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RESEARCH ARTICLE

Evaluation of the Bioactivities of Commercially Available Cold-Pressed Borage Fixed Oil (*Borago officinalis*) and Geranium Essential Oil (*Pelargonium graveolens*)

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Evaluation of the Bioactivities of Commercially Available Cold-Pressed Borage Fixed Oil (Borago officinalis) and Geranium Essential Oil (Pelargonium graveolens)

SUMMARY

This study aimed to determine the antimicrobial, antibiofilm, and cytotoxic effects of commercially available oils of Borago officinalis and Pelargonium graveolens, specifically a cold-pressed fixed oil and an essential oil, respectively. The antimicrobial effects of the tested oils were determined using the disk diffusion method and microdilution assay. Subsequently, the antibiofilm effect of the tested oils was determined against Pseudomonas aeruginosa and Staphylococcus aureus using a biofilm inhibition assay. Finally, the cytotoxic effect of these plant oils was tested using the MTT assay on DLD-1 and CCD-18Co cell lines. According to the antimicrobial activity results, the B. officinalis cold-pressed fixed oil showed no antimicrobial effect when compared with gentamicin, which was used as the control. In contrast, the essential oil of P. graveolens exhibited its strongest antimicrobial effect against Salmonella enterica serovar Typhimurium (S. Typhimurium) among Gram-negative bacteria. Among Gram-positive bacteria, the highest antibacterial effects were observed in the following order: Micrococcus luteus > S. aureus > Enterococcus faecalis > Bacillus subtilis. According to the antibiofilm results, the essential oil of P. graveolens inhibited S. aureus biofilm by 77% and P. aeruginosa biofilm by 56%, whereas the B. officinalis cold-pressed fixed oil showed only a low level of biofilm inhibition. Preliminary IC_{50} estimates based on the MTT assay were 73.73 µg/mL for P. graveolens essential oil and approximately 83.25 µg/mL for B. officinalis cold-pressed fixed oil.

Keywords: *Antimicrobial effect, antibiofilm, Borago officinalis, cytotoxicity, Pelargonium graveolens*

Ticari Olarak Satılan Soğuk Preslenmiş Hodan Sabit Yağı (Borago officinalis) ve Sardunya Uçucu Yağının (Pelargonium graveolens) Biyoaktivitelerinin Değerlendirilmesi

ÖZ

Bu çalışmada, Borago officinalis ve Pelargonium graveolens bitkilerinden ticari olarak temin edilen yağların, sırasıyla soğuk preslenmiş sabit yağ ve uçucu yağ olmak üzere, antimikrobiyal, antibiyofilm ve sitotoksik etkilerinin belirlenmesi amaçlanmıştır. İlk olarak, test edilen yağların antimikrobiyal etkileri disk difüzyon ve mikrodilüsyon testleri kullanılarak incelenmiştir. İkinci olarak, test edilen yağların antibiyofilm etkisi Pseudomonas aeruginosa ve Staphylococcus aureus üzerinde biyofilm inhibisyon testi ile değerlendirilmiştir. Son olarak, bu bitkisel yağların sitotoksik etkileri DLD-1 ve CCD-18Co hücre hatları üzerinde MTT testi kullanılarak araştırılmıştır. Antimikrobiyal aktivite sonuçlarına göre, kontrol olarak kullanılan gentamisin ile karşılaştırıldığında B. officinalis soğuk preslenmiş sabit yağının herhangi bir antimikrobiyal etki göstermediği belirlenmiştir. Buna karşın, P. graveolens uçucu yağı Gram-negatif bakteriler arasında en güçlü antimikrobiyal etkiyi Salmonella enterica serovar Typhimurium (S. Typhimurium) üzerinde göstermiştir. Gram-pozitif bakteriler arasında ise en yüksek antibakteriyel etkinin şu sırayla olduğu saptanmıştır: Micrococcus luteus > S. aureus > Enterococcus faecalis > Bacillus subtilis. Antibiyofilm sonuçlarına göre, P. graveolens uçucu yağı S. aureus biyofilm oluşumunu %77, P. aeruginosa biyofilm oluşumunu ise %56 oranında inhibe ederken, B. officinalis soğuk preslenmiş sabit yağı yalnızca düşük düzeyde biyofilm inhibisyonu göstermiştir. MTT testi temelinde yapılan ön IC_{50} tahminleri, P. graveolens uçucu yağı için 73,73 µg/mL ve B. officinalis soğuk preslenmiş sabit yağı için yaklaşık 83,25 µg/mL olarak belirlenmiştir.

