Journal of Research in Pharmacy

Research Article

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Phenolic Profile, Antimicrobial Activity, and *In Vivo* Effects of *Inula montbretiana* DC. in the *Galleria mellonella* Model

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Received: 6 June 2026 / Revised: 15 September 2026 / Accepted: 30 October 2026

ABSTRACT: This study aimed to evaluate the *in vitro* antimicrobial activities of chloroform (IMC), ethyl acetate (IMEA), and ethanol (IME) extracts obtained from the aerial parts of *Inula montbretiana* DC. by percolation. Antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae,* and *Candida albicans* was assessed using agar well diffusion and microdilution methods. The *in vivo* effects of the extracts were investigated in *Galleria mellonella* larvae infected with *C. albicans, S. epidermidis,* or *K. pneumoniae*. In the microdilution assay, IMEA exhibited the broadest antimicrobial spectrum, with the lowest minimum inhibitory concentration (MIC) against *C. albicans* (0.11 mg/mL), followed by *K. pneumoniae* (0.21 mg/mL), whereas IME showed activity only against *C. albicans*. In the agar well diffusion assay, IMEA produced an inhibition zone of 12.31 ± 0.16 mm against *S. epidermidis* and that of IME was comparatively stronger against *K. pneumoniae*, with an inhibition zone of 14.43 ± 0.25 mm. In the infection model, IMC at 456 µg/mL and IMEA at 2120 µg/mL significantly improved survival in *C. albicans*-infected larvae, with approximately 40% survival at 120 h. IMC at 456 µg/mL also significantly improved survival in *S. epidermidis*-infected larvae, with approximately 40% survival at 120 h. The extracts showed limited efficacy against *K. pneumoniae*. HPLC-DAD analysis of IMEA and IME identified phenolic acids, including 4-hydroxybenzoic, chlorogenic, and caffeic acids, and flavonoids, including luteolin and apigenin. These findings demonstrate solvent-dependent antimicrobial activity and suggest protective effects of selected extracts in the *G. mellonella* infection model.

KEYWORDS: *I. montbretiana*; HPLC-DAD; phenolic compounds; antimicrobial activity; *Galleria mellonella*.

1. INTRODUCTION

Antimicrobial resistance (AMR) refers to the reduced effectiveness of treatments against microbial pathogens and is a major global public health threat. Multidrug resistance has become a global concern owing to the emergence and rapid dissemination of resistant pathogens. According to the World Health Organization (WHO), AMR is associated with substantial mortality worldwide and could cause up to 10 million deaths annually by 2050 [1]. Alternative therapeutic strategies, including antibiotic-adjuvant combinations, bacteriophage therapy, antimicrobial peptides, and phytochemicals, are being explored because of the limited development of new antibiotics in recent years [2]. Invertebrate models have gained increasing importance in biomedical research. *Galleria mellonella* larvae are widely used for *in vivo* studies because of their cost-effectiveness, short life cycle, and suitability for high-throughput screening. Additionally, the absence of strict ethical constraints enhances their applicability as complementary experimental models [3].

Plant extracts have attracted considerable attention in the context of controlling emerging antibacterial resistance. Phytobioactive compounds are plant secondary metabolites that are abundantly present in medicinal plants and have notable therapeutic potential [4]. The genus *Inula* (Asteraceae) comprises over 100 species distributed across Africa, Asia, and Europe, particularly in the Mediterranean region. This genus is widely used in various traditional medicine systems, especially in Traditional Chinese Medicine. The aerial

How to cite this article: Dikpınar T, Kasarcı B, Taşkın T, Damar-Çelik D, Rayaman P, Şenkardeş İ. Phenolic profile, antimicrobial activity, and *in vivo* effects of *Inula montbretiana* DC. in the *Galleria mellonella* model. J Res Pharm. 2026; 30(6): 1543-1552.

© 2026 Marmara University Press
ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1965335

parts and roots of *Inula* species have been used to treat various ailments, including rheumatoid arthritis, gastrointestinal disorders, respiratory tract infections, malaria, dysentery, and hepatitis. In addition, these species possess pharmacological activities such as anthelmintic, antiseptic, expectorant, diuretic, and hypotensive effects. Phytochemical investigations have shown that *Inula* species are rich in monoterpenes, sesquiterpenes, diterpenes, sesquiterpene lactones, flavonoids, and various phenolic compounds [5].

The available literature on *Inula montbretiana* remains limited, particularly regarding the combined evaluation of the antimicrobial activities of extracts with different polarities obtained via percolation, their *in vivo* effects in the *Galleria mellonella* infection model, and their phenolic profiles. Therefore, this study aimed to investigate the *in vitro* antimicrobial activity of chloroform, ethyl acetate, and ethanol extracts obtained from the aerial parts of *I. montbretiana* using agar well diffusion and broth microdilution assays. Their *in vivo* effects were further evaluated in the *G. mellonella* infection model, and the phenolic profiles of the ethyl acetate and ethanol extracts were characterized using HPLC-DAD.

