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Antimicrobial activities of different extracts from *Centaurea iberica*

Centaurea iberica bitkisinden elde edilen farklı ekstrelerin antimikrobiyal aktiviteleri

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ABSTRACT

Across different regions of the world, plants have long been valued for their wide range of functional properties. Aromatic plants, in particular, are cultivated to obtain natural compounds used in cosmetic scenting, food enhancement, and preservation. Within this category, species belonging to the Asteraceae family have gained increasing attention in recent years due to their expanding relevance in numerous applied disciplines. The objective of this study was to evaluate the antibacterial and antifungal potential of different solvent extracts (*n*-hexane, ethyl acetate, and methanol) and an infusion of *Centaurea iberica* L. against five bacterial (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853) and two yeast (*Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019) strains using the microdilution method. The minimum inhibitory concentration (MIC) values for all tested microorganisms ranged between 78.125 and 5000 µg/mL. According to the results, all studied extracts exhibited stronger antifungal activity than antibacterial activity. Among them, the ethyl acetate extract demonstrated the most potent antifungal effect with MIC values between 78.125 and 156.25 µg/mL, whereas the infusion extract showed the weakest overall activity. In conclusion, further phytochemical investigations are required to identify the secondary metabolites responsible for these activities, which may contribute to the development of novel antifungal agents.

Keywords: Antibacterial, antifungal, *Centaurea*, Asteraceae

ÖZET

Bitkiler dünyanın farklı bölgelerinde, geniş işlevsel özellikleri nedeniyle uzun zamandır değer görmektedir. Özellikle aromatik bitkiler, kozmetik ürünlerde koku verici olarak, gıdalarda lezzet artırıcı ve koruyucu doğal bileşiklerin elde edilmesi amacıyla yetiştirilmektedir. Bu kategoride yer alan Asteraceae familyasına ait türler, çeşitli uygulamalı disiplinlerde artan önemleri nedeniyle son yıllarda giderek daha fazla ilgi görmektedir. Bu çalışmanın amacı, *Centaurea iberica* L. bitkisinin farklı çözücü ekstrelerinin (*n*-heksan, etil asetat ve metanol) ve infüzyonunun beş bakteri (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853) ve iki maya (*Candida albicans* ATCC 10231 ve *Candida parapsilosis* ATCC 22019) suşuna karşı mikrodilüsyon yöntemi kullanılarak antibakteriyel ve antifungal potansiyelinin değerlendirilmesidir. Tüm test mikroorganizmaları için minimum inhibitör konsantrasyon (MİK) değerleri 78,125-5000 µg/mL aralığında değişmiştir. Elde edilen bulgulara göre, incelenen tüm ekstreler antibakteriyel aktiviteye kıyasla daha güçlü antifungal aktivite göstermiştir. Tüm ekstreler arasında etil asetat ekstresi, 78,125-156,25 µg/mL aralığındaki MİK değerleriyle en güçlü antifungal aktiviteyi sergilerken, genel olarak en zayıf aktivite infüzyon ekstresinde görülmüştür. Sonuç olarak, bu biyolojik aktivitelerden sorumlu ikincil metabolitleri tanımlayabilmek için daha ileri fitokimyasal araştırmalara ihtiyaç duyulmakta olup, bu bileşikler yeni antifungal ajanların geliştirilmesine katkı sağlayabilir.

Anahtar kelimeler: Antibakteriyel, antifungal, *Centaurea*, Asteraceae

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Plants have been used for their therapeutic properties throughout human history. Phytochemical studies aim to identify and characterize the bioactive compounds responsible for the medicinal effects of plants, investigate their pharmacological mechanisms, and support the development of reliable plant-based pharmaceuticals (Mat, 2010). One of the largest members of the plant kingdom, the Asteraceae family has gained increasing attention in recent years due to its diverse medicinal applications in the pharmaceutical industry (Gülsoy-Toplan et al., 2022; Nadaf et al., 2025). *Centaurea* L. is a valuable genus within the Asteraceae family, widely recognized for its medicinal and economic importance. The genus includes more than 700 species that are widely distributed across Asia, Europe, tropical Africa, and North America (Fattaheian-Dehkordi et al., 2021). In Türkiye, they are represented by 174 species, 126 of which are endemic (Özdemir Nath & Kultur, 2023). The species are commonly referred to by various local names, including 'Peygamber çiçeği, Çobankaldıran, Zerdali dikeni, Timurdikeni, Çoban dikeni, and Acımık' (Baytop,1997; Özdemir Nath & Kultur, 2023). *Centaurea* species have been used in traditional medicine for the management of several disorders such as gynecological, dermatological, antidiabetic activity, gastrointestinal, cardiovascular problems, parasitic and microbial infections (Fattaheian-Dehkordi et al., 2021; Khan et al., 2011; Koca et al., 2009). In Anatolian folk medicine they are commonly utilized to relieve inflammation and pain, treat hemorrhoids, abscesses, various infectious diseases and wounds, and reduce fever (Baytop, 1999). These ethnopharmacological applications have prompted scientific interest in exploring the phytochemical profile of *Centaurea* species and validating their bioactivities through modern pharmacological studies.