Anahtar Kelimeler: *Antimikrobiyal etki, antibiyofilm, Borago officinalis, sitotoksisite, Pelargonium graveolens*

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INTRODUCTION

Following the discovery of antibiotics, they have been widely prescribed for the treatment of bacterial infections worldwide. However, the subsequent development of bacterial resistance due to antibiotic use has become a global public health crisis (AlSheikh et al., 2020). In addition to antibiotic resistance, drug side effects can lead to poor patient compliance and, consequently, treatment failure. This situation drives patients to seek natural alternatives to antibiotics (Berida et al., 2024). Many plants containing bioactive molecules such as saponins, terpenoids, phenolic compounds, and alkaloids exhibit significant antimicrobial and antibiofilm activities through various mechanisms and have important implications for public health (Abdallah, Alhatlani, de Paula Menezes, & Martins, 2023). Borage (*Borago officinalis* L.) and Geranium (*Pelargonium graveolens* L'Hér.) are medicinal plants with a long history of use in traditional medicine and are notable for their antimicrobial potential (Asadi-Salmani, Bahmani, & Rafieian-Kopaei, 2014; Verma, Padalia, & Chauhan, 2016).

B. officinalis is an annual herbaceous plant belonging to the family Boraginaceae and is traditionally used for food and medicinal purposes (Alçay, 2019). Its seeds are particularly valued as a source of oil rich in γ-linolenic acid (Bouali, Zaiter, Dicko, Nasri, & Ouarda, 2026). *P. graveolens*, belonging to the family Geraniaceae, is an aromatic perennial plant widely cultivated for its fragrant essential oil, which is commonly used in perfumery and cosmetic products (Prasad et al., 2019). The chemical composition of these plant-derived oils varies depending on the plant material, geographic origin, cultivation conditions, and extraction method (dos Santos et al., 2025).

The flavonoids and phenolic compounds in the *B. officinalis* plant confer anti-inflammatory, cytotoxic, antioxidant, and wound-healing properties (Ibrahim & Alshammaa, 2023). Seed and leaf extracts of the plant have been reported to exhibit antimicrobial activity against *S. aureus* (Yaghmour, Garajah, Kayali, & Al-Rimawi, 2021). Similarly, the *P. graveolens* plant has also been shown to be effective against *S. aureus* and is used traditionally for gastrointestinal ailments as well as respiratory tract infections (Ennaifer, Bouzaiene, Messaoud, & Hamdi, 2020). However, the vast majority of studies in the existing literature have focused on extracts or oils prepared under controlled laboratory conditions. In contrast, the therapeutic use of these plants among the public is largely through commercial plant oils obtained directly from pharmacies or herbalists. The production, storage, and standardization conditions of these commercial products can vary from brand to brand, and consequently, their efficacy can be inconsistent (Tisserand & Young, 2014). Comprehensive and comparative studies on the antimicrobial potential of these readily available commercial oils are quite limited.

Despite the increasing number of studies investigating plant-derived oils, most research has focused on laboratory-prepared extracts. However, commercially available products may differ significantly in composition and biological activity due to variations in production and storage. We hypothesize that the commercially available cold-pressed fixed oil of *B. officinalis* and essential oil of *P. graveolens* may exhibit different levels of antimicrobial, antibiofilm, and cytotoxic activities compared to those reported in the literature. Although these products differ in their extraction methods and chemical nature, both are commercially available plant-derived oil products used by consumers and may therefore be evaluated comparatively in terms of their biological activities.

This study aimed to fill this significant gap in the literature. The primary objective of this preliminary study was to evaluate the *in vitro* antimicrobial activity of a selected commercial cold-pressed fixed oil of *B. officinalis* and an essential oil of *P. graveolens* sold in the Turkish market against *S. aureus* and *P. aeruginosa*, which are among the most common causative agents of bacterial sinusitis, as well as other pathogenic bacteria. Furthermore, the study aimed to compar-

atively demonstrate their efficacy levels against standard antibiotics using scientific data (Özel & Cingi, 2012). In addition, the cytotoxic activity of the tested oils was evaluated on the human colorectal adenocarcinoma cell line (DLD-1) and the normal colon cell line (CCD-18Co).

