Kastamonu Uni., Orman Fakültesi Dergisi, 2024, 24(2): 158-167 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1557416

Antimicrobial, Anti-biofilm, Anti-swarming, Anti-quorum Sensing Activities, and Cytotoxicity of Propolis Samples from Northeast of Türkiye

Merve CORA[1*], Ülkü Zeynep ÜREYEN ESERTAŞ[2], İnci DURUKAN[1]

[1]Karadeniz Technical University, Faculty of Medicine, Department of Medical Microbiology, Trabzon, TÜRKİYE
[2]Ağrı İbrahim Çeçen University, Faculty of Medicine, Department of Medical Microbiology, Ağrı, TÜRKİYE
*Corresponding Author: mcora@ktu.edu.tr

Received Date: 20.11.2023 Accepted Date: 08.05.2024

Abstract

Aim of study: Studies on propolis have increased as it has been revealed that it contains biologically active molecules. In the current study, it was aimed to analyze biological activity, and cytotoxicity of ethanolic extract of three different propolis samples from Türkiye.

Material and methods: The antibacterial activity of the extracts against 14 microorganisms was assessed using the agar well diffusion method and the microdilution method. *Chromobacter violeceum* was used in quorum-sensing assay, and *Pseudomonas aeruginosa* PAO1 strain was used in swarming and biofilm assays. Using the MTT test, the cytotoxic effect of the extracts was examined on the lung adenocarcinoma cell line (A549), pancreatic tumoral cell line (AR42J), breast cancer cell line (MDA-MB-231), and normal epithelial cell line (Vero).

Main results: All propolis extracts were effective against 8/14 microorganisms included in the study. While all propolis extracts have shown anti-quorum sensing activity, there was not any anti-swarming and anti-biofilm activity in each sample. It was demonstrated that every propolis sample had a dose-dependent cytotoxic effect on the examined cell lines.

Research highlights: Due to the biological activity shown by the propolis samples included in the study, it is considered that it has the potential to influence the creation of novel medications in the future.

Keywords: Biofilm, Cytotoxicity, Propolis, Quorum sensing.

Türkiye'nin Kuzeydoğusundan Alınan Propolis Örneklerinin Antimikrobiyal, Anti-biyofilm, Anti-swarming, Anti-quorum Sensing Aktiviteleri ve Sitotoksisitesi

Öz

Çalışmanın amacı: Biyolojik aktif moleküller içerdiğinin anlaşılmasıyla birlikte propolis ile ilgili yapılan çalışmaların sayısı son yıllarda artmıştır. Bu çalışmada üç farklı Türkiye propolisinin etanol ekstraktlarının biyolojik aktiviteleri ve sitotoksik özelliklerinin araştırılması amaçlanmıştır.

Materyal ve yöntem: Ekstraktların antimikrobiyal özellikleri 14 mikroorganizma kullanılarak agar kuyucuk ve mikrodilüsyon yöntemleri ile araştırıldı. Anti-quorum sensing aktivitesi için *Chromobacter violaceum*, anti-swarming ve anti-biyofilm aktivite değerlendirilmesi için *Pseudomonas aeruginosa* PAO1 izolat kullanıldı. Ekstraktların sitotoksik etkileri MTT yöntemi ile pankreatik tümöral hücre hattı (AR42J), akciğer adenokarsinoma hücre hattı (A549), meme kanseri hücre ahttı (MDA-MB-231) ve normal epitel hücre hattı (Vero) kullanılarak araştırıldı.

Temel sonuçlar: Çalışmaya dahil edilen tüm ekstraktrın 8/14 mikroorganizmaya karşı etkili olduğu tespit edildi. Ayrıca tüm ekstraktlarda anti-quorum sensing aktivitesi görülürken, ekstraktlarda anti-swarming ve anti-biyofilm aktivitesi görülmedi. Tüm ekstraktların hücre hatları üzerine doza bağımlı sitotoksik etki gösterdiği belirlendi.

Araştırma vurguları: Çalışmada kullanılan propolis örneklerinin gösterdiği biyolojik aktivite nedeniyle gelecekte yeni ilaçların geliştirilmesinde rol oynayabileceği düşünülmektedir.

Anahtar Kelimeler: Biyofilm, MTT, Propolis, Sitotoksisite.

Citation (Atıf): Cora, M., Ureyen Esertas, U. Z., & Durukan, I. (2024). Antimicrobial, Anti-biofilm, Anti-swarming, Anti-quorum Sensing Activities, and Cytotoxicity of Propolis Samples from Northeast of Türkiye. *Kastamonu University Journal of Forestry Faculty*, 24 (2), 158-167.

