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ORIGINAL ARTICLE

GC-MS profiling and biological activities of lipophilic extracts from *Fritillaria imperialis* L. and *Fritillaria pinardii* Boiss. bulbs

Onur Esen[1,2], Gülbahar Özge Alim Toraman[3], Murat Ihlamur[4,5], Fatmanur Gürbüzkol Yılmaz[6,7], Derya Doğanay[8], Firdevs Demirel[3] and Gülaçtı Topçu[3,9*]

[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Biruni University, Zeytinburnu, İstanbul 34025, Türkiye
[2]Department of Pharmacognosy and Natural Products Chemistry, Institute of Health Sciences, Bezmialem Vakif University, Fatih, İstanbul 34093, Türkiye
[3]Department of Pharmacognosy, Faculty of Pharmacy, Bezmialem Vakif University, Fatih, İstanbul 34093, Türkiye
[4]Vocational School, Biruni University, Zeytinburnu, İstanbul 34025, Türkiye
[5]Biruni University Advanced Technology and Research Center (B@MER), Biruni University, Zeytinburnu, Istanbul 34025, Türkiye
[6]Department of Pharmacognosy, Faculty of Pharmacy, Biruni University, Zeytinburnu, İstanbul 34025, Türkiye
[7]Department of Pharmacognosy, Institute of Health Sciences, Marmara University, Maltepe, İstanbul 34844, Türkiye
[8]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Health Sciences, Üsküdar, İstanbul 34668, Türkiye
[9]Drug Application and Research Center (DARC), Bezmialem Vakif University, Fatih, Istanbul 34093, Türkiye

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Abstract: The biological activities of *Fritillaria* species have largely been attributed to their steroidal alkaloids; however, the contribution of lipophilic constituents remains poorly understood. In this study, petroleum ether and diethyl ether extracts obtained from the bulbs of *F. imperialis* and *F. pinardii* were evaluated for their chemical composition and biological activities. GC–MS analysis revealed that fatty acid methyl esters were the predominant constituents, with linoleic acid methyl ester being the major compound in both species. The extracts displayed weak antibacterial activity but notable antifungal effects, particularly against Candida species. MTT assays showed low cytotoxicity toward J774 murine macrophage cells, whereas the extracts decreased HT-29 colorectal cancer cell viability under the tested conditions. In addition, the petroleum ether extracts exhibited strong 5-lipoxygenase inhibitory activity, surpassing indomethacin in some cases. The findings demonstrate that lipophilic metabolites contribute significantly to the biological potential of *Fritillaria* species and suggest that non-polar constituents should be considered alongside alkaloids as important bioactive components. To the best of our knowledge, this is the first report on the biological activities of lipophilic extracts from *F. pinardii* bulbs.

Keywords: *Fritillaria imperialis*, *F. pinardii*, antimicrobial activity, cytotoxic activity, anti-inflammatory activity

1 Introduction

Fritillaria is a medicinal plant genus belonging to the Liliaceae family and has been widely utilized in Traditional Chinese Medicine (TCM) for centuries. It is traditionally employed to clear heat, moisten the lungs, alleviate cough, and reduce phlegm accumulation. Historical records indicate that its medicinal application can be traced back to the Han Dynasty (220 BCE) (Borjigin et al., 2023). To date, around 175 species of the genus *Fritillaria* have been identified globally (WCVP, 2026). Türkiye represents one of the principal centers of diversity for the genus *Fritillaria*, with 56 recorded species, of which 31 are endemic. The genus is particularly well represented in Anatolia, especially in the Eastern and Southeastern Anatolian regions, which constitute important areas of distribution for *Fritillaria* taxa. Recent taxonomic investigations have confirmed the prominent role of Türkiye as a global hotspot of *Fritillaria* diversity. Furthermore, the documented number of *Fritillaria* taxa in the country continues to rise as new species are discovered and formally described (Rix, 1984; Tekşen, 2018; Sonay et al., 2025).

Fritillaria imperialis is one of the most notable members of the genus due to its considerable ornamental importance. The

*Corresponding Author: Gülaçtı Topçu. Email: gtopcu@bezmialem.edu.tr

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species occurs naturally across a broad geographical range extending from Türkiye, Iran, and Iraq to the Himalayan region (Chen et al., 2020; Hao et al., 2013). Within Türkiye, it is predominantly distributed in the Eastern Anatolian region (Tekşen, 2018). Phytochemical investigations have revealed that *F. imperialis* contains a diverse array of bioactive constituents, including steroidal alkaloids, flavonoids, saponins, and phenolic compounds (Hao et al., 2013; Chen et al., 2020; Kumar et al., 2025). *Fritillaria pinardii* is an important geophytic species native to Türkiye, where it occurs predominantly in the Eastern Anatolian region (Tekşen, 2018). Ethnobotanical investigations have documented its traditional use in certain local communities, particularly for wound treatment and as a nutritional resource (Korkmaz et al., 2014; Mükemre et al., 2015).