MATERIALS AND METHODS

Chemicals and Oil Products

Phosphate buffer, dimethylsulfoxide (DMSO), fetal bovine serum and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and D-(+)-Glucose monohydrate were purchased from Sigma–Aldrich (Steinheim, Germany). Dulbecco's Modified Eagle Medium (DMEM) was obtained from Merck (Darmstadt, Germany). Tryptic Soy Agar (TSA), Sabouraud Dextrose Agar, Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB) and crystal violet were purchased from Merck (Darmstadt, Germany). Tryptone Soya Broth (TSB) was obtained from BD Difco™ (Le Pont de Claix, France).

In this study, a commercially available cold-pressed fixed oil of *B. officinalis* and a commercially available essential oil obtained by steam distillation of *P. graveolens* were purchased from Polente Natural (Istanbul, Türkiye). The oils were specifically selected based on their commercial availability and widespread use among consumers in the Turkish market. These selection criteria aimed to evaluate the biological activities of products directly accessible to the public, reflecting real-life usage scenarios rather than laboratory-standardized plant extracts. The oils were stored at 4 °C in the dark until use to preserve their stability. All assays were conducted in one independent biological experiment. Antibiofilm and cytotoxicity assays were performed in triplicate using technical replicates within this experiment, whereas antimicrobial assays were performed once.

Antimicrobial Activity

Growth Conditions of Microorganisms

The antimicrobial effect of the tested oils was evaluated using the Kirby-Bauer disk diffusion method and the broth microdilution assay. To this end, their effects were tested against a selection of microorganisms, comprising four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 51299, and *Micrococcus luteus* NRRL B-4375), four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, and *Salmonella enterica* serovar Typhimurium ATCC 14028), and one fungal species (*Candida albicans* ATCC 10231). Bacterial strains from stock cultures were revived by inoculating them onto TSA and incubating at 37 °C for 24 hours, while the fungal strain was grown on Sabouraud Dextrose Agar (SDA) at 27 °C for 48 hours.

Disk Diffusion Assay

For the disk diffusion assay, bacterial and fungal suspensions were initially prepared in a sterile 0.9% sodium chloride solution. The microbial inoculum was adjusted to the 0.5 McFarland turbidity standard. Under aseptic conditions, the bacterial suspensions were uniformly spread onto MHA plates, whereas the *C. albicans* suspension was spread onto SDA plates using sterile cotton swabs. Sterile blank disks (6 mm in diameter) were then loaded with 50 µL of undiluted tested oils (100%, v/v) and carefully placed on the inoculated agar using sterile forceps. Gentamicin (30 µg) was used as the reference antibiotic for the bacterial strains. No reference antifungal drug was included in the original experiment. For *C. albicans*, the disk diffusion assay was performed according to the method described by Tran, Graham, and Adukwu (2020). Following incubation at 37 °C for 24 h for bacteria and 30 °C for 48 h for *C. albicans*, the diameters of the inhibition zones were measured in millimeters (mm).

Microdilution Assay

To determine the Minimum Inhibitory Concentration (MIC) of the tested oils required to inhibit microbial growth, a broth microdilution assay was

conducted using a two-fold serial dilution procedure. For bacterial strains, the assay was performed in Mueller–Hinton Broth (MHB) according to broth microdilution approach, whereas the procedure for C. albicans was adapted from the essential-oil susceptibility method described by Tran et al. (2020). Briefly, 100 µL of MHB was dispensed into each well of a sterile U-bottom 96-well microplate. For *C. albicans*, the assay was performed using SDB. Subsequently, the tested oils, prepared using 10% (v/v) DMSO, were subjected to a two-fold serial dilution series. The initial final concentration of the tested oils in the first well was 20% (v/v), followed by 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, 0.0391, 0.0195, and 0.0098% (v/v). Microbial suspensions were prepared and adjusted to a final density of approximately 5×10^5 CFU/mL for bacteria and 0.5×10^3–2.5×10^3 CFU/mL for *C. albicans*. The microplates were then incubated under appropriate conditions. Following incubation, the minimum concentration of the tested oils that completely inhibited visible microbial growth was designated as the MIC.