Introduction

With the studies carried out in the last century, many drugs have been developed against microorganisms that cause disease in humans. However, the increasing use of drugs causes microorganisms to develop resistance to drugs and thus the treatment of diseases becomes difficult. As a result of this situation, natural compounds that have been used traditionally in treatment of diseases for centuries are coming to the fore again, with the hope of discovering new bioactive compounds and antimicrobial agents (Angiolella et al. 2018).

One of the natural products that has become increasingly popular in recent years is propolis, which bees produce by collecting resin and wax from plants and mixing them with their saliva (Wojtacka, 2022). Propolis, which was used for various purposes such as preservative, embalming, and wound antiseptic in ancient civilizations, was used as herbal medicine in the Middle Ages. With the developing technology in last decade, it has been observed that propolis has more than 500 compounds, but there are different components in each propolis depending on the raw material of propolis collected by bees from various parts of plants, geographical location, botanical resources, season and bee species (et al. 2022; Bankova, 2005). Accordingly, an interest has emerged in a more in-depth understanding of the biological activity of propolis and its various components (Huang et al. 2014).

Pancreatic cancer has a 5-year survival rate and is a very deadly cancer. Pancreatic cancer is predicted to surpass breast cancer, which currently ranks third in the European Union for cancer-related mortality. Type 2 diabetes, obesity, and tobacco use are among the risk factors for pancreatic cancer. Most of the patients are diagnosed with 70 years and above. Although only 10-25 %of patients survived after surgery for 5 years, surgery remains the only treatment that offers curative potential. Although supportive treatments are used during disease, new approaches to healing the disease are proceeding (Mizrahi et al. 2020).

The current study looked at the three distinct ethanolic propolis extracts' anti-bacterial, anti-biofilm, anti-quorum sensing, and anti-swarming properties. Analyzing the extracts' cytotoxicity against the lung adenocarcinoma cell line A549, the pancreatic cancer cell line AR42J, and the breast cancer cell line MDA-MB-231 was another goal.

Material and Methods

Preparation of Ethanolic Propolis Extracts

Three different propolis samples from Türkiye (from Ardahan City, Uzungöl district of Trabzon City, and Pazar district of Rize City) those were chemically analyzed in previous study were included in the study (Cora et al. 2023). Each propolis sample and 70% ethanol were shaken separately in a sterile falcon at 250 rpm for 24 hours without heat. After filtering the mixture using filter paper, the solvent was removed with an evaporator and a lyophilizer. Dry matter was dissolved with sterile dimethyl sulfoxide (DMSO). In the experiments, DMSO was used at a concentration (below 0.5%) that would not damage the cells.

Microorganisms Used in the Study

Propolis extracts' antibacterial efficacy was evaluated against *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Acinetobacter haemolyticus* ATCC 19002, *Bacillus cereus* ATCC 14579, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Chromobacterium violaceum* ATCC 12472, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Mycobacterium smegmatis* ATCC 607, *Klebsiella pneumoniae* ATCC 13883, *Candida parapsilosis* ATCC 22019, and *Candida albicans* ATCC 10231.

Antimicrobial Activity of Propolis Extracts Agar Well Diffusion Assay

The methodology described by Denev et al. was used to carry out the agar well diffusion technique (Denev et al. 2014). Suspensions of 0.5 McFarland density for bacteria, and 1 McFarland density for Candida species were prepared with phosphate-buffered saline (PBS) from microorganisms grown in appropriate media. *M. smegmatis* suspension was prepared in Brain-Herat Infusion Broth (BHIB). *M. smegmatis*,

bacteria suspensions, and Candida species were spread on Brain-Heart Infusion Agar (BHIA), Mueller Hinton Agar (MHA), and MHA with 2% glucose respectively. The wells that were opened in the medium with a 6 mm diameter were filled with 50 µL of extracts at a concentration of 100 mg/mL. DMSO was used as a negative control, and amphotericin B, ampicillin, ciprofloxacin, and gentamicin were used as a positive control for Candida species, Gram-positive bacteria, *M. smegmatis*, and Gram-negative bacteria, respectively. For twenty-four hours, cultures were incubated at 37°C. But Candida species incubated for two days, and *M. smegmatis* for three days. Zone diameters of <6 mm were considered ineffective, 6-14 mm were considered moderately effective, and 15 mm and above were considered as high activity (Balouiri et al. 2016). The experiment was repeated twice.

Minimum Inhibition Concentration (MIC) Assay

The MIC values of microorganisms were established whose effects were detected in the agar well diffusion method. Suspensions of 0.5 McFarland density for bacteria, 1 McFarland density for Candida species were prepared with PBS from microorganisms grown in appropriate media. *M. smegmatis* suspension was prepared in BHIB. BHIB was used for *M. smegmatis* and MHB-II was used for other bacteria in the MIC test (Woods et al 2003; Murray et al. 2009). 50 µL of medium was added to the wells. 50 µL of extracts at a concentration of 10 mg/mL were added to the first wells and serially diluted. DMSO at the same concentration in the extracts was used as a negative control, amphotericin B, ciprofloxacin, ampicillin, and gentamicin were used as a positive control for Candida species, *M. smegmatis*, Gram-positive bacteria, and Gram-negative bacteria, respectively. The wells containing medium were used as sterility control. The wells were filled with the test bacteria at a concentration of $5x10^5$ CFU/mL. Plates were incubated for 24 hours at 37°C. There were two iterations of the experiment.