Steroidal alkaloids are considered the principal contributors to the biological activities observed in Fritillaria species (Hao et al., 2013). Among these compounds, isosteroidal alkaloids including peimine, peiminine, imperialine, verticine, and verticinone have demonstrated diverse bioactivities, encompassing antimicrobial, antioxidant, anti-inflammatory, cytotoxic, and neuroprotective properties (Shang et al., 2018; Kumar et al., 2025). Although the biological activities of Fritillaria species are primarily associated with their alkaloid content, limited studies have revealed that extracts devoid of alkaloids may also contribute to the observed pharmacological effects through diverse biological activities (Li et al., 2010; Borjigin et al., 2023).

In this study, lipophilic fractions were obtained from the bulbs of *F. imperialis* and *F. pinardii*, and their antimicrobial, cytotoxic and anti-inflammatory activities were evaluated. To the best of our knowledge, all of these biological activities were investigated for *F. pinardii* for the first time in the present study.

2 Materials and Methods

2.1 Plant Material

Bulbs of *F. imperialis* and *F. pinardii* were collected from the Turkish *Fritillaria* Collection Garden maintained by the Erzincan Horticultural Research Institute. Voucher specimens were authenticated and deposited under herbarium numbers F30009 and F44044, respectively. The harvested bulbs were collected in August 2022, transported to the laboratory, dried after slicing, and pulverized for subsequent experimental analyses.

2.2 Preparation of Plant Extracts

Air-dried and powdered bulbs of *F. imperialis* (317 g) and *F. pinardii* (160 g) were extracted by maceration using methanol as the solvent. Following solvent evaporation, crude methanol extracts were obtained with extraction yields of 7.57% and 9.38%, respectively. For the preparation of non-alkaloidal fractions, 2 g of each dried methanol extract was dissolved in 3% H_2SO_4 solution (pH 2–3). The acidic solutions were subsequently subjected to successive liquid–liquid partitioning in a separatory funnel using petroleum ether followed by diethyl ether, yielding the lipophilic fractions. The yields of petroleum ether/diethyl ether extracts were 16.32%/9.33% for *F. imperialis* and 11.8%/10.99% for *F. pinardii*, respectively (Kusman Saygi et al., 2023). The abbreviations of the extracts used in the activity assays are given below:

FiP: *F. imperialis* petroleum ether extract
FiD: *F. imperialis* diethyl ether extract
FpP: *F. pinardii* petroleum ether extract
FpD: *F. pinardii* diethyl ether extract

2.3 GC-MS Analysis

Petroleum ether extracts were methylated according to the method described by Christie (2003), with minor modifications. Briefly, 50 mg of extract was dissolved in 1 mL of toluene, followed by the addition of 2 mL of 1% H_2SO_4 in methanol. The reaction mixture was incubated at 50°C overnight. After cooling, 5 mL of 5% NaCl solution was added, and the resulting methyl ester derivatives were extracted with hexane (5 mL × 3). The organic phase was washed with 2% $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and concentrated prior to GC–MS analysis. Compound identification was achieved by matching the acquired mass spectra against the NIST and Wiley Mainlib mass spectral libraries and by comparison with data available in the PubChem database and the relevant literature.

2.4 Antimicrobial Activities

The tested microorganisms included *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* (MRSA) ATCC 43300, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, *Aspergillus fumigatus* ATCC 204305, a clinical isolate of A. *fumigatus*, *Candida albicans* ATCC 10231, a clinical isolate of *C. albicans*, *Candida tropicalis* ATCC 750, and *Candida parapsilosis* ATCC 22019. Bacterial and yeast strains were cultured on TSA and SDA, respectively, and incubated for 24–48 h at 37°C (bacteria) or 27°C (yeasts). Microbial suspensions were prepared in Mueller–Hinton Broth (MHB) and adjusted to 0.5 McFarland using a densitometer (BioSan), corresponding to approximately 10^6 CFU/mL (CLSI, 2012; CLSI, 2023).