2. RESULTS

2.1. Antimicrobial activity

2.1.1. The agar well diffusion method

The findings indicated that extracts exhibited more pronounced antimicrobial activity against Gram-positive bacteria than Gram-negative bacteria (**Table 1**). Among all tested bacteria, IMEA extract showed the highest activity, producing an inhibition zone of 12.31 ± 0.16 mm against *S. epidermidis*. In addition, IMEA demonstrated moderate antimicrobial activity against *P. aeruginosa*, *E. coli*, and *K. pneumoniae*. IMC was particularly effective against *S. epidermidis* and *K. pneumoniae*, whereas no inhibitory effect was observed against *P. aeruginosa* or *P. vulgaris*. IME extract exhibited activity only against *K. pneumoniae* and *C. albicans*, showing a comparatively stronger inhibitory effect against *K. pneumoniae* than the other extracts. All extracts demonstrated moderate antifungal activity against *C. albicans*.

Table 1. Inhibition zones of *I. montbretiana*

Extract& Control	Microorganisms						
	S. aureus ATCC 25923	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*S. epidermidis* ATCC 12228	*K. pneumoniae* ATCC 4352	*P. vulgaris* ATCC 13315	*C. albicans* ATCC 90028
IMC	10.51±0.34	-	8.06±0.05	11.82±0.09	11.73±0.08	-	10.11±0.09
IMEA	10.84±0.67	11.71±0.43	11.23±0.18	12.31±0.16	11.81±0.64	-	9.01±0.07
IME	-	-	-	-	14.43±0.25	-	8.72±0.66
*	31.72±0.51	28.22±0.10	30.13±0.41	42.41±0.27	28.62±0.83	18.44±0.16	-
**	-	-	-	-	-	-	22.41±0.38

The results are presented in mm.
(-): Not observed.
*: Meropenem 10 µg/mL, **: Amphotericin B 100 µg/mL

2.1.2. Minimum inhibitory concentration (MIC) method

MIC and MBC (Mininum bactericidal concentration) results presented in **Supplemantary Table 1** indicate that the antimicrobial activity of *I. montbretiana* extracts varies considerably depending on the extraction solvent. IMEA exhibited the broadest spectrum and the strongest antimicrobial activity among all tested extracts except *P. vulgaris*. In particular, it showed low inhibitory values against *C. albicans* and *K. pneumoniae*, indicating a high antimicrobial potential. IMC exhibited a more limited activity profile, showing notably low MIC and MBC values against *S. epidermidis*. IME showed activity only against *C. albicans*, indicating a selective antifungal effect. Overall, *I. montbretiana* extracts were particularly effective against

Gram-positive bacteria and *C. albicans*, and IMEA was the most active extract in terms of both antimicrobial spectrum and activity level.

2.2. *In vivo G. mellonella* larvae model

2.2.1. Survival analysis of G. mellonella larvae infected with bacterial and fungal pathogens

Survival analysis indicated that the pathogenicity of the microorganisms in *G. mellonella* larvae was dependent on the inoculum. For *C. albicans*, McFarland 0.5 suspensions caused no mortality, whereas McFarland 1.0 resulted in approximately 20% mortality within 24 h of incubation. In contrast, McFarland 2.0 caused complete larval mortality within 24 h of infection. For *K. pneumoniae*, suspensions prepared at 1×10^7 CFU/mL did not cause mortality. McFarland 2.0 suspensions resulted in complete mortality within 24 h, whereas McFarland 1.0 caused approximately 90% mortality within 24 h and complete mortality by 72 h. McFarland 0.5 suspensions caused approximately 50% mortality within 24 h. For *S. epidermidis*, McFarland 0.5 suspensions caused no mortality, whereas McFarland 1.0 and 2.0 caused approximately 10% and 20% mortality, respectively. Significant differences in survival rates were observed between the selected inoculum and the other tested inoculum levels for each microorganism (**Figure 1**). Based on these findings, McFarland 2.0 for *C. albicans*, McFarland 3.0 for *S. epidermidis*, and McFarland 1.0 for *K. pneumoniae* were selected for the subsequent infection experiments.