Biofilm Inhibition Assay (Crystal Violet Method)

Biofilm quantification was performed according to the crystal violet method, with some modifications (Stepanović et al., 2007). The biofilm inhibition activity was evaluated using *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 strains. Bacterial cultures were adjusted to a concentration of approximately 1×10^8 CFU/mL in TSB supplemented with 1% glucose to promote biofilm formation. Briefly, 100 µL of the bacterial suspension was added to each well of a sterile 96-well microplate, followed by the addition of the tested oils at sub-inhibitory concentrations (1/2 × MIC). Wells containing only TSB broth were used as the negative control, while wells containing bacterial suspension without oils served as the positive control. The plates were incubated statically at 37 °C for 24 hours to allow biofilm formation. After incubation, the supernatant was carefully removed, and non-adherent cells were washed away by rinsing the wells three times with sterile PBS (pH 7.2). The plates were dried at 60 °C for 60 minutes to fix the biofilms. The fixed biofilms were stained with 200 µL of 0.5% (w/v) crystal violet solution for 15 minutes, followed by three washes with sterile distilled water to remove excess dye. The plates were further dried at 37 °C for 30 minutes, and the crystal violet bound to the biofilms was solubilized by adding 200 µL of 96% ethanol for 15 minutes at room temperature. Finally, the optical density (OD) of each well was measured at 570 nm using a microplate reader. The percentage of biofilm inhibition was calculated using the following formula:

$$\text{Biofilm Inhibition } (\%) = (1 - (OD_{570}(\text{Test}))/OD_{570}(\text{Positive Control})) \times 100$$

Cytotoxic Activity (MTT assay)

In this study, DLD-1 (human colorectal adenocarcinoma, ATCC CCL-221) and CCD-18Co (normal colon, ATCC CRL-1459) cell lines were obtained from the Cell Culture Laboratory of Amasya University Central Research Laboratory. DLD-1 cells were cultured in RPMI-1640 medium, whereas CCD-18Co cells were maintained in MEM medium. All media were supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Upon reaching appropriate confluency, cells were subcultured into 75 cm^2 flasks. Stock solutions of the oils were prepared by dissolving the oils in DMSO. Working concentrations (1000–31 µg/mL) were obtained by serial dilution and used for the MTT assay. Accordingly, cytotoxicity concentrations are expressed in µg/mL, whereas MIC values are expressed as % (v/v), reflecting the distinct experimental conditions of the assays. For the MTT assay, cells were seeded into 96-well plates and allowed to attach for 24 hours. After treatment with the oils at different concentrations, MTT solution was added and incubated for 2 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as the percentage of the mean absorbance

of treated cells relative to that of the control cells. Dose–response curves were plotted using Microsoft Excel, and IC_{50} values were estimated from the logarithmic slope plots.

RESULTS AND DISCUSSION

The antimicrobial effects of the tested oils were evaluated against eight bacterial strains and one yeast species using the disk diffusion method and microdilution assay. The results are presented in Table 1.

Table 1. Antimicrobial activity (inhibition zones and MIC values) of *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil

	B. officinalis		*P. graveolens*		CN
Microorganisms	Disk diffusion (mm)	MIC %	Disk diffusion (mm)	MIC %	Disk diffusion (mm)
E. coli ATCC 25922	ND	>20	12	2.5	19
P. aeruginosa ATCC 27853	ND	20	12	5	18
*S. Typhimurium ATCC 14028	ND	>20	20	1.25	22
K. pneumoniae ATCC 700603	ND	>20	9	2.5	16
S. aureus ATCC 25923	ND	20	18	5	18
B. subtilis ATCC 6633	ND	>20	12	10	22
E. faecalis ATCC 51299	ND	20	12	5	19
M. luteus NRRL B-4375	ND	20	25	1.25	28
C. albicans ATCC 10231	ND	20	22	2.5	ND

ND: Not detected, CN: Gentamicin (30 µg), * *Salmonella enterica* serovar Typhimurium

The cold-pressed fixed oil of *B. officinalis* did not exhibit any antimicrobial effect in the disk diffusion assay, as no inhibition zones were observed against the tested microorganisms. However, MIC results revealed that at a high concentration (20%, v/v), the oil demonstrated antimicrobial effects against certain bacterial and fungal species (*P. aeruginosa*, *S. aureus*, *E. faecalis*, *M. luteus*, and *C. albicans*).

The essential oil of *P. graveolens* exhibited a measurable antimicrobial effect against the tested microorganisms in both the disk diffusion assay, with inhibition zones ranging from 9 mm to 25 mm, and the broth microdilution method, at concentrations between 1.25% and 10%. Among the Gram-negative bacteria, the essential oil of *P. graveolens* exhibited the strongest antimicrobial activity against *S.* Typhimurium, while its effects on other Gram-negative strains were comparatively weaker. In contrast, among the Gram-positive bacteria, the highest antimicrobial activity was observed against *M. luteus*. The oil demonstrated moderate activity against *S. aureus* and *E. faecalis*, whereas its activity against *B. subtilis* was relatively weak. Similarly, differences in antifungal activity against *C. albicans* were observed between the two tested oil products. *P. graveolens* essential oil exhibited moderate antifungal activity, whereas the antifungal effect of *B. officinalis* cold-pressed fixed oil was largely negligible.