Anti-Quorum Sensing Activity Assay

The *C. violaceum* ATCC 12472 strain producing violacin pigment naturally was used in the assay. 50 µL of overnight *C. violaceum* culture in LB adjusted to 0.5 McFarland density was inoculated onto 5 mL LB soft agars. After the soft agar was poured onto the LB agar in the petri dish and allowed to dry, 50 µL of each extract's sub-Mic concentration was added to the wells that had been opened. The results were evaluated by identifying areas in petri dishes where bacterial growth was present but pigment formation was suppressed. (McLean et al. 2004). An equivalent amount of DMSO of the extracts in the wells was used as a negative control.

Anti-Biofilm Activity Assay

To determine the inhibition of the extracts on biofilm development, *P. aeruginosa* PAO1 strain was incubated in LB broth at 175 rpm at 37°C for 8 hours. Bacteria suspension adjusted at 0.5 McFarland density was used in the experiments by diluting it to 1%. For each extract, 40 µL of extract, 125 µL of LB medium, and 35 µL of bacterial suspension were placed on the microplate in triplicate. As negative controls, wells with just bacterial suspension and medium were employed. Following a 24-hour incubation period at 37°C, microplates were thoroughly cleaned three times using deionized water. The wells were filled with 0.3% crystal violet. The microplates were cleaned three times with distilled water after 15 minutes. 95% ethanol was added to the wells. The measurement was taken in a spectrophotometer at 570 nm after 15 minutes (Kolayli et al. 2022). The average of three separate trials was used to create the graph.

Anti-Swarming Activity Assay

The extracts were added to the autoclaved but not solidified LB agar and poured onto the LB agar in the petri dish. Using a sterile needle loop, the *P. aeruginosa* PAO1 strain, which was cultured on LB agar, was inserted in the center of the solidified agar and incubated at 37°C for 16–18 hours. The diameter of the spread from the site of inoculation to the perimeter was measured to track the swarming activity (Rashid and

Kornberg, 2000, Rice et al. 2005). The results were analyzed by comparing the measurements with the plate that just included PAO1 strain without extract.

Cytotoxicity Assay

Cell culture

The study includes the culture collection of the following cell lines: normal epithelial cell line (Vero), lung adenocarcinoma cell line (A549), breast cancer cell line (MDA-MB-231), and pancreatic tumor cell line (AR42J) that were kept in the Medical Microbiology Department at Karadeniz Technical University. Originally, the American Type Culture Collection (ATCC, USA) provided the cell lines. The cell lines A549, AR42J, and Vero were kept in Dulbecco's Modified Eagles media (DMEM), whereas the MDA-MB-231 cell line was kept in RPMI 1640 media with 1% penicillin/streptomycin solution and 10% fetal bovine serum added. Cultures were incubated at 37°C with 5% CO_2.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl-Tetrazolium Bromide (MTT) Assay

The MTT method was modified and used to determine the cytotoxic effect of the extract on cancer cell lines (Mosmann, 1983). Cells removed from flasks by trypsinization were counted. 5000 cells per well of a 96-well sterile cell culture plate were seeded in 200 µL of medium. After 24 hours, the plates were taken from the incubator and the media in the wells were removed. 100 µL of extracts at doses ranging from 3.12 to 400 µg/mL were added to the wells. Three wells were used for each concentration. Untreated wells were used as control. Following the time frame, MTT dye was added to each well with a final concentration of 0.5 mg/mL, and the plates were incubated for 3.5 hours at 37°C. After the incubation period, the media in the wells were removed. Following the addition of 100 µL of DMSO to each well, the plates were shaken gently for 45 minutes. Using a microplate reader, the absorbance of the plates was measured at 570 nm. Cell viability in treated wells was calculated as % concerning control wells. The experiment was repeated three times. IC_{50} values and selectivity index (SI) of the extracts were calculated (Shamsuzzaman et al 2013; Demir et al. 2016).

Statistical Analysis

The data's normal distribution was ascertained using the Kolmogorov-Smirnov test. One-way ANOVA was used to assess intergroup differences, and a *p*-value of less than 0.05 was considered significant.