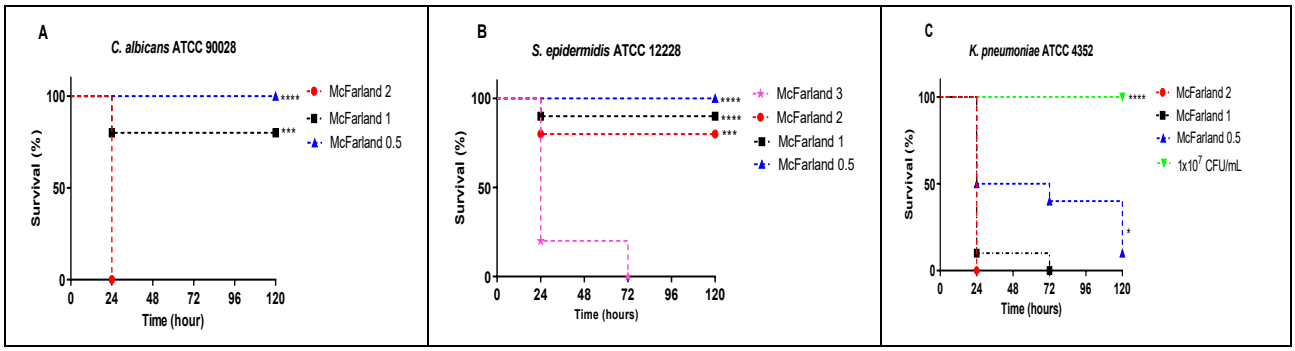

Figure 1. Ka**plan–Meier survival curves of** *G. mellonella* **larvae following infection with different microbial inocula.** Ten larvae were used for each inoculum group (n = 10). Larvae were injected with 10 µL microbial suspensions adjusted to different McFarland standards or CFU concentrations and monitored at 24, 48, 72, 96, and 120 h post-infection. Based on the survival profiles, McFarland 2.0 for *C. albicans*, McFarland 3.0 for *S. epidermidis*, and McFarland 1.0 for *K. pneumoniae* were selected for subsequent infection experiments. Pairwise survival comparisons were performed between the selected inoculum for each microorganism and the other tested inoculum levels using the log-rank (Mantel–Cox) test. **(A)** *C. albicans* ATCC 90028, ***p < 0.001 and ****p < 0.0001 vs McFarland 2.0. **(B)** *S. epidermidis* ATCC 12228, ***p < 0.001 and ****p < 0.0001 vs McFarland 3.0. **(C)** *K. pneumoniae* ATCC 4352, *p < 0.05 and ****p < 0.0001 vs McFarland 1.0.

2.2.2. Effects of the extracts on G. mellonella infected with bacterial and fungal pathogens

The concentrations used in the infection experiments were selected based on a preliminary toxicity analysis performed in *G. mellonella* larvae (**Supplementary Figure 1 and 2**). The survival rates of *G. mellonella* larvae infected with *C. albicans*, *S. epidermidis*, and *K. pneumoniae* varied depending on the type and concentration of the extracts used. IMC at 456 µg/mL and IMEA at 2120 µg/mL showed the highest larval survival against *C. albicans* infection, maintaining approximately 40% survival at 120 h post-treatment. Both treatments significantly improved survival compared with the infection control ($p < 0.01$ and $p < 0.05$, respectively), whereas IME and the lower concentrations of IMC and IMEA showed no significant improvement in survival. Against *S. epidermidis* infection, IMC at 456 µg/mL maintained the highest observed larval survival, with approximately 40% survival at 120 h, and significantly improved survival compared with the infection control ($p < 0.05$). IMEA showed limited protective effects, whereas low-dose IMC treatment resulted in complete mortality by 72 h post-treatment. For *K. pneumoniae* ATCC 4352, both ethyl acetate concentrations resulted in approximately 50% larval survival at 24 h, but complete mortality occurred by 48 h, with no significant difference from the infection control (**Figure 2**).

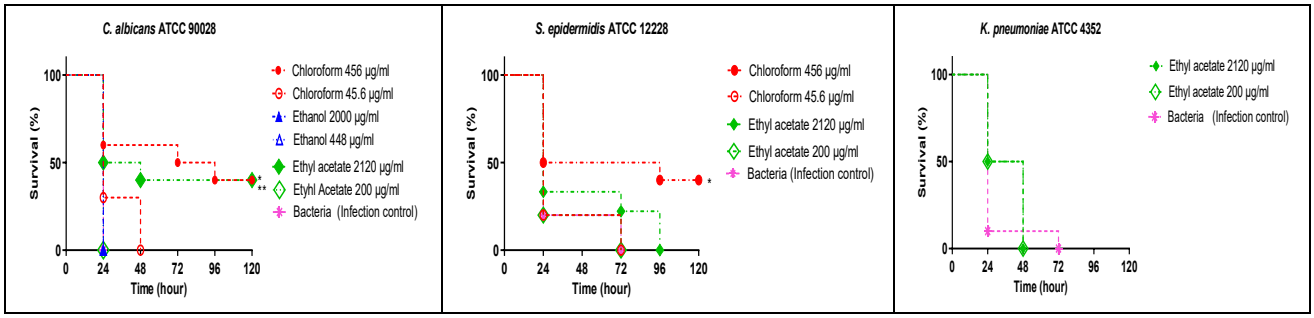

Figure 2. Kaplan–Meier survival curves of *G. mellonella* **larvae infected with** *C. albicans*, *S. epidermidis*, **and** *K. pneumoniae* **following treatment with** *I. montbretiana* **extracts.** Ten larvae were used for each treatment group (n = 10). **(A)** *C. albicans* ATCC 90028, **(B)** *S. epidermidis* ATCC 12228, and **(C)** *K. pneumoniae* ATCC 4352. Survival curves of the treatment groups were compared with the corresponding infection control using the log-rank (Mantel–Cox) test. *p < 0.05 and **p < 0.01 vs infection control.

2.3. Phytochemical content analysis by HPLC-DAD

Table 2 presents the distribution and amounts of the identified phenolic and flavonoid compounds in IME and IMEA, and their chemical structures are illustrated in **Figure 3**. The results indicate that the extraction solvent significantly influences the chemical profile of the constituents. Chlorogenic acid was identified as the predominant compound in IME, followed by lower levels of caffeic acid and luteolin. These findings suggest that IME is relatively rich in phenolic acids, whereas its flavonoid content is limited. In contrast, a different chemical profile was observed for IMEA. Chlorogenic acid was not detected, whereas 4-OH benzoic acid, caffeic acid, luteolin, and apigenin were detected. The results indicate that the extraction solvent may influence the chemical composition of the extracts, as differences were observed in the concentrations of the identified phenolic and flavonoid compounds.