The antibiofilm activity of plant oils is considered an important mechanism in combating the resistance developed by biofilm-forming bacteria against antibiotics and disinfectants. The data obtained in this study indicate different levels of biofilm inhibition by the *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil against *P. aeruginosa* and *S. aureus*. Under the tested conditions, *P. graveolens* essential oil showed higher observed biofilm inhibition, with inhibition values of 77% for *S. aureus* and 56% for *P. aeruginosa*. In contrast, *B. officinalis* cold-pressed fixed oil showed only low levels of biofilm inhibition against both bacterial strains (Figure 1.).

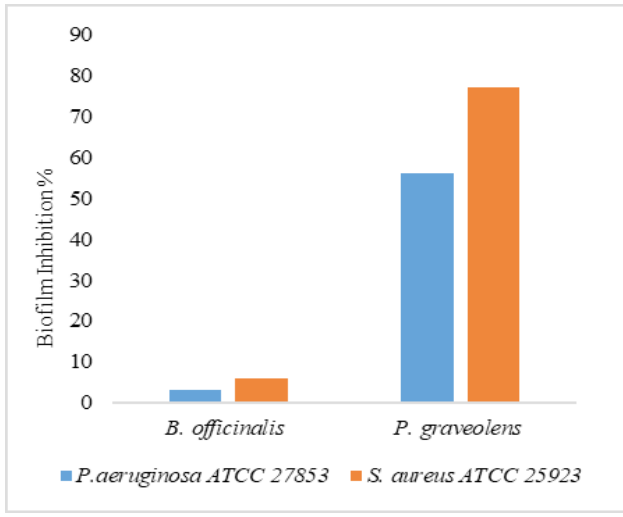
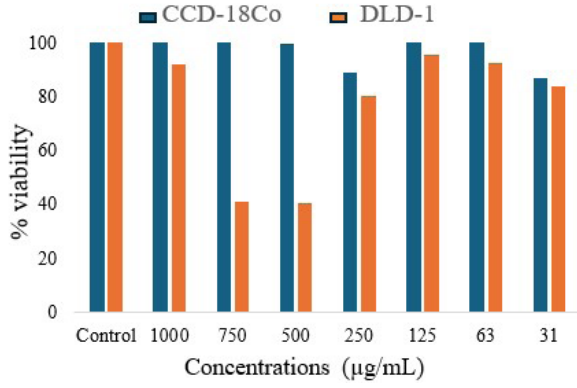
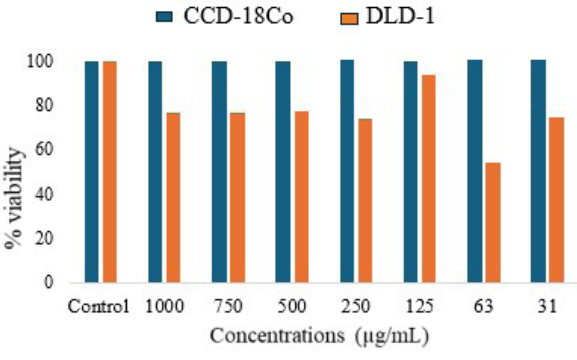


Figure 1. Biofilm inhibition percentages of *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923

The cytotoxic effects of the cold-pressed fixed oil of *B. officinalis* and the essential oil of *P. graveolens* were determined by the MTT assay on DLD-1 and CCD-18Co cell lines. The results are shown in Figure 2 and Table 2.



(a)



(b)

Figure 2. Cytotoxic effects of (a) *B. officinalis* cold-pressed fixed oil and (b) *P. graveolens* essential oil on DLD-1 and CCD-18Co cells

Table 2. Cell viability percentages of DLD-1 cells following treatment with *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil at different concentrations

	B. officinalis		*P. graveolens*	
Concentrations	Absorbance	% Viability	Absorbance	% Viability
Control	0.994	100	0.947	100
1000 µg/mL	0.912	91.780	0.729	76.939
750 µg/mL	0.405	40.764	0.723	76.306
500 µg/mL	0.402	40.472	0.734	77.467
250 µg/mL	0.797	80.221	0.703	74.195
125 µg/mL	0.947	95.271	0.891	94.036
63 µg/mL	0.916	92.183	0.509	53.794
31 µg/mL	0.831	83.621	0.708	74.765

IC_{50} values were estimated using logarithmic trendlines fitted to the dose–response data in Microsoft Excel. For IC_{50} estimation, cell viability values exceeding 100% were capped at 100%.