Results

The Results of Agar Well Diffusion Test and MIC

All propolis extracts included in the study were found moderately effective against *E. faecalis*, *B. subtilis*, *S. aureus*, *C. violaceum*, *B. cereus*, *M. smegmatis*, *C. albicans*, and *C. parapsilosis* in agar well diffusion test. On the other hand, they were ineffective against *P. aeruginosa*, *E. aerogenes*, *A. haemolyticus*, *E. coli*, *S. typhimurium*, and *K. pneumoniae*. It was determined that the MIC value of propolis extracts against microorganisms varied between 0.312 and 10 mg/mL. The MIC concentrations of the propolis extracts against the microorganisms that were effective in the agar well method were summarized in Table 1.

Anti-Biofilm, Anti-quorum Sensing, Anti-Swarming Activity Assay Results

While all propolis extracts have shown anti-quorum sensing activity, there was not any anti-swarming and anti-biofilm activity in each sample. It has even been observed that all propolis extracts increase biofilm formation (Figure 1).

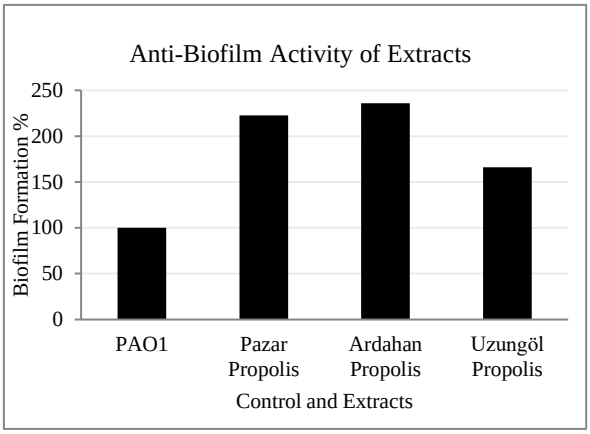

Figure 1. Anti-biofilm activity of ethanolic propolis extracts against *P. aeruginosa* PAO1 strain

Table 1. MIC concentrations (mg/mL) of propolis samples against tested microorganisms

Samples	Microorganisms used in MIC assay							
	B.cereus	*B.subtilis*	*E.faecalis*	*S.aureus*	*C.violaceum*	*C.albicans*	*C.parapsilosis*	*M.smegmatis*
Ardahan Propolis	0.625±0	0.625 ±0	0.312±0	0.625±0	1.25±0	0.625±0	1.25±0	1.875±0.88
Uzungöl Propolis	0.9375±0.44	0.625±0	0.4685±0.22	0.9375±0.44	1.875±0.88	0.625±0	1.25±0	1.875±0.88
Pazar Propolis	0.9375±0.44	1.25±0	0.625±0	1.875±0.88	10±0	2.5±0	2.5±0	2.5±0

Table 2. IC_{50} and selectivity index results of the extracts

Sample	IC_{50} (µg/mL)				Selectivity Index		
	MDA-MB-231	A549	AR42J	Vero	MDA-MB-231	A549	AR42J
Pazar Propolis	210.2	322.9	353.6	347.1	1.7	1.1	0.9
Ardahan Popolis	242.1	301.9	377.9	250.8	1.0	0.8	0.7
Uzungöl Propolis	260	303.6	313.5	321.2	1.2	1.1	1.0

Cytotoxicity Assay Results

In MTT assay it was determined that the IC_{50} values of Pazar propolis ethanolic extract (PPEE), Ardahan propolis ethanolic extract (APEE), and Uzungöl propolis ethanolic extract (UPEE) in the three cancer cell lines studied ranged between 210.2-353.6 µg/mL, 242.1-377.9 µg/mL, 260- 313.5 µg/mL, respectively. The IC_{50} values of PPEE, APEE, and UPEE in normal epithelial cells were calculated as 347.1 µg/mL, 250.8 µg/mL, and 321.2 µg/mL, respectively. When the selectivity indexes of the extracts were evaluated, it was determined that the SI values of PPEE, APEE and UPEE varied between 0.9-1.7, 0.7-1.0, 1.0-1.2. The IC_{50} and SI values of the extracts were summarized in Table 2.

The effects of PPEE on A549 cells at 200 µg/mL and above, on MDA-MB-231 cells at 50 µg/mL and above, on AR42J cells at 100 µg/mL and above were found to be statistically significant. The effects of APEE on A549 and Ar42J cells at 100 µg/mL and above, on MDA-MB-231 cells at 50 µg/mL and above were found to be statistically significant. The effects of UPEE on A549 cells at 100 µg/mL and above, on MDA-MB-231 and AR42J cells at 50 µg/mL and above were found to be statistically significant. The cytotoxicity of the extracts on cell lines was summarized in Figure 2.