Table 2. Concentrations of identified phenolic and flavonoid compounds in IME and IMEA extracts

	Retention time (min)	Wavelength λ (nm)	IME (µg/mL ± SD)	IMEA (µg/mL ± SD)
4-OH Benzoic acid	25.532	330	-	0.99±0.03
Chlorogenic acid	27.781	330	11.00±1.40	-
Caffeic acid	30.264	330	4.75±0.01	4.94±0.02
Luteolin	47.482	330	2.72±0.13	6.59±0.05
Apigenin	49.803	330	-	3.79±0.07

(-): Not detected
SD: Standard deviation

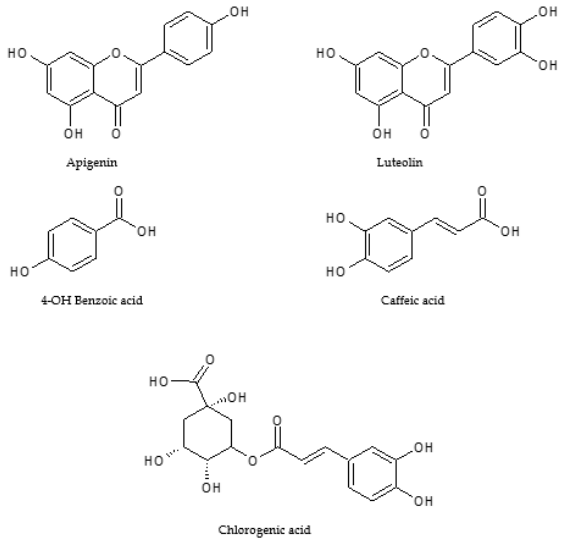

Figure 3. Chemical structures of the phenolic and flavonoid compounds identified in IME and IMEA

3. DISCUSSION

This study investigated the antimicrobial activities, *in vivo* effects using a *G. mellonella* infection model, and phenolic content profiles of *I. montbretiana* extracts. IMEA showed the highest inhibition zone diameters against *S. aureus* and *S. epidermidis*, whereas IME showed the highest inhibition zone diameter against *K. pneumoniae*. Although IMC exhibited pronounced activity against *C. albicans*, its antibacterial activity was limited. In the agar well diffusion assay, IME was effective only against *K. pneumoniae* and *C. albicans*, indicating a more restricted antimicrobial profile. These findings suggest that differences in chemical composition may contribute to the variation in biological activities among the extracts.

HPLC-DAD analysis revealed differences in the phytochemical profiles of the extracts obtained using different solvents. The chemical profile of IME was characterized by higher levels of polar phenolic acids, whereas IMEA contained higher levels of flavonoid aglycones, consistent with its higher flavonoid content. Such variations in the distribution of compounds not only reflect differences in chemical composition but may also account for the observed differences in biological activities. The results of *G. mellonella* model demonstrated that extracts did not exhibit significant toxicity at low and moderate doses; however, increased mortality was observed at high doses. The partial protective effects of IMEA and IMC against *C. albicans* and *S. epidermidis* infections in infected larvae support the *in vivo* potential of these extracts.

In the literature, methanol and ethyl acetate extracts of *I. viscosa* showed the highest antifungal and antibiofilm activities, with an MIC of 64 µg/mL against *C. albicans* and up to 90% biofilm inhibition at 512 µg/mL, whereas chloroform extracts exhibited lower activity. In the *G. mellonella* infection model, extracts administered at 25, 12.5, and 6.25 µg/larva caused no mortality in uninfected larvae. While all untreated infected larvae died within 5 days, survival rates in extract-treated larvae ranged from 60% to 80%. HPLC-DAD-ESI/MS analysis identified caffeic acid, galloylquinic acid, rosmarinic acid, two di-*O*-caffeoylquinic acid isomers, and flavonoids such as quercetin, luteolin, naringin, and apigenin as major constituents [6]. These findings highlight the influence of extraction solvents on biological activity, consistent with the differences in antimicrobial activity observed among the *I. montbretiana* extracts in our study. HPLC-MS/MS analysis of *I. oculus-christi* revealed 5-caffeoylquinic acid, 1,5-dicaffeoylquinic acid, luteolin/kaempferol, apigenin, and rutin as the main constituents. The extracts were tested against *C. utilis*, and the lowest MIC value (0.03 mg/mL) was reported for the 5% infusion and ethyl acetate extracts [7].

LC-MS analysis revealed that chlorogenic acid (2284.1 ± 11 µg/g) and caffeic acid (234.0 ± 2.1 µg/g) were the major phenolic constituents of the *I. helenium* root ethanol extract. In addition, several flavonoids, including chrysin, luteolin, luteolin-7-*O*-glucoside, naringenin, and hesperetin, were detected in the extract. The antimicrobial activity results demonstrated that the extract exhibited activity against all tested pathogenic microorganisms. The antimicrobial effect was more pronounced against Gram-positive bacteria, with the highest inhibition zone diameters observed for *Bacillus cereus* (18.00 mm) and *S. aureus* (16.33 mm). Furthermore, the calculated MBC/MIC ratios of ≤4 supported the bactericidal activity of the extract against the tested bacterial strains [8]. Chlorogenic acid (3.5 mg/g extract) was identified as a major compound in *I. graveolens* leaves using LC-MS/MS. In addition, salicylic acid (0.055 mg/g extract), caffeic acid (0.054 mg/g extract), protocatechuic acid (0.026 mg/g extract), hesperidin (0.016 mg/g extract), and rutin (0.0160 mg/g extract) were detected in methanol extract [9].