According to the MTT assay results, the IC_{50} values of *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil were estimated to be 83.25 and 73.73 µg/mL, respectively. These values should be interpreted as preliminary estimates because the observed cell viability responses did not consistently follow a concentration-dependent pattern, particularly for *B. officinalis* cold-pressed fixed oil. Although the three technical replicates yielded consistent results within the same experiment, further independent biological experiments are needed to confirm the reproducibility of the findings and the reliability of the IC_{50} estimates. In the CCD-18Co cell line, neither oil produced a consistent reduction in cell viability across the tested concentrations.

Plant-derived oils have been utilized for centuries in pharmacological preparations, complementary medicine practices, and for maintaining food quality naturally (Prabuseenivasan, Jayakumar, & Ignacimuthu, 2006). According to the European Pharmacopoeia, essential oils are odorous products, usually of complex composition, obtained from botanically defined herbal materials by steam distillation, dry distillation, or a suitable mechanical process without heating (European Pharmacopoeia, 2022). In this context, the essential oil of *P. graveolens* fits this definition, while the oil of *B. officinalis* is a fixed oil typically obtained by cold-pressing. Essential oils, due to their volatility and lipophilic nature, generally exhibit biological activity by disrupting cell membrane integrity, leading to leakage of cellular contents and inhibition of cellular respiration (Jawale et al., 2012). Furthermore, essential oils represent potential sources of novel antimicrobial compounds, particularly effective against certain microbial pathogens, given the side effects and resistance issues associated with synthetic antimicrobials (Chouhan, Sharma, & Guleria, 2017; Wińska et al., 2019). Several studies have demonstrated the antimicrobial effects of extracts from *B. officinalis* and essential oils from *P. graveolens* (Aliakbarlu & Tajik, 2012; de Lima et al., 2024; Kačániová et al., 2024; Sánchez-Hernández et al., 2023).

In our study, the commercially available *B. officinalis* cold-pressed fixed oil showed no antimicrobial activity in the disk diffusion assay, while only a minimal inhibitory effect was observed at high concentrations in the microdilution assay. The discrepancy between the two methods suggests that *B. officinalis* cold-pressed fixed oil may not diffuse effectively in agar-based media due to its non-polar nature and

high molecular weight components. This difference may be due to variations in the chemical composition of *B. officinalis* cold-pressed fixed oil (Borowy, Chwil, & Kapłan, 2017; Hasdemir, Kesbiç, Cravana, & Fazio, 2023). Studies in the literature have generally been aimed at determining the antimicrobial properties of *B. officinalis* extracts obtained with various solvents, while it has been found that there is a lack of data on the antimicrobial activity of *B. officinalis* cold-pressed fixed oil. Considering the literature's studies on *B. officinalis* extracts, low antimicrobial activity was found, as was the case with the *B. officinalis* cold-pressed fixed oil in our study. According to Yaghmour et al., the inhibition zone (12 mm) of the seed extract using *B. officinalis* seed oil and leaf extract against *S. aureus* was higher than the inhibition zone of the leaf extracts (7.5 mm), but no significant effect was observed against *E. coli* and *C. albicans* for both extracts. Similarly, Kumavat & Mishra (2021) investigated the antibacterial effect of nanoparticles synthesized from *B. officinalis* leaf extract (Kumavat & Mishra, 2021). The study reported limited activity, showing an 8 mm inhibition zone against *E. coli*, whereas no notable antibacterial effects were detected against *S. typhi*, *B. subtilis*, or *S. aureus*. Chemically, the cold-pressed fixed oil of *B. officinalis* differs from the essential oil of *P. graveolens*. Borage seed oil is widely recognized as a source of γ-linolenic acid, an omega-6 polyunsaturated fatty acid, which is considered one of its characteristic constituents (Tanwar, Goyal, Kumar, Rasane, & Sihag, 2020). The biological properties reported for borage oil may therefore be related, at least in part, to its fatty acid composition (Asadi-Samani et al., 2014). Nevertheless, because the fatty acid composition of the commercial product examined in the present study was not determined, the contribution of specific constituents to the observed antimicrobial, antibiofilm, or cytotoxic effects cannot be established from the present data.