Numerous studies have demonstrated that *Centaurea* species contain a diverse range of secondary metabolites, including phenolics, steroids, terpenoids, essential oil, fatty acids, and sesquiterpene lactones, which contribute to their broad spectrum of biological activities (Albayrak et al., 2017; Astari et al., 2013; Al Easa & Rizk, 1992; Erel et. al, 2013; Shoeb et al., 2007). The extracts of several *Centaurea* species showed the remarkable antibacterial, antifungal, anticancer, antioxidant, and anti-inflammatory activities (Arif et al., 2004; Khammar & Djeddi, 2012; Teneva et al., 2024). Additionally, it is observed that sesquiterpene lactone-rich fractions exhibit notable antimicrobial properties (Aktumsek et al., 2013). In this context, numerous *Centaurea* species have been extensively investigated for their antimicrobial properties against various pathogenic microorganisms (Güven et al., 2005).

Centaurea iberica Trevir. ex Sprengel commonly known as Iberian star-thistle, is an annual herb native to the Eastern Mediterranean and Southwestern Asia, including Türkiye, Iran, Iraq, and surrounding regions (Bibi et al., 2023). Multiple studies have reported that *C. iberica* is rich in secondary metabolites such as lignans, terpenoids, anthocyanins, flavonoids, phenolic acids, stilbenes, xanthophylls, and carotenes (Arif et al., 2004). The antimicrobial, anticancer, antioxidant, and anti-inflammatory activities of *C. iberica* extracts collected from different regions have been demonstrated in several previous studies (Alper et al., 2021; Koca et al., 2009). It is widely used in Turkish folk medicine for promoting wound healing, treating insect and snake bites, and relieving abdominal pain (Erol & Tuzlacı, 1997).

Antibiotic resistance has emerged as one of the most critical global health challenges. Evidence indicates that resistance has developed against nearly all commercially available antimicrobial agents, and these trends continue to escalate (Kwapong et al., 2020). As a result, therapeutic failures in the treatment of several infectious diseases are becoming increasingly frequent. Moreover, microbial contamination represents another serious concern in the food, cosmetic, pharmaceutical, and beverage industries (Rathee et al., 2023). Consequently, there is a growing scientific interest in identifying natural antimicrobial candidates capable of overcoming resistance issues associated with conventional preservatives. Extensive studies have confirmed the potential antibacterial and antifungal properties of plant-derived extracts and secondary metabolites (Savoia, 2012). Therefore, the well-documented antimicrobial potential of *Centaurea* species highlights their importance as valuable candidates for developing alternative therapeutic agents.

The long-standing ethnobotanical usage of the plant has led researchers to investigate its antimicrobial and anti-inflammatory potential. Therefore, the current investigation aimed to assess the antibacterial and antifungal potential of different solvent extracts obtained from *C. iberica* collected in northwestern Türkiye. The findings of this study contribute to the ongoing investigations into natural antimicrobial agents and may provide a basis for future research focusing on the isolation of bioactive constituents and their possible application in antimicrobial drug development.

Materials and methods

Plant material

The aerial parts of *C. iberica* were collected during the flowering stage from Çatalca (Durusu Mevkii), İstanbul, located in the northwestern region of Türkiye, in 2025. The specimen was identified by one of us (Dr. Bahar Gürdal), and the voucher was deposited in the Herbarium of the Faculty of Pharmacy, İstanbul University (ISTE No.: 119164). The plant materials were air-dried at room temperature and stored in a dark place until use.

Preparation of extracts

The aerial parts of *C. iberica* were powdered using a laboratory-type mill and then extracted with different solvents in sequence *n*-hexane, ethyl acetate, and methanol using an ultrasonic bath at a plant material-to-solvent ratio of 1:10 (g/mL) at 25 ± 0.1 °C for 30 min. Following extraction, the extracts were filtered and the solvents were removed under reduced pressure at temperatures below 40 °C. To obtain the aqueous extract, 10.0 g of the dried aerial parts were infused in 200 mL of distilled water at 80 °C for 30 min, then lyophilized and stored at −20 °C until analysis.

Test microorganisms

The American Type Culture Collection (ATCC) standard strains of three Gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Staphylococcus epidermidis* ATCC 12228); two Gram-negative bacteria (*Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853) and two yeasts (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019) are used in the experiments. The microorganism cultures utilized in the study were obtained from the culture collection of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Health Sciences.

Preparation of test microorganisms

The bacteria and yeasts stored at −80 °C were activated one day before the experiment. The dilution approach was used to introduce bacteria into TSA (Tryptic Soy Agar) and yeasts into SDA (Sabouraud Dextrose Agar) medium for activation. Using colonies obtained, the bacterial and yeast densities were adjusted to 0.5 McFarland (10^8 cfu/mL) in a McFarland device (BioSan) with the aid of a sterile inoculating loop. The 0.5 McFarland (BioSan) (10^8 cfu/mL) standard suspension of test isolates was diluted 100X with Mueller Hinton Broth (MHB) medium for bacteria and RPMI medium for yeasts to a concentration of 10^6 cfu/mL (CLSI, 2015; CLSI, 2023).