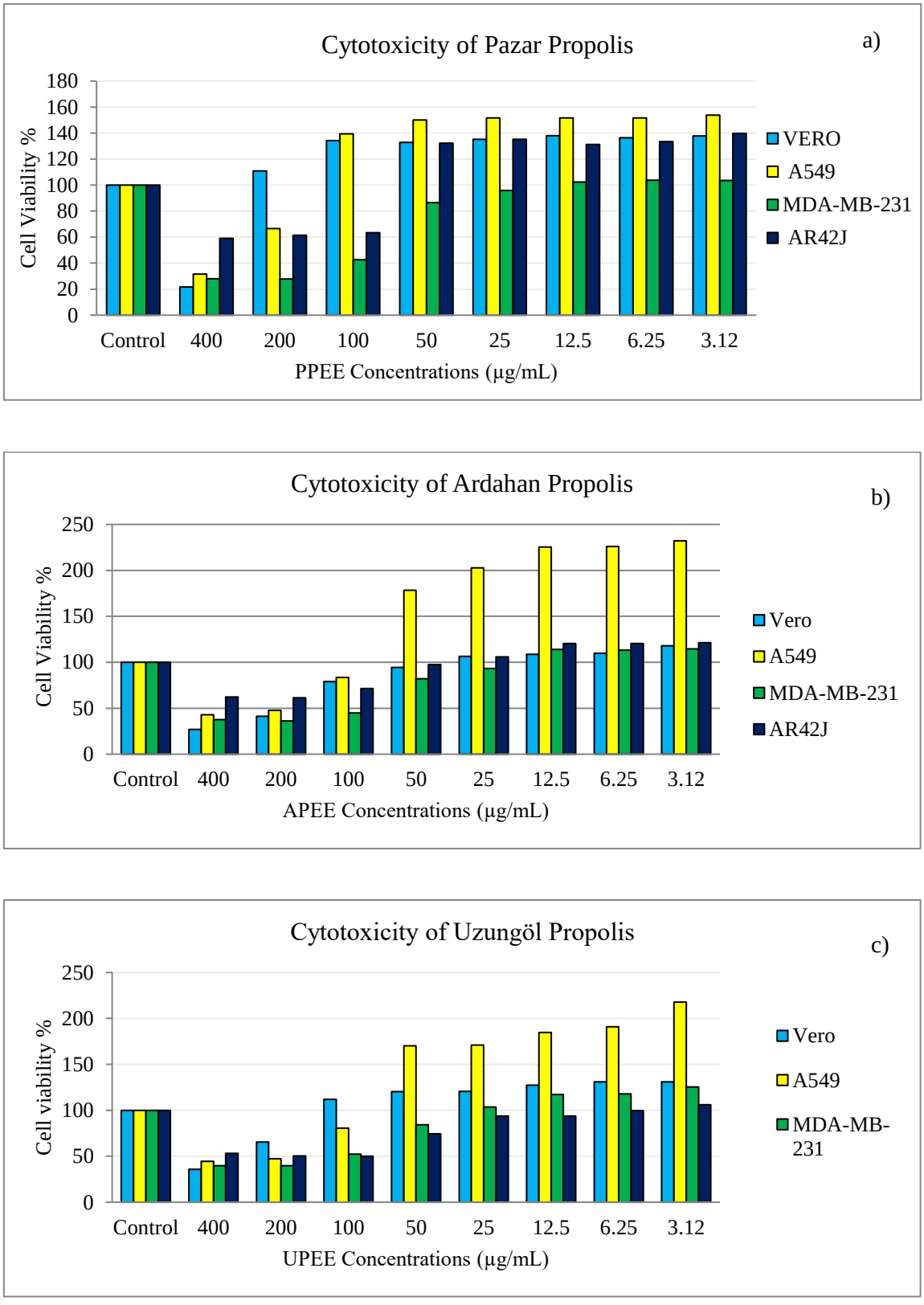

Figure 2. The cytotoxic effect of Pazar (a), Ardahan (b), and Uzungöl (c) propolis extracts on different cell lines

Discussion

There is an increase in the number of studies evaluating the biological activities of natural substances such as propolis due to the resistance developed against drugs, and the greater availability and popularity of natural substances. Since ancient times, propolis has been used alone or combined with other natural ingredients in strengthening immunity and the treatment of diseases (Ożarowski and Karpiński, 2023).