In previous studies, *I. montbretiana* has been investigated for its phytochemical composition and diverse biological activities. Methanolic extracts were reported to contain a total phenolic content of 190.9 mg GAE/g in roots and 119.4 mg GAE/g in aerial parts [10]. Similarly, ethanol extracts have been reported to exhibit a total phenolic content of 46.15 µg GAE/mL [11]. From the flowering aerial parts, 9β-hydroxyparthenolide-type sesquiterpene lactones were isolated, some of which were identified as novel compounds. The dichloromethane extract exhibited strong antiprotozoal activity against *Trypanosoma brucei rhodesiense* (IC_{50}: 3.38 µg/mL) [12]. In addition, methanol flower extract exhibited antimicrobial activity against *E. faecalis* and *C. tropicalis* (MIC: 50 µg/mL) [10]. Furthermore, the ethanol extract demonstrated strong urease-inhibitory activity, with a lower IC_{50} value compared to thiourea, indicating potent enzymatic inhibition [11]. Regarding antioxidant capacity, root extracts exhibited higher activity than those of flowers and leaves, and CUPRAC analysis confirmed an increase in reducing capacity with increasing concentration [10, 11].

The use of *G. mellonella* as an alternative *in vivo* model has gained popularity because of its low cost, easy handling, and innate immune system that has functional similarities to the mammalian innate immune response [2]. The current study found that IMC at a concentration of 456 µg/mL and IMEA at 2120 µg/mL provided the highest survival rates for larvae infected with *C. albicans*. Both treatments significantly enhanced

survival compared to the infection control group. Additionally, IMC at 456 µg/mL also significantly improved larval survival against *S. epidermidis* infection. Similar to the findings of this study, previous research using the *G. mellonella* infection model has shown that antimicrobial compounds can delay larval mortality and moderately enhance survival owing to their antimicrobial properties [13, 14, 15]. Additionally, the antifungal activity of *Rosmarinus officinalis* extracts has been evaluated using the *G. mellonella* model, where therapeutic administration following infection prolonged larval survival [14, 16].

These findings indicate that the phytochemical profiles and biological activities of *Inula* species vary depending on the extraction solvent and the morphological part of the plant used. However, this study has some limitations. The biological activities were evaluated using *in vitro* assays and the *G. mellonella* infection model. Therefore, further studies using mammalian models are needed to confirm the observed effects and clarify the underlying mechanisms of action.

4. CONCLUSION

This study demonstrates that *I. montbretiana* exhibits varying *in vitro* antimicrobial activities against *C. albicans* and relevant bacterial strains. These effects were further supported by *in vivo* evaluation using *G. mellonella* infection model, where selected extracts showed partial improvement in larval survival. Phytochemical profiling by HPLC-DAD revealed differences in phenolic composition among the extracts, which may contribute to their varying biological activities. However, the findings are limited to *in vitro* assays and the *G. mellonella* model and require further validation in mammalian models. Overall, *I. montbretiana* represents a promising natural source of compounds with antimicrobial potential. Further studies focusing on bioassay-guided fractionation and mechanistic investigations are warranted to better elucidate the active constituents and their modes of action.

5. MATERIALS AND METHODS

5.1. Plant material

Inula montbretiana DC. was collected in July 2023 from Mersin, Türkiye. It was identified by Dr. İsmail Şenkardeş and deposited at the Herbarium of Faculty of Pharmacy, Marmara University (MARE No: 23091). The aerial parts of the plant were powdered.

5.2. Extraction

Powdered aerial parts (50 g) were subjected to sequential extraction in a percolator using 750 mL of chloroform, ethyl acetate, and ethanol, respectively. Each solvent was percolated until it became colorless. First, the obtained extracts were treated with anhydrous Na_2SO_4 and then concentrated using a rotary evaporator under reduced pressure at temperatures not exceeding 50 °C. The crude extracts were weighed, and their percent yields were calculated (**Table 3**). They were used for antimicrobial activity assays and chemical content analyses.

Table 3. Amounts and yields of extracts

Plant	Solvent	Extract code	Amount (g)	% Yield
Inula montbretiana	Chloroform	IMC	1.58	3.16
	Ethyl acetate	IMEA	0.26	0.52
	Ethanol	IME	0.77	1.54

5.3. Antimicrobial activity

The antimicrobial activity of the extracts was initially determined using the agar well diffusion assay. Subsequently, the minimum inhibitory concentration (MIC) was determined for the extracts exhibiting antimicrobial activity in the agar well-diffusion assay. The bacterial strains *Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, and *Klebsiella pneumoniae* ATCC 4352, and the yeast strain *Candida albicans* ATCC 90028 were used in this study.