In contrast to *B. officinalis* cold-pressed fixed oil, the present study revealed that *P. graveolens* essential oil demonstrated pronounced antimicrobial activity in both the disk diffusion and microdilution assays. The biological activity observed for the *P. graveolens* essential oil may be related to the chemical constituents commonly reported for this species (Balabanova, Zheleva-Dimitrova, & Gevrenova, 2025). Previous studies have shown that *P. graveolens* essential oils are characterized predominantly by monoterpenes and oxygenated monoterpenes, with citronellol, geraniol, and linalool frequently reported among the major constituents (Al-Mijalli et al., 2022; Ennaifer et al., 2020; Jaradat et al., 2022). However, the chemical composition of the commercial essential oil used in the present study was not determined; therefore, these constituents are discussed only as possible contributors to the observed biological effects and should not be interpreted as analytically confirmed components of the tested product. In the disk diffusion assay, the *P. graveolens* essential oil produced the largest inhibition zone against *M. luteus* (25 mm), followed by *S.* Typhimurium (20 mm) and *S. aureus* (18 mm) (Table 1). It has been suggested that Gram-negative bacteria exhibit higher resistance to plant oils compared to Gram-positive species. This phenomenon is largely attributed to the structural complexity of the Gram-negative cell wall, whose unique organization hinders the penetration of phenolic compounds present in essential oils (Sakkas et al., 2016). Consistent with our findings (1.25% v/v), Rathore, Mukhia, Kumar and Kumar, (2023) reported that *P. graveolens* essential oil had a strong antimicrobial effect on *M. luteus* (1%) in a microdilution assay. Similarly, Al-Mijalli et al. (2022) reported that *P. graveolens* essential oil exhibited the strongest antimicrobial activity against *B. subtilis*, a Gram-positive bacterium, with an inhibition zone of 14 mm in the disk diffusion assay and a minimum inhibitory concentration of 0.5% in the microdilution assay. In another study, Kačániová et al. (2024) found that *P. graveolens* essential oil showed the highest antimicrobial activity against *S. enterica*, a Gram-negative bacterium. Consistent with these findings, our study also demonstrated that among Gram-negative bacteria, *P. graveolens* essential oil exhibited the strongest antimicrobial activity against *S. enterica* serovar Typhimurium.

Many studies have investigated the antibiofilm activity of essential oils (Maggio et al., 2025; Reichling, 2020; Srinivasan, Jin, Lin, & Zhao, 2025; Touati, Mairi, Ibrahim, & Idres, 2025). Bacterial cells are frequently embedded within dense extracellular polymeric matrices, which may hinder the penetration and efficacy of bactericidal agents. Gram-negative bacteria, in particular, exhibit intrinsic resistance mechanisms that provide protection against a broad spectrum of antimicrobial compounds. Within this framework, essential oils have been shown not only to inhibit the synthesis of extracellular polysaccharides and proteins involved in biofilm formation but also to facilitate the disruption of the biofilm architecture itself. The subsequent degradation of this protective barrier renders previously shielded bacterial cells more vulnerable, thereby allowing essential oils to exert direct antimicrobial effects (Palomares-Navarro et al., 2022; Ruppé et al., 2015; Yang et al., 2025). In the present study, the antibiofilm activities of the *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil were evaluated against two strong biofilm-forming strains, *P. aeruginosa* and *S. aureus*, using a biofilm inhibition assay. Under the tested conditions, *P. graveolens* essential oil showed higher observed inhibition of biofilm formation than *B. officinalis* cold-pressed fixed oil for both bacterial strains. Conversely, *B. officinalis* cold-pressed fixed oil displayed only a limited capacity to interfere with biofilm formation, suggesting that its antibiofilm potential is relatively weak. In line with our findings, Wafa et al. (2023) reported that *P. graveolens* essential oil exhibited notable antibiofilm activity against *S. aureus* even at concentrations below the MIC (20, 10, and 5 µL/mL, $p < 0.05$). Their study further indicated that at a concentration of 20 µl/mL, the oil significantly inhibited biofilm formation by a strong biofilm-producing *S. aureus* strain, achieving up to 91.4% inhibition ($p < 0.01$). In contrast, in our experiments, *P. graveolens* essential oil reduced *P. aeruginosa* biofilm formation by 56%. However, Wafa et al. (2023) observed that subinhibitory concentrations of the oil had no measurable effect on *P. aeruginosa* biofilm development.