Realizing that bees use propolis to sterilize the inside of the hive, researchers began to investigate the antibacterial potential of propolis (Belmehdi et al. 2022). It was determined that ethanolic extracts from southern Sonora, Mexico propolis presented antibacterial activity against *Escherichia coli* O157, *Staphylococcus aureus*, *Escherichia coli* O157:H7, and *Salmonella typhimurium* bacteria (Portela- Márquez et al. 2022). Brazillian red propolis ethanolic extract had activity against *Parvimonas micra*, *Fusobacterium nucleatum*, *Prevotella melaninogenica*, *Prevotella nigrescens*, *Prevotella intermedia*, and *Porphyromonas gingivalis* in a study conducted in Brazil (Neto et al. 2022). The effect of the ethanolic extract obtained from Pazar, Ardahan, and Uzungöl propolis against 14 microorganisms was evaluated in the current study and it was found to be effective against *S. aureus*, *B. cereus*, *E. faecalis*, *B. subtilis*, *M. smegmatis*, *C. violaceum*, *C. albicans*, and *C. parapsilosis* in different concentrations. Additionally, it was observed that the highest MIC values were in Pazar propolis. It was determined that Pazar propolis, which had the lowest total phenolic content (TPC) and total flavonoid content (TFC) in the analyses performed in another study, showed the highest MIC values in the current study (Cora et al. 2023). On the other hand, although Uzungöl propolis had higher TFC and TPC values than Ardahan propolis, the reason for the lower MIC values in Ardahan propolis was thought to be due to the higher amount of phenolic compounds (such as chrysin, pinocembrin, caffeic acid phenethyl ester) evaluated in high-performance liquid chromatography (HPLC) analysis in Ardahan propolis (Cora et al. 2023).

Bacterial biofilms are multicellular life forms that are encased in an extracellular polymeric matrix that they generate to enhance their resistance to external stimuli (Akcelik and Akcelik, 2022). In different studies, it has been shown that Hungarian propolis, Chinese propolis, and Thai propolis which were prepared with different solvents, were effective against *S. aureus* biofilm, *S. mutans* biofilm, and *E. coli* biofilm, respectively (Bouchelaghem et al.2022; Yuan et al. 2022; Mukaide et al. 2022). In the current study, ethanolic extract of three propolis samples were tested against *P.aeruginosa* biofilm. However, it has been determined that extracts do not prevent biofilm formation, but rather increase it. It was thought that the reason why the effects of the propolis extracts on bacterial biofilm differ from each other is that the components in propolis vary depending on various properties.

Quorum sensing is a cell-cell signaling mechanism in several bacteria that controls the expression of group behaviors. Quorum sensing bacteria secrete diffusible signal molecules in the presence of signal as a response by changing genome-wide gene expression (Rattray and Brown, 2023). Propolis is a compound that inhibits quorum sensing mechanism (Mokrani et al. 2023). Quorum sensing inhibition properties of Algerian propolis ethanolic extract and two trademark ethanolic propolis extract were demonstrated (Kolayli et al. 2022; Mokrani et al. 2023). In the current study, anti-quorum sensing activity of PPEE, APEE, and UPEE was exhibited against *Chromobacterium violaceum* ATCC 12472 strain. HPLC analysis of propolis samples showed that they contained similar phenolic compounds, albeit at different ratios (Cora et al. 2023). Therefore, it was thought that the fact that all propolis samples showed anti-quorum sensing activity and did not show anti-swarming and anti-biofilm activity were related to their phenolic profiles.

Propolis and its components, which are effective at different stages of cancer development processes, thus show anticancer properties. In addition, it is also used by patients to diminish the side effects of chemotherapy and radiotherapy (Forma &

Bryś, 2021). However, since the components of each propolis are different from each other, the anticancer properties of every propolis sample need to be investigated. The anticancer activity of two Lebanese propolis extracts prepared by ethanol investigated against MDA-MB-231 human breast cancer cells and HCT-116 human colorectal cancer cells. The IC_{50} value of each sample was found to be 22.3 and 61.7 µg/mL for breast cancer cells, and 33.3 and 50.9 µg/mL for colorectal cancer cells, respectively (AlDreini et al. 2023). The IC_{50} value of Malaysian propolis ethanolic and aquatic extract was found 31.25 and 120 µg/mL respectively against HeLa cells (Gapar et al. 2023). In addition, the cytotoxic effect of Cyprus, Egyptian, and Algerian propolis extracts was shown in breast cancer cell lines (MDA-MB-231 and MCF-7), human breast cancer cell line (MDA-MB-231), and human pancreatic PANC-1 cell line, respectively (Aboghrip et al. 2023; Nasirli et al. 2023; Tatlisulu & Ozgor, 2023). In the current study, the IC_{50} values of PPEE, APEE, and UPEE in the three cancer cell lines studied ranged between 210.2-353.6 µg/mL, 242.1-377.9 µg/mL, 260-313.5 µg/mL, respectively. The IC_{50} values of PPEE, APEE, and UPEE in normal epithelial cells were found to be 347.1 µg/mL, 250.8 µg/mL, and 321.2 µg/mL, respectively. All of the propolis samples included in the study were found to be most effective against the MDA-MB-231 cell line and least effective against the AR42J cell line. These results were found to be compatible with the chemical analyses of propolis samples. However, the level of effect of propolis samples on cell lines is different from each other. Considering that only 19 phenolic compounds in propolis samples were analyzed, it was thought that it would be appropriate to analyze more compounds to better explain the difference between propolis samples (Cora et al. 2023).

