values.

No microbial contaminations were observed for both formulations. Propolis and boric acid-containing gel formulation was found to exert similar antimicrobial and antifungal effectiveness to that of the WHO-recommended formulation I (Table 3).

Table 3. Antimicrobial and antifungal efficacy of the conventional and F2 gel formulations

Test organism	Contact time (min)	WHO-recommended formulation I (logR)	F2 (logR)
Enterococcus hirae	1	>5.19	>5.19
Escherichia coli	1	>5.3	>5.3
Pseudomonas aeruginosa	1	>5.27	>5.27
Staphylococcus aureus	1	>5.22	>5.22
Aspergillus brasiliensis	1	>4.13	>4.13
Candida albicans	1	>4.06	>4.06

DISCUSSION

Hand disinfectants can be both in gel or liquid solution formulation and provide cleaning without rinsing. Liquid formulations are often used in spray applications. Gel formulations are generally prepared using hydroalcoholic gel systems. A hydroalcoholic gel formulation basically consists of at least 40% alcohol and gelling agent such as Carbomer. Carbomer or Carbopol is a synthetic polymer of acrylic acid and contains 56 to 68% (w/w) carboxylic acid groups. Carbomer polymers have good bioadhesion properties and are used in various topical pharmaceutical formulations. Triethanolamine is added to Carbomer based hydroalcoholic gel formulations to neutralize the carboxylic groups of the Carbomer and forming gel.

Carbomers are typically used in topical formulations between 0.5 - 3% concentration. In this study, Carbomer concentration was selected in the range of 0.5 to 1% and the gel characteristics were evaluated (Table 1). A neutralizing agent is needed for thickening of Carbomer gel formulations. The addition of neutralizing agent increases the viscosity around pH 6-7 and the viscosity begins to decrease after pH 9. In this study, triethanolamine was used as a neutralizing agent, which reacts with the carboxylic groups of the Carbomer. As expected, the viscosity of the formulations increased as Carbomer concentrations (Table 2). This increment affected the textural parameters.

Mechanical properties of topical gel formulation have an important role in terms of daily use. Texture analyzer is a useful tool for determining the textural profile of gel formulations.

Different textural parameters such as hardness, adhesiveness, cohesiveness, and springiness values of the gel formulations were evaluated. A typical TPA test consists of two compression cycles. In the first cycle, the probe goes downward and contacts with the gel sample. After achieving the minimum trigger force, the probe moves at set speed. Hardness (N or g) is the first maximum force and provides information about the applicability of the gel formulation. Adhesiveness, the amount of work needed to overcome attractive forces between probe and gel, is calculated from the negative force area of the first cycle. The textural parameters of the hydroalcoholic gel formulations showed a growing correlation between the amounts of Carbomer. Hardness and adhesiveness of the gel formulations dramatically increased after the addition of gelling agent (Table 2). Hurler et al. reported the hardness of Carbopol hydrogels (0.5 %) around 300 g (17). In the current study, the hardness of F2 formulation (0.5 % Carbomer concentration) was found 121.8 g. The preparation of the Carbomer gels with water caused to higher viscosity than the hydroalcoholic gels due to the level of hydrogen bonding in the different systems. The adhesiveness of gel formulations increases with the Carbomer concentration (Table 2). Similarly, Tan et al. reported that the increased Carbopol concentration increased the adhesiveness of the formulation due to the chemical interaction between Carbopol and probe (18).

After the first compression cycle of TPA, the probe moves up to initial position and repeats the same movement for the second compression cycle after a wait period. Springiness of gel formulation is related to the elasticity of gel and calculated from the ratios of two compression times or distance. Cohesiveness is another important textural parameter and related to the gel integrity. This parameter is calculated from the ratios of two positive compression areas. Carbomer-containing gel formulations are useful for increasing the contact time of mucosal drug delivery systems. High cohesiveness values are acceptable due to full structural recovery following gel application (19). In our study, higher Carbomer concentration decreased the cohesiveness values (Table 2). Springiness of the gel formulations increased with the Carbomer concentration. Lower springiness values indicate higher product elasticity (20). Therefore, F2 formulation was found to be more successful in terms of springiness. In a study, Carbopol hydrogels were prepared, and the formulation with low firmness and high adhesiveness was found suitable due to the ease of product removal from the container and ease of application onto substrate (21). In our study, increasing the Carbomer concentration from 0.5% to 1% increased the firmness and work of shear values around 3 times higher (Table 2). Therefore, F2 formulation was found desirable in terms of spreadability. Work of bioadhesion values is a of gel formulation, which is another important bioadhesion parameter (22,23). F1 formulation showed very low (near-zero) work of bioadhesion values. Addition of Carbomer increased the work of bioadhesion values. Shin et al. reported that if the viscosity of Carbopol containing formulation is increased by adjusting the pH, the bioadhesive strength increases (24).

The aim of this study was to develop a disinfectant gel formulation with antimicrobial/antifungal properties similar to that of the WHO-recommended solution with lower alcohol amount. Although there are some alcohol-free disinfectant formulations, the use of alcohol-based disinfectants was recommended by WHO and FDA during the pandemic period. WHO-recommended formulations I and II contain ethanol and isopropyl alcohol, respectively. In this study, an alternative solution to ethanol-based disinfectant formulation of WHO was developed. Isopropyl alcohol was reported to be effective against enveloped viruses, whereas ineffective against similar non-enveloped viruses due to its lipophilic nature as compared to ethanol (25). Although there are studies showing that the use of alcohol-based disinfectants causes skin problems such as dry skin (26,27), some multicenter studies report that alcohol-based disinfectant use does not cause any skin problems (28,29). As a result of characterization studies, F2 formulation designed in the present study was proceeded to the bactericidal and fungicidal activity tests. Bactericidal and fungicidal activities of F2 formulation and WHO-recommended formulation I were comparatively tested. Both formulations showed more than 5 log and 4 log reduction towards bacteria and fungi, respectively (30). Both formulations were found to have bactericidal and fungicidal effect at very short contact time as 1 minute (Table 3). The addition of propolis and boric acid to formulation helps maintain the bacteriostatic and fungistatic effects. F2 formulation was found successful and exhibited similar level of antibacterial and antifungal activity compared to that of WHO-recommended formulation I (31).

CONCLUSION

Propolis and boric acid-containing hydroalcoholic gel formulations were successfully prepared and characterized. Increased viscosity decreased the ease of use of the formulation. Therefore, 0.5% Carbomer concentration was selected for the final formulation. The final formulation showed similar antibacterial and antifungal activity compared to WHO-recommended formulation I. Based on the results, we propose that the final formulation designed in this study could be used as a safe and effective hand disinfectant.

Conflict of interest
No conflict of interest was declared by the authors.

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