Determination of minimum inhibitory concentration (MIC)

The microdilution method was used to determine the minimum inhibitory concentration (MIC) values of the test plants used in the investigation (CLSI, 2015; CLSI, 2023). To prepare them for testing for antimicrobial activity, the study's paste-like extracts were diluted with dimethyl sulfoxide (DMSO) to a concentration of 10 mg/mL. "U" type, sterile, flat-bottomed 96-well microtiter (Brand) plates were used in the microdilution procedure. With the exception of the first well, each well received 50 microliters of RPMI medium for *Candida* species and 50 microliters of MHB medium for bacteria. The first and second starting wells were then filled with 50 µL of plant extracts that had been prepared to a final concentration of 10 mg/mL. An automatic pipette was then used to perform serial dilutions, starting from well number 2 and continuing to well number 11, to obtain concentrations ranging from 10 mg/mL to 0.0097 mg/mL. Lastly, each well received 50 µL of a bacterial suspension with a density of 10^6 cfu/mL. In order to control growth, the final well was left empty. The control group, tigecycline, and voriconazole followed the same process, producing serial concentrations ranging from 512 µg/mL to 0.5 µg/mL. As a negative control, DMSO was employed. Every plate made in this way was incubated for 24 hours at 37 °C in an incubator. Following a 24-hour period, each well received 50 µL of a 2 mg/mL 2,3,5-triphenyltetrazolium chloride (TTC-Merck) solution, which was then incubated for 30 min. The MIC values were calculated by looking at the color changes in the plates. The results were averaged after the study was conducted three times.

Results and discussion

The aerial parts of *C. iberica* were subjected to sequential solvent extraction to obtain crude fractions, and their respective yields are shown in Table 1.

Table 1. The yield of the extracts (beginning with 10 g of material)

Samples	Yield (%)
n-hexane extract	1.05
Ethyl acetate	1.69
Methanol	7.8
Infusion	1.52

The antimicrobial activities of the extracts were evaluated by using the minimum inhibitory concentration (MIC) values in a micro-dilution method. The MIC values of samples compared with the standard drugs are presented in Table 2. According to our results, the MIC values of *C. iberica* extracts were determined to range from 625–5000 µg/mL for Gram-positive bacteria, 625–3750 µg/mL for Gram-negative bacteria, and 78.125–1250 µg/mL for yeast strains. The extracts showed moderate to mild antimicrobial activities compared with the standards. Based on the MIC values, the extracts exhibited stronger antimicrobial activity against yeasts than against bacteria. The results of the antibiotic and antifungal control groups were within the expected activity range (Table 2). The study showed that the tested extracts had higher MIC values compared to the antimicrobial agents used as positive controls. Accordingly, it can be stated that the extract activities were lower compared to the antimicrobial agents.

Table 2. The antimicrobial activity of the various extracts from *Centaurea iberica* (MIC, µg/mL)

	Extracts				
Microorganisms	***n*-hexane (10000 µg/mL)**	**Ethyl acetate (10000 µg/mL)**	**Methanol (10000 µg/mL)**	**Infusion (10000 µg/mL)**	**Tigecycline/ Voriconazole (512 µg/mL)**
Gram-Positive Bacteria					
S. aureus ATCC 29213	2500	1250	2500	5000	0.5
S. epidermidis ATCC 12228	5000	1250	5000	5000	1
E. faecalis ATCC 29212	2500	625	1250	2500	0.5
Gram-Negative Bacteria					
P. aeruginosa ATCC 27853	2500	1250	2500	2500	32
A. baumannii ATCC 19606	2500	625	3750	3750	2
Yeasts					
C. albicans ATCC 10231	1250	**156.25**	1250	2500	4
C. parapsilosis ATCC 22019	1250	**78.125**	2500	2500	4

Among the extracts, the MIC values of the *n*-hexane extract ranged between 1250–5000 µg/mL, while the ethyl acetate extract exhibited MIC values of 78.125–1250 µg/mL. The MIC values of the methanol extract were 1250–5000 µg/mL, and those of the infusion extract ranged from 2500–5000 µg/mL.

Evaluation of these findings revealed that the ethyl acetate extract showed the highest antimicrobial activity, as indicated by the lowest MIC values, whereas the infusion extract displayed the weakest activity (Figure 1).

When the antimicrobial activity of the extracts was evaluated individually against the tested bacterial and yeast strains, it was observed that the *n*-hexane extract exhibited the lowest MIC values against yeasts. This indicates that the extract is more effective against fungal microorganisms than against bacteria.

Furthermore, when the antibacterial activity was examined within the bacterial group, the *n*-hexane extract demonstrated a stronger effect on Gram-negative strains compared to Gram-positive strains (Figure 2).