The antimicrobial assay demonstrated that the propolis samples included in the study were found ineffective against Gram-negative bacteria. It is among the possibilities that differences in cell wall structure may prevent propolis from affecting Gram-negative microorganisms. According to the antimicrobial activity experiments, it was thought that, depending on the type of microorganism, the propolis samples studied could be used as a product development or supplement against the species in which they were effective. In cytotoxicity experiments, the effect of propolis on cell lines was revealed. Although the propolis samples included in this study had a dose-dependent cytotoxic effect on the cell lines studied, it was thought that they should not be used alone in anticancer studies because of having a cytotoxic effect on normal cells.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: M.C., Ü.Z.Ü.E. and İ.D.; Investigation: M.C.; Material and Methodology: M.C., Ü.Z.Ü.E. and İ.D.; Supervision: M.C., Ü.Z.Ü.E. and İ.D.; Visualization: M.C. and Ü.Z.Ü.E.; Writing-Original Draft: M.C. and Ü.Z.Ü.E.; Writing-review & Editing: M.C. All authors have read and agreed to the published version of manuscript.

Conflict of Interest:

The author has no conflicts of interest to declare.

Funding

The author declared that this study has received no financial support.

References

Aboghrip, M.K., El-Gayar, K.E., Abbas, M.S., Ahmed, A.E., Soliman, A.S. 2023. Some chemical studies on cytotoxicity of antioxidants from propolis extracts against human pancreatic PANC-1 cancer cell. *Egyptian Journal of Chemistry*. 6(11), 151-161.

Akçelik, N., Akçelik, M. 2022. What makes another life possible in bacteria? Global regulators as architects of bacterial biofilms. *World Journal of Microbiology and Biotechnology*. 38, 236.

AlDreini, S., Fatfat, Z., Abou Ibrahim, N., Fatfat, M., Gali-Muhtasib, H., Khalife, H. 2023. Thymoquinone enhances the antioxidant and

anticancer activity of Lebanese propolis. *World Journal of Clinical Oncology*. 14(5), 203-214.

Angiolella, L., Sacchetti, G., Efferth, T. 2018. Antimicrobial and antioxidant activities of natural compounds. *Evidence-Based Complementary and Alternative Medicine*. 2018, 1945179.

Balouiri, M., Sadiki, M., Ibnsouda, S.K. 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 6(2), 71-79.

Bankova, V. 2005. Chemical diversity of propolis and the problem of standardization. Journal of *Ethnopharmacology*. 100, 114–117.

Belmehdi, O., El Menyiy, N., Bouyahya, A., El Baaboua, A., El Omari, N., Gallo, M., Montesano, D., Naviglio, D., Zengin, G., Senhaji, N.S., Goh, B.H., Abrini, J. 2022. Recent advances in the chemical composition and biological activities of propolis. *Food Reviews International*. 39(9), 6078-6128.

Bouchelaghem, S., Das, S., Naorem, R.S., Czuni, L., Papp, G., Kocsis, M. 2022. Evaluation of total phenolic and flavonoid contents, antibacterial and antibiofilm activities of Hungarian propolis ethanolic extract against *Staphylococcus aureus*. *Molecules*. 27(2), 574.

Cora, M., Buruk, C.K., Unsal, S., Kaklikkaya, N., Kolayli, S. 2023. Chemical analysis and in vitro antiviral effects of northeast Türkiye propolis samples against HSV-1. *Chemistry and Biodiversity*. 20(8), e202300669.

Demir, S., Aliyazicioglu, Y., Turan, I., Misir, S., Mentese, A., Yaman, S.O., Akbulut, K., Kilinc, K., Deger, O. 2016. Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. *Nutrition and Cancer*. 68(1), 165-172.

Denev, P., Kratchanova, M., Ciz, M., Lojek, A., Vasicek, O., Nedelcheva, P., Blazheva, D., Toshkova , R., Gardeva, E., Yossifova, L., Hyrsl, P., Vojtek, L. 2014. Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. *Food Chemistry*. 157, 37–44.

Forma, E. & Bryś, M. 2021. Anticancer activity of propolis and its compounds. *Nutrients*. 13(8), 2594.

Gapar, N.A.A., Asem, N., Hapit, N.H.A., Mas, M., Omar, E.A. 2023. Phytochemical profiling and anticancer activities of ethanolic and aqueous propolis extract of *Trigona apicalis*. *Journal of Health and Translational Medicine*. 2023(1), 141-148.

Huang, S., Zhang, C.P., Wang, K., Li, G.Q., Hu, F.L. 2014. Recent advances in the chemical composition of propolis. *Molecules.* 19, 19610–19623.