Antimicrobial activity was evaluated using the disk diffusion method. Extracts were dissolved in ethanol (8 mg/mL) and sterilized through a 0.22 µM membrane filter. Microbial suspensions (0.1 mL) were spread onto the appropriate agar media, and discs impregnated with 20 µL of extract solution were placed on the surface. Bacterial strains were cultured on Mueller–Hinton agar, while fungal strains were grown on Sabouraud Dextrose Agar. Ceftriaxone and Piperacillin/Tazobactam served as antibacterial positive controls, whereas Voriconazole was used as the antifungal control. Plates were incubated at 37°C for bacteria and 27°C for fungi for 18–24 h. Antimicrobial activity was determined by measuring inhibition zone diameters (mm) using a digital

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caliper. Distilled water was used as the negative control, and all assays were performed in triplicate (CLSI, 2012; Mostafa et al., 2018; CLSI, 2023).

2.5 Cytotoxic Activity

HT-29 human colorectal adenocarcinoma cells and J774 murine macrophage cells were obtained from the cryobank of the Biruni University Advanced Technology and Research Center (B@MER), Istanbul, Türkiye. HT-29 human colorectal adenocarcinoma cells were cultured in DMEM-High Glucose, whereas J774 murine macrophage cells were cultured in RPMI-1640 medium. Both media were supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, and cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cytotoxic activity was evaluated using the MTT assay. Cells were seeded in 96-well plates at a density of 1×10^5 cells/mL and exposed to extracts at concentrations of 10–100 µg/mL for 48 h. Following treatment, MTT solution (10 µL, 10 mg/mL) was added and incubated for 3 h. Formazan crystals were dissolved with 100 µL DMSO, and absorbance was measured at 570 nM after 30 min incubation in the dark (Edmondson et al., 1988; van Meerloo et al., 2011; Petkov et al., 2021). All assays were performed in triplicate, and cell viability was calculated using the formula given below.

$$\text{Cell Viability (\%)} = (\text{Sample absorbance/Control absorbance}) \times 100$$

2.6 Anti-Inflammatory Activity

Anti-inflammatory activity was evaluated by the soybean lipoxygenase (5-LOX) inhibition assay described by Phosrithong and Nuchtavorn (2016) with minor modifications adapted to a 96-well microplate format. Briefly, 10 µL of extract, standard, or test substance at different concentrations was mixed with 20 µL ethanol, 20 µL borate buffer, and 25 µL type V soybean lipoxygenase solution (20,000 U/mL). After incubation at 25°C for 5 min, 100 µL of 0.6 mM linoleic acid solution was added, and the change in absorbance at 234 nM was monitored for 6 min. Indomethacin served as the positive control. The percentage inhibition was calculated as [(Control Absorbance − Sample Absorbance)/Control Absorbance] × 100 (Phosrithong & Nuchtavorn, 2016; Yıldırım et al., 2019; Gürbüzkol & Bitiş, 2025). All experiments were performed in triplicate.

2.7 Statistical Analyses

Results are presented as mean ± standard deviation (SD). Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism version 10.

3 Results and Discussion

3.1 GC-MS Analysis

The chemical constituents of the petroleum ether extracts and their relative abundances, as determined by GC–MS analysis, are presented in Table 1.

F. imperialis and *F. pinardii* exhibited similar fatty acid methyl ester profiles, with 9,12-Octadecadienoic acid (Z,Z) being the major constituent in both species (39.51% and 32.27%, respectively). Other major components common to both species were hexadecanoic acid and 11-octadecenoic acid. These results indicate a shared lipid profile with minor species-specific variations.

3.2 Antimicrobial Activity

The petroleum ether and diethyl ether extracts of *F. imperialis* and *F. pinardii* showed generally weak antibacterial activity. Limited inhibition was observed only against *Staphylococcus aureus* ATCC 29213 and MRSA ATCC 43300, while no activity was detected against the tested Gram-negative bacteria. As expected, the inhibition zones produced by the extracts were markedly lower than those of the reference antibiotics, ceftriaxone and piperacillin–tazobactam.