5.3.1. Agar well diffusion method

Microbial suspensions were prepared in 0.85% physiological saline solution (PSS) from colonies grown on solid culture media. Bacterial suspensions were adjusted to a concentration of 10^8 CFU/mL and

yeast suspensions to 10^6 CFU/mL according to the McFarland 0.5 turbidity standard. From these suspensions, inoculation was performed onto Mueller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for yeast using sterile swabs to obtain a uniform surface distribution of the inoculum. Wells with a diameter of 5 mm were aseptically prepared on agar surfaces using a sterile punch. A volume of 50 µL of each extract, dissolved in its respective solvent, was added to the wells. In addition, meropenem (10 µg/well) was used as a positive control for bacteria and amphotericin B (100 µg/well) for yeast, while the solvent and physiological saline solution were used as negative controls. The Petri dishes were incubated at 37 °C for 18-24 h for bacterial growth and at 35 °C for 24-48 h for yeast growth. At the end of the incubation period, the zones of growth inhibition were measured in millimeters. All experiments were performed in triplicate, and the mean values were calculated [17, 18].

5.3.2. MIC determination for bacteria

Bacterial suspensions were prepared from 24 h bacterial culture colonies and adjusted to the McFarland 0.5 turbidity standard in accordance with the Clinical and Laboratory Standards Institute standards. The suspensions were diluted to obtain a final inoculum concentration of 5×10^5 CFU/mL. A volume of 100 µL of cation-adjusted Mueller-Hinton broth (CAMHB) was dispensed into sterile U-bottom microdilution plates. Subsequently, 100 µL of the dissolved extract was added to the first well, followed by serial dilutions. Thereafter, 5 µL of bacterial suspension was added to the wells containing the extracts and the plates were incubated at 37 °C for 24 h. At the end of the incubation period, the lowest concentrations of samples showing no visible growth were determined as MIC. Growth controls of all bacteria and a CAMHB medium control were included in the assays. Solvent controls were also tested under the same conditions. CAMHB and solvents were used as negative controls, and meropenem was used as a positive control [19].

5.3.3. MIC determination for yeast

In the present study, 100 µl of RPMI-1640 medium was placed in each well of the U-based microdilution plate. Dilutions of the tested extracts were prepared in RPMI-1640 medium in U-bottom microdilution plates. *C. albicans* ATCC 90028 was inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37 °C for 24 h. Subsequently, yeast suspension was prepared in RPMI-1640 and adjusted to the McFarland 0.5 (1×10^6 -5 $\times10^6$ yeast/mL) turbidity standard. Suspensions were further diluted with RPMI-1640 to obtain a final concentration of 5×10^2–2.5×10^3 yeast cells/mL, and 100 µL of yeast suspension was added to the wells containing the extracts. The prepared plates were incubated at 37 °C for 24-48 h. The plates were evaluated and reincubated for 24 h, followed by a second evaluation at 48 h. The lowest concentration at which no visible growth was observed was considered the MIC. Growth controls for *C. albicans* and RPMI-1640 were included. RPMI-1640, solvents, and amphotericin B were used as negative and positive controls, respectively [20, 21, 22].

5.3.4. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) determination

In the present study, MBC and MFC values were determined from the MIC assay wells that had no visible microbial growth. Here 5 µL aliquot from each selected well was inoculated onto the surface of MHA plates for bacteria and SDA plates for yeast. Then the bacterial plates were incubated at 37 °C for 24 h and the yeast plates were incubated at 37 °C for 48 h. The MBC and MFC were defined as the lowest extract concentrations showing no microbial growth on the corresponding agar plates [23].

5.4. *In vivo G. mellonella* larvae model

In vivo toxicity experiments were conducted using larvae, following the method described by Wijesinghe et al. [24]. The experimental and control groups (n = 10 per group) were kept separately in petri dishes. Larvae exhibiting significant melanization or any noticeable color change, as well as those outside the 0.2–0.3 g body weight range were excluded from the study. The larvae were inoculated with 10 µL of IMEA (concentrations of 2, 20, 200, 2120, and 5000 µg/mL), IME (concentrations of 4, 48, 448, 2000, and 5000 µg/mL), or IMC (concentrations of 4.56, 45.6, 456, 2000, and 5000 µg/mL) via the last left proleg, following surface disinfection with 70% ethanol. The larvae were then transferred to Petri dishes and incubated at 30 °C. Evaluations were performed at 24, 48, 72, 96, and 120 h. All experiments were performed in duplicate.

5.4.1. Survival analysis of G. mellonella larvae infected with bacterial and fungal pathogens

G. mellonella larvae were reared on a natural diet containing honeybee nest residues at 28 °C under dark conditions. Late-stage instar larvae with body weights ranging from 0.2 to 0.3 g were selected for the

experiments. *C. albicans* was cultured on SDA and *S. epidermidis* and *K. pneumoniae* strains were cultured on Tryptic Soy Agar (TSA) at 37 °C. For lethal dose determination, 10 µL of microbial suspensions were injected using an insulin syringe. *C. albicans* suspensions were adjusted to McFarland standards of 0.5, 1.0, and 2.0. *K. pneumoniae* suspensions were prepared at $1x10^7$ CFU/mL and adjusted to McFarland standards of 0.5, 1.0, and 2.0. *S. epidermidis* suspensions were adjusted to McFarland standards of 0.5, 2.0, and 3.0. Larvae were incubated at 37 °C in sterile Petri dishes, and survival was monitored at 24, 48, 72, 96, and 120 h post-infection [25, 14].