Kolayli, S., Ureyen Esertas, U.Z., Kara, Y. 2022. The antimicrobial, anti-quorum Sensing, and anti-biofilm activities of ethanolic propolis extracts used as food supplements. *Biology Bulletin*. 49(Suppl 3), 21-30.

McLean, R.J.C., Pierson, L.S., Fuquac, C. 2004. A simple screening protocol for the identification of quorum signal antagonist. *Journal of Microbiological Methods*. 58(3), 351–360.

Mizrahi, J.D., Surana, R., Valle, J.W., Shroff, R.T. 2020. Pancreatic cancer. *The Lancet*. 395, 2008-2020.

Mokrani, M., Zellagui, A., Hadjab, W., Öztürk, M., Ceylen, O., Bensouici, C. 2023. Bioactive molecules from Algerian propolis with therapeutic potential against the oral pathogen *Enterococcus faecalis* and *Chromobacterium violaceum* CV026. *Nova Biotechnologica et Chimica*. 22(1), e1599.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 65, 55-63.

Mukaide, K., Shimamura, Y., Masuda, S., Vongsak, B., Kumazawa, S. 2022. Antibacterial and antibiofilm activities of Thailand propolis against *Escherichia coli*. *Natural Product Communications*. 17(4).

Murray, P.R., Baron, E.J., Pfaller, M.A., Jorgensen, J.H., Yolken, R.H. 2009. Manual of Clinical Microbiology. Volume 1, 9th Edition. ASM Press, Washington, DC. 390-770.

Nasirli, F., Daikh, A., Doğan, N.M., Arslan, S., Mutlu, D., Segueni, N. 2023. In vitro evaluation of cytotoxic activity of Algerian propolis against human breast adenocarcinoma (MDA-MB-231) cells and investigation of its potential mechanism of action. *Current Bioactive Compounds*. 19(1), 28-38.

Neto, N.F.O., Bonvicini, J.F.S., Souza, G.L., Santiago, M.B., Veneziani, R.C.S., Ambrósio, S.R., Bastos,J.K., Silva, M.J.B., Martins, C.H.G., Moura, C.C.G., Turrioni, A.P. 2022. Antibacterial activity of Brazilian red propolis and in vitro evaluation of free radical production. *Archives of Oral Biology*. 143, 105520.

Ożarowski, M., Karpiński, T.M. 2023. The effects of propolis on viral respiratory diseases. *Molecules*. 28(1), 359.

Portela-Márquez, M.A., Ruiz Cruz, S., Chaidez-Quiroz, C., Silva-Beltrán, N.P., Morán-Palacio, E.F. 2022. Phenolic compounds, antihemolytic, anti-inflammatory and antibacterial activity of propolis from southern Sonora. *Biotecnia*. 2022. 24(3), 77-86.

Rashid, M.H., Kornberg, A. 2000. In organic polyphosphate is needed for swimming, swarming, and twitching motilities of Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences of the United States of America*. 97(9), 4885-4890.

Rattray, J.B., Brown, S.P. 2023. Beyond Thresholds: Quorum-sensing as quantitatively varying reaction norms to multiple environmental dimensions. *Israel Journal of Chemistry*. 63: e202200109.

Rice, S.A., Koh, K.S., Queck, S.Y., Labbate, M., Lam, K., Kjelleberg, S. 2005. Biofilm formation and sloughing in Serratia marcescens are controlled by quorum sensing and nutrient cues. *Journal of Bacteriology*. 187(10), 3477-3485.

Shamsuzzaman, Dar A.M., Yaseen, Z., Alam, K., Hussain, A., Gatoo, M.A. 2013. Steroidal pyrimidines: synthesis, characterization, molecular docking studies with DNA and in vitro cytotoxicity. *Journal of Molecular Structure*. 1045, 62-71.

Tatlısulu, S. & Özgör, E. 2023. Identification of Cyprus propolis composition and evaluation of its antimicrobial and antiproliferative activities. *Food Bioscience*. 51, 102273.

Wojtacka, J. 2022. Propolis contra pharmacological interventions in bees. *Molecules*. 27(15), 4914.

Woods, G.L., Brown-Elliott, B.A., Desmond, E.P., Hall, G.S., Heifets, L., Pfyffer, G.E., Ridderhof, J.C., Wallace, R.J., Warren, N.C., Witebsky, F.G. 2003. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; Approved Standard. *NCCLS Doc.* M24-A, 18, 20-31.

Yuan, J., Yuan, W., Guo, Y., Wu, Q., Wang, F., Xuan, H. 2022. Anti-biofilm activities of Chinese poplar propolis essential oil against *Streptococcus mutans*. *Nutrients*. 14(16):3290.

Zullkiflee, N., Taha, H., Usman, A. 2022. Propolis: Its role and efficacy in human health and diseases. *Molecules*. 19;27(18), 6120.