In contrast, the extracts exhibited more pronounced antifungal activity, particularly against *Candida* species. The

Table 1. Chemical composition of the petroleum ether extracts of *F. imperialis* and *F. pinardii* determined by GC–MS analysis

No	Retention time	Compund name	Formula	Molecular weight (g/mol)	*F. imperialis* (Area %)	*F. pinardii* (Area %)
1	7.53	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	216.28	1.17	1.32
2	7.83	2-Octanol, 8,8-dimethoxy-	$C_{10}H_{22}O_3$	190.28	1.33	–
3	8.75	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242.40	–	2.08
4	10.66	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	14.56	22.16
5	11.40	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.48	4.16	1.95
6	12.77	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	39.51	32.27
7	12.82	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	17.38	15.43
8	13.06	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.50	9.87	8.70
9	13.60	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	$C_{20}H_{38}O_2$	310.52	2.30	–
10	15.97	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.56	5.85	4.83
11	20.79	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.61	–	6.34

Table 2. Antimicrobial activities of *F. imperialis* and *F. pinardii* extracts against tested microbial strains

Microorganism	FiP	FiD	FpP	FpD	Control
S. aureus ATCC 29213	10	0	0	0	30 (C1*)/33 (C2**)
MRSA ATCC 43300	0	10	10	0	25 (C1*)/35 (C2**)
K. pneumoniae ATCC 700603	0	0	0	0	20 (C1*)/25 (C2**)
E. coli ATCC 259212	0	0	0	0	18 (C1*)/32 (C2**)
A. baumannii ATCC 19606	0	0	0	0	25 (C1*)/30 (C2**)
A. fumigatus ATCC 204305	12	13	9	0	33 (C3***)
A. fumigatus (Clinic)	0	0	0	12	40 (C3***)
C. albicans ATCC 10231	15	0	0	0	15 (C3***)
C. albicans (Clinic)	15	20	10	18	10 (C3***)
C. tropicalis ATCC 750	25	30	10	30	40 (C3***)
C. parapsilosis ATCC 22019	10	12	20	20	30 (C3***)

strongest effects were observed against *C. tropicalis* ATCC 750, with *F. imperialis* diethyl ether extract (FiD) and *F. pinardii* diethyl ether extract (FpD) producing inhibition zones of 30 mm. In addition, *F. imperialis* diethyl ether extract (FiD) showed notable activity against the clinical isolate of *C. albicans* (20 mm), exceeding the activity of the reference antifungal agent voriconazole. Overall, the results indicate that the non-polar extracts of both *Fritillaria* species possess greater antifungal than antibacterial potential (Table 2).

The pronounced antifungal activity observed in the lipophilic extracts may be attributed to their enrichment in non-polar constituents, particularly fatty acids, fatty acid esters, terpenoids, and other hydrophobic metabolites. These compounds are known to interact readily with fungal cell membranes, leading to alterations in membrane permeability, disruption of ergosterol-associated functions, and ultimately inhibition of fungal growth. Previous studies have demonstrated that lipid-soluble phytochemicals can penetrate fungal cell envelopes and induce membrane damage and oxidative stress, thereby contributing to their antifungal effects. Furthermore, several fatty acids, including linoleic and oleic acid derivatives commonly detected in lipophilic plant extracts, have been reported to suppress fungal growth through inhibition of spore germination and disruption of cell wall and membrane integrity. Therefore, the notable antifungal activity of the petroleum ether and diethyl ether extracts may be associated with the presence of these lipophilic bioactive constituents (Guimarães & Venâncio, 2022; López-Arellanes et al., 2025; Mohamed, 2026).

3.3 Cytotoxic Activities

All extracts showed low cytotoxicity toward J774 murine macrophage cells over the tested concentration range. The mean cell viability values remained well above the 80% viability threshold at all concentrations. At 80 µg/mL, the viability values were 94.78 ± 1.43% for FiP, 97.27 ± 1.46% for FiD, 96.72 ± 1.74% for FpP, and 97.80 ± 2.64% for FpD. Similarly, at the highest tested concentration of 100 µg/mL, the viability values were 94.55 ± 2.26% for FiP, 96.06 ± 1.59% for FiD, 96.13 ± 2.30% for FpP, and 97.45 ± 0.67% for FpD. Among