5.4.2. Effects of the extracts on G. mellonella infected with bacterial and fungal pathogens

Larvae (n = 10) infected with a 10 µL suspension of *C. albicans* adjusted to a McFarland 2.0 standard through the left proleg using a sterile insulin syringe were incubated at 37 °C for 2 h. Following the incubation period, 10 µL of IMEA (200 and 2120 µg/mL), IME (448 and 2000 µg/mL), and IMC (45.6 and 456 µg/mL) were injected into the right proleg, and the larvae were further incubated at 37 °C. For *S. epidermidis* infection experiments, larvae were inoculated with bacterial suspensions adjusted to a McFarland 3.0 standard and subsequently treated with IMC (45.6 and 456 µg/mL) and IMEA (200 and 2120 µg/mL). For *K. pneumoniae* infection experiments, larvae were inoculated with bacterial suspensions adjusted to a McFarland 1 standard and subsequently treated with IMEA (200 and 2120 µg/mL) [14].

5.5. HPLC-DAD analysis

The phenolic compounds present in the biologically active extracts were analyzed using HPLC-DAD. Analyses were performed using an Agilent 1260 Infinity I system equipped with a ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of 0.1% phosphoric acid solution (pH 2.18) as solvent A and 100% acetonitrile as solvent B. The column temperature was maintained at 40 °C, and the flow rate and injection volume were set at 0.5 mL/min and 10 µL, respectively. The gradient elution program was applied as follows: 1-10 min, 100 % solvent A; 10-20 min, 93 % A + 7 % B; 20-30 min, 88 % A + 12 % B; 30-40 min, 78 % A + 22 % B; 40-45 min, 65 % A + 35 % B; 45-55 min, 45 % A + 55 % B; and 55-60 min, 93 % A + 7 % B. The analysis was terminated after 62 min [26]. The limits of detection (LOD) and quantification (LOQ) of the analytical method were determined based on the calibration data obtained from five replicate measurements (**Table 4**). LOD and LOQ were calculated using the following equations:

LOD= Mean+3 x SD (standard deviation)
LOQ= Mean+10 x SD

Table 4. LOD and LOQ of the analyzed compounds

Compounds	Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)
4-OH Benzoic acid	Y=524.8x+94.91	0.9898	0.71	1.03
Chlorogenic acid	Y=14.7x-519.6	0.9965	0.65	1.56
Caffeic acid	Y=7.359x-446.0	0.9673	1.20	2.52
Luteolin	Y=46.23x+1.017	0.9728	1.11	3.54
Apigenin	Y=9.314x-814.4	0.9683	1.52	3.74

The chemical structures of the identified compounds were drawn using ChemSketch (ACD/Labs) software.

5.6. Statistical analysis

Survival data from experiments conducted with *G. mellonella* were analyzed using Kaplan–Meier survival analysis. Differences between the survival curves were assessed using the log-rank test (Mantel–Cox) in GraphPad Prism version 8.0 (San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

Acknowledgements: This study was supported by TUBITAK, 2209-A (project number: 1919B012335549).

Author contributions: Concept – T.D., B.K.; Design – T.D., B.K., T.T., D.D.Ç., P.R; Supervision – T.D., B.K.; Resources – T.D., B.K., T.T., D.D.Ç., P.R., İ.Ş; Materials – D.D.Ç, P.R.; Data Collection and/or Processing – T.D., B.K.; Analysis and/or Interpretation – T.D., B.K., T.T., D.D.Ç., P.R.; Literature Search – T.D., B.K., T.T., D.D.Ç., P.R.; Writing – T.D., B.K., T.T., D.D.Ç., P.R., İ.Ş.; Critical Reviews – T.D., B.K., T.T., D.D.Ç., P.R., İ.Ş.

Conflict of interest statement: The authors have no conflict of interest to declare within this article.

REFERENCES

[1] Biharee A, Sharma A, Kumar A, Jaitak V. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia. 2020; 146: 104720. https://doi.org/10.1016/j.fitote.2020.104720

[2] Me´nard G, Rouillon A, Cattoir V, Donnio PY. *Galleria mellonella* as a suitable model of bacterial infection: past, present and future. Front Cell Infect Microbiol. 2021; 11: 782733. https://doi.org/10.3389/fcimb.2021.782733

[3] Singkum P, Suwanmanee S, Pumeesat P, Luplertlop N. A powerful *in vivo* alternatıve model in scientific research: *Galleria mellonella*. Acta Microbiol Immunol Hung. 2019; 66 (1): 1-25. https://doi.org/10.1556/030.66.2019.001

[4] Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, Rasool G, Egbuna C, Mtewa AG, Uche CZ, Aslam MA. Phytobioactive compounds as therapeutic agents for human diseases: a review. FSN. 2023; 11(6): 2500-2529. https://doi.org/10.1002/fsn3.3308

[5] Seca AM, Grigore A, Pinto DC, Silva AM. The genus *Inula* and their metabolites: from ethnopharmacological to medicinal uses. J Ethnopharmacol. 2014; 154(2): 286-310. http://dx.doi.org/10.1016/j.jep.2014.04.010

[6] Asraoui F, El Mansouri F, Cacciola F, Brigui J, Louajri A, Simonetti G. Biofilm inhibition of *Inula viscosa* (L.) Aiton and *Globularia alypum* L. extracts against *Candida* infectious pathogens and *in vivo* action on *Galleria mellonella* model. Adv Biol. 2023; 7: 2300081. https://doi.org/10.1002/adbi.202300081