Chemotherapeutic agents often give rise to tumor phenotypes characterized by multidrug resistance (MDR). These cellular characteristics are often associated with impairments at one or more stages of apoptotic signaling, mainly due to increased drug efflux mediated by ATP-binding cassette (ABC) transporters that rely on cellular energy (Bhalla, Gupta, & Jaitak, 2013; Mohamed et al., 2023). Recent studies have demonstrated that secondary metabolites found in essential oils may counteract or reverse mechanisms of drug resistance (Guo et al., 2022; Khajehdehi, Khalaj-Kondori, & Baradaran, 2022; Machado, Da Fonseca, Duarte, Robbs, & De Sousa, 2022). In this study, we determined the cytotoxic effects of the *B. officinalis* cold-pressed fixed oil and *P. graveolens* essential oil using the MTT test. The present study represents the first investigation into the cytotoxic effects of *B. officinalis* cold-pressed fixed oil, whereas previous studies have primarily examined the cytotoxicity of *B. officinalis* extracts prepared with various solvents. This scarcity of data may be attributed to the fact that *B. officinalis* contains only small amounts of oil in its aerial parts, including seeds, leaves, and flowers (Slama, Slougui, Ounnas, Benaissa, & Bataiche, 2025). When the cytotoxic effect of *P. graveolens* essential oil was compared with the literature, our findings were found to be consistent with previous reports. For instance, Jaradat et al. (2022) investigated the cytotoxic effect of *P. graveolens* essential oil on different cancer cell lines. The results they obtained determined that the highest effect was observed against MCF-7 (32.71 ± 1.25 µg/mL), followed by Hep3B and HeLa cancer cells, respectively. Another study reported that exposure to *P. graveolens* essential oil at 100 µg/mL resulted in nearly complete cell death in different cell lines (Fahmy et al., 2023). However, direct comparisons with previous studies should be interpreted cautiously because of differences in cell lines, oil composition, and experimental conditions. In the present study, the estimated IC_{50} value of 73.73 µg/mL should be considered preliminary, as cell viability did not fall below 50% at any of the tested concentrations. Further independent biological experiments are needed to confirm this estimate and assess the reproducibility of the observed cytotoxic response.

CONCLUSION

In conclusion, this study analyzed the antimicrobial, antibiofilm, and cytotoxic effects of two commercially available oil products: a cold-pressed fixed oil of *B. officinalis* and an essential oil of *P. graveolens*. The essential oil of *P. graveolens* showed antimicrobial activity and inhibited biofilm formation. In the MTT assay, cell viability in DLD-1 cells varied across the tested concentrations but did not fall below 50%. Therefore, the estimated IC_{50} value should be interpreted cautiously. Further independent biological experiments are needed to better understand the effects of this essential oil on colon carcinoma cells. The biological activities observed for *P. graveolens* essential oil may be associated with its major components, including monoterpenes and monoterpene-derived alcohols such as citronellol, geraniol, and linalool. Therefore, future studies should include chemical characterization (e.g., GC–MS analysis) to standardize the essential oil and directly correlate its composition with the observed bioactivity. The results indicate that the tested *P. graveolens* essential oil may have potential for further investigation as a natural antimicrobial agent, provided that its efficacy is confirmed by *in vivo* studies. This study may provide valuable insights for researchers working in the fields of medicinal plants and pharmacology.

LIMITATIONS

One of the main limitations of this study is that only one commercial product was analyzed for each type of oil. Although the findings provide valuable insights into the potential biological activities of the tested products, substantial variation in chemical composition and biological activity may exist among commercially available products due to differences in production methods, storage conditions, and raw material sources. Therefore, the present findings cannot be generalized to all commercially available *B. officinalis* and *P. graveolens* oils. In addition, the lack of chemical characterization of the tested oils limits the ability to associate the observed biological activities with specific chemical constituents. It should also be noted that the antifungal evaluation against *C. albicans* was conducted without a reference antifungal agent, which limits direct comparison with a standard antifungal treatment. Finally, as the findings are based exclusively on in vitro experiments, they cannot be directly extrapolated to in vivo conditions or clinical efficacy.

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AUTHOR CONTRIBUTION STATEMENT

Conceived the study: H.Y., A.A.B., and C.B. Experiment and data processing: A.A.B and E.Ç. Drafted the manuscript: H.Y., E.Ç., and C.B. Revised the manuscript: H.Y., A.A.B., E.Ç., and C.B. All authors confirm that they have read and approved the completed manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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