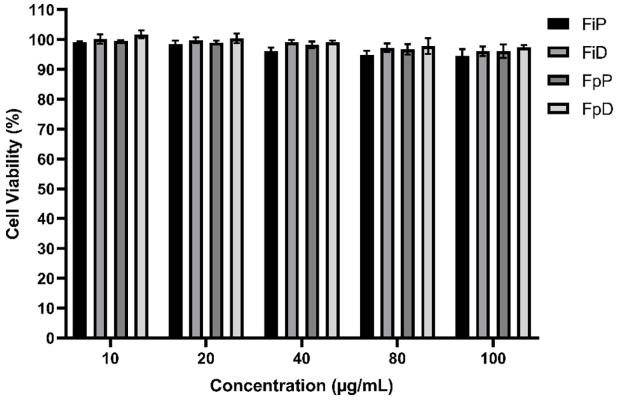


Figure 1. Effects of *F. imperialis* and *F. pinardii* petroleum ether and diethyl ether extracts on J774 murine macrophage cell viability after 48 h exposure. Data are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. *p < 0.05 was considered statistically significant

the tested extracts, FiP produced the lowest mean viability value at 100 µg/mL; however, none of the extracts reduced J774 cell viability below 80%. These findings indicate that the lipophilic extracts were well tolerated by J774 murine macrophage cells and did not exhibit marked cytotoxicity under the experimental conditions (Figure 1).

In contrast to their low cytotoxicity toward J774 murine macrophage cells, the extracts reduced the MTT-derived viability of HT-29 colorectal cancer cells. All extracts decreased HT-29 cell viability below 80% even at the lowest tested concentration of 10 µg/mL. At this concentration, cell viability values were 63.87 ± 2.64% for FiP, 73.31 ± 2.20% for FiD, 70.36 ± 1.85% for FpP, and 69.37 ± 3.64% for FpD. The strongest reduction in HT-29 cell viability was observed at 80 µg/mL, particularly for FpP, which decreased cell viability to 47.20 ± 3.22%. At the same concentration, the viability values were 57.44 ± 1.10% for FiP, 61.62 ± 2.21% for FiD, and 58.17 ± 2.27% for FpD ($p < 0.05$). Since FiP, FiD, and FpD did not reduce cell viability below 50% within the tested concentration range, their IC_{50} values were considered

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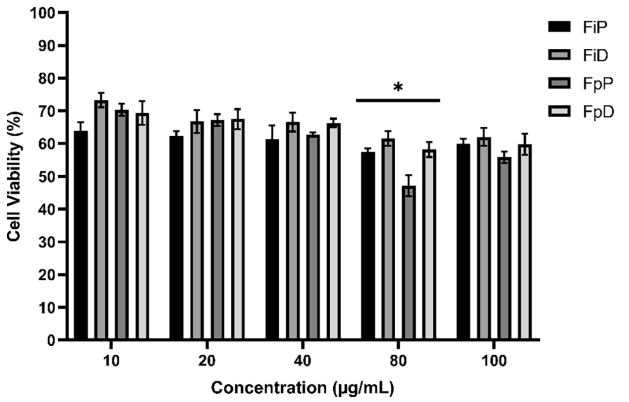


Figure 2. Effects of *F. imperialis* and *F. pinardii* petroleum ether and diethyl ether extracts on HT-29 colorectal cancer cell viability after 48 h exposure. Data are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. *p < 0.05 was considered statistically significant

to be higher than 100 µg/mL. Although FpP reduced cell viability below 50% at 80 µg/mL, a formal IC_{50} value was not calculated because the dose–response pattern was not strictly concentration-dependent. Overall, FpP showed the most pronounced inhibitory effect on HT-29 cell viability, with the lowest cell viability value observed at 80 µg/mL (Figure 2).

The reduction in HT-29 cell viability observed in the MTT assay may be associated with the presence of fatty acid derivatives and other hydrophobic constituents capable of affecting cellular metabolic activity and membrane-associated processes. Previous studies have reported that fatty acids and their derivatives may suppress cancer cell proliferation and reduce cell viability in different tumor cell lines, while lipophilic diterpenes such as phytol have also been associated with antiproliferative effects (Pejin et al., 2014; Pacheco et al., 2018). Therefore, the decrease in HT-29 cell viability observed for the petroleum ether and diethyl ether extracts may be related to the combined effects of these lipophilic metabolites. However, since no mechanistic assays were performed in the present study, the contribution of these constituents to the observed cytotoxic effects should be interpreted cautiously. Evaluation of additional cancer and non-cancerous cell lines in future studies would provide a more comprehensive understanding of the cytotoxic profile of these extracts.