[7] Saltan N, Köse YB, Göger F, İşcan G. Phenolic profile, antioxidant and anticandidal activities of *Inula oculus-christi* L. from Turkey. J Res Pharm. 2022; 26(4): 799-808. http://doi.org/10.29228/jrp.177

[8] Buza V, Niculae M, Hanganu D, Pall E, Burtescu RF, Olah N-K, Matei-Laţiu M-C, Vlasiuc I, Iozon I, Szakacs AR, et al. Biological activities and chemical profile of *Gentiana asclepiadea* and *Inula helenium* ethanolic extracts. Molecules. 2022; 27(11): 3560. https://doi.org/10.3390/molecules27113560

[9] Erenler R, Atalar MN, Yıldız İ, Geçer EN, Yıldırım A, Demirtaş İ, Alma MH. Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*. J Integr Anatol Med. 2023; 4(3) :3-10. https://doi.org/10.53445/batd.1278048

[10] Gökbulut A, Özhan O, Satılmış B, Batçıoğlu K, Günal S, Şarer E. Antioxidant and antimicrobial activities, and phenolic compounds of selected *Inula* species from Turkey. Nat Prod Commun. 2013; 8(4): 475-478. http://doi.org/10.1177/1934578x1300800417

[11] Sevindik E, Aydın S, Paksoy MY, Sökmen BB. Anti-urease, total phenolic content and antioxidant activities of some *Inula* L. (Asteracea) taxa in Turkey. Genetika. 2020; 52(3): 825-834. https://doi.org/10.2298/GENSR2003825S

[12] Gökbulut A, Kaiser M, Brun R, Sarer E, Schmidt TJ. 9β-hydroxyparthenolide esters from *Inula montbretiana* and their antiprotozoal activity. Planta Med. 2012; 78(3): 225-229. https://doi.org/10.1055/s-0031-1280371

[13] Souza Dos Santos B, Bezerra Filho CM, Alves do Nascimento Junior JA, et al. Anti-staphylococcal activity of *Syagrus coronata* essential oil: Biofilm eradication and *in vivo* action on *Galleria mellonela* infection model. Microb Pathog. 2019; 131: 150-157. https://doi.org/10.1016/j.micpath.2019.04.009

[14] Kaskatepe B, Aslan Erdem S, Ozturk S, Safi Oz Z, Subasi E, Koyuncu, M, Vlaini´c J, Kosalec I. Antifungal and anti-virulent activity of *Origanum majorana* L. essential oil on *Candida albicans* and *in vivo* toxicity in the *Galleria mellonella* larval model. Molecules. 2022; 27: 663. https://doi.org/10.3390/molecules27030663

[15] Alnezary FS, Almutairi MS, Alhifany AA, Almangour TA. Assessing *Galleria mellonella* as a preliminary model for systemic *Staphylococcus aureus* infection: Evaluating the efficacy and impact of vancomycin and *Nigella sativa* oil on gut microbiota. Saudi Pharm J. 2023; 31(12): 101824. https://doi.org/10.1016/j.jsps.2023.101824

[16] Meccatti VM, Figueiredo-Godoi LMA, Pereira TC, et al. The biocompatibility and antifungal effect of *Rosmarinus officinalis* against *Candida albicans* in *Galleria mellonella* model. Sci Rep. 2022; 12(1): 15611. http://doi.org/10.1038/s41598-022-19425-9

[17] Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp. 1990; 15: 113-115.

[18] Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard, 2006. M7-A7 Wayne, PA.

[19] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 30th Ed. 2020. M100 Wayne, PA.

[20] Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; 4th informational supplement, 2012. M27-S4. Wayne, PA.

[21] Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev. 2020; 33(3): e00069-19. https://doi.org/10.1128/CMR.00069-19

[22] Rayaman P, Şafak S, Rayaman E, Şenkardeş İ, Taşkın T. Investigation of antimicrobial, antibiofilm and antiurease activities of *Teucrium polium* L. extracts. TURJAF. 2025; 13(s2): 3598-3603. https://doi.org/10.24925/turjaf.v13is2.3598-3603.8226

[23] Kebede B, Shibeshi W. In vitro antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. Vet Med Sci. 2022; 8(4): 1802-1815. https://doi.org/10.1002/vms3.772

[24] Wijesinghe GK, Maia FC, de Oliveira TR, et al. Effect of *Cinnamomum verum* leaf essential oil on virulence factors of *Candida* species and determination of the *in-vivo* toxicity with *Galleria mellonella* model. Mem Inst Oswaldo Cruz. 2020; 115: e200349. http://doi.org/10.1590/0074-02760200349

[25] Fuchs BB, Eby J, Nobile CJ, El Khoury JB, Mitchell AP, Mylonakis E. Role of filamentation in *Galleria mellonella* killing by *Candida albicans*. Microbes Infect. 2010; 12(6): 488-496. http://doi.org/10.1016/j.micinf.2010.03.001

[26] Natividade MMP, Corrêa LC, de Souza SVC, Pereira GE, de Oliveira Lima LC. Simultaneous analysis of 25 phenolic compounds in grape juice for HPLC: method validation and characterization of São Francisco Valley samples. Microchem J. 2013; 110: 665-674. http://doi.org/10.1016/j.microc.2013.08.010