3.4 Anti-Inflammatory Activity

The anti-inflammatory activities of *F. imperialis* and *F. pinardii* petroleum ether and diethyl ether extracts were evaluated using the 5-LOX inhibition assay (Table 3). Among the tested samples, the petroleum ether extract of *F. imperialis* (FiP) exhibited the strongest inhibitory activity, with an IC_{50} value of 4.215 ± 1.26 µg/mL. Similarly, the petroleum ether extract of *F. pinardii* (FpP) showed potent activity (IC_{50} = 10.61 ± 8.5 µg/mL). Both petroleum ether extracts were assigned to the same statistical group as the reference drug indomethacin (IC_{50} = 21.04 ± 0.48 µg/mL), indicating no significant difference in their inhibitory effects ($p > 0.05$). In contrast, the diethyl ether extracts displayed lower activity, with FiD and FpD exhibiting IC_{50} values of 50.44 ± 2.95 and 108.6 ± 2.82 µg/mL, respectively.

The potent 5-lipoxygenase inhibitory activity observed in the petroleum ether extracts of *F. imperialis* and *F. pinardii* may be attributed to the enrichment of lipophilic constituents such as fatty acid derivatives, terpenoids, and phytosterols. Lipophilic plant metabolites have been reported to suppress the lipoxygenase pathway and reduce leukotriene biosynthesis, thereby contributing to anti-inflammatory activity (Breu et al., 1992; Schneider & Bucar, 2005). The strong inhibition observed for both petroleum ether extracts, which exceeded that of the reference compound indomethacin, suggests that non-polar constituents play a significant role in the anti-inflammatory potential of these extracts.

Several biological effects attributed to Fritillaria species have been linked to their rich content of steroidal alkaloids. Among these, isosteroidal alkaloids such as peimine, peiminine, imperialine, verticine, and verticinone have been reported to possess a wide range of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, cytotoxic, and neuroprotective properties. In addition, these compounds have been shown to modulate key biological processes, such as apoptosis, inflammatory signaling pathways, and cell cycle progression (Hao et al., 2013; Shang et al., 2018; Qu et al., 2022; Kumar et al., 2025). However, the findings of the present study suggest that the biological potential of *Fritillaria* species cannot be attributed solely to their alkaloid content. The significant activities observed in the petroleum ether and diethyl ether extracts indicate that lipophilic constituents may also contribute substantially to the overall bioactivity profile of these species. Therefore, non-polar metabolites should be considered alongside steroidal alkaloids as important contributors to the pharmacological properties of *Fritillaria*.

Table 3. Anti-inflammatory (Anti-5-LOX) activities of *F. imperialis* and *F. pinardii* extracts expressed as IC_{50} values (µg/mL)

Sample	FiP	FİD	FpP	FpD	Indomethacin
IC_{50}	4.215 ± 1.26[a]	50.44 ± 2.95[a,b]	10.61 ± 8.5[a]	108.6 ± 2.82[b]	21.04 ± 0.48[a]

Note: *Different superscripts indicate statistically significant differences between groups ($p < 0.05$). There is no statistically significant difference between values sharing at least one common superscript ($p > 0.05$).

4 Conclusion

This study investigated the antimicrobial, cytotoxic, and anti-inflammatory activities of lipophilic extracts obtained from the bulbs of *F. imperialis* and *F. pinardii*. To the best of our knowledge, this is the first report evaluating the biological activities of the petroleum ether and diethyl ether extracts of these species. The results revealed antifungal activity, reduced HT-29 colorectal cancer cell viability, and 5-lipoxygenase inhibitory activity, suggesting that lipophilic constituents may contribute to the observed biological effects of both species. These findings further suggest that, in addition to the well-known steroidal alkaloids, non-polar metabolites may also contribute to the pharmacological properties of Fritillaria species. Overall, the results indicate that the lipophilic extracts of *F. imperialis* and *F. pinardii* contribute to the biological activity profile of these species; however, further phytochemical, mechanistic, and pharmacological studies are required to identify the active constituents and clarify their biological relevance.

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Author Contributions

Carried out experimental studies: OE; GC-MS studies: GOAT, FD; Antimicrobial activity: DD, OE; Cytotoxic aktivity: MI, OE; Anti-inflammatory activity: FGY, OE; Design of the study: GT; Writing and reading the draft manuscipt: OE, GT.

Availability of Data and Materials

The authors declare that should any raw data files be needed about the further data of the study, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

ORCID

Onur Esen: 0000-0001-8754-0293
Gülbahar Özge Alim Toraman: 0000-0002-7816-7551
Murat Ihlamur: 0000-0002-0458-5638
Fatmanur Gürbüzkol Yılmaz: 0009-0005-0404-4646
Derya Doğanay: 0000-0001-9147-4110
Firdevs Demirel: 0009-0006-2323-4936
Gülaçtı Topçu: 0000-0002-7946-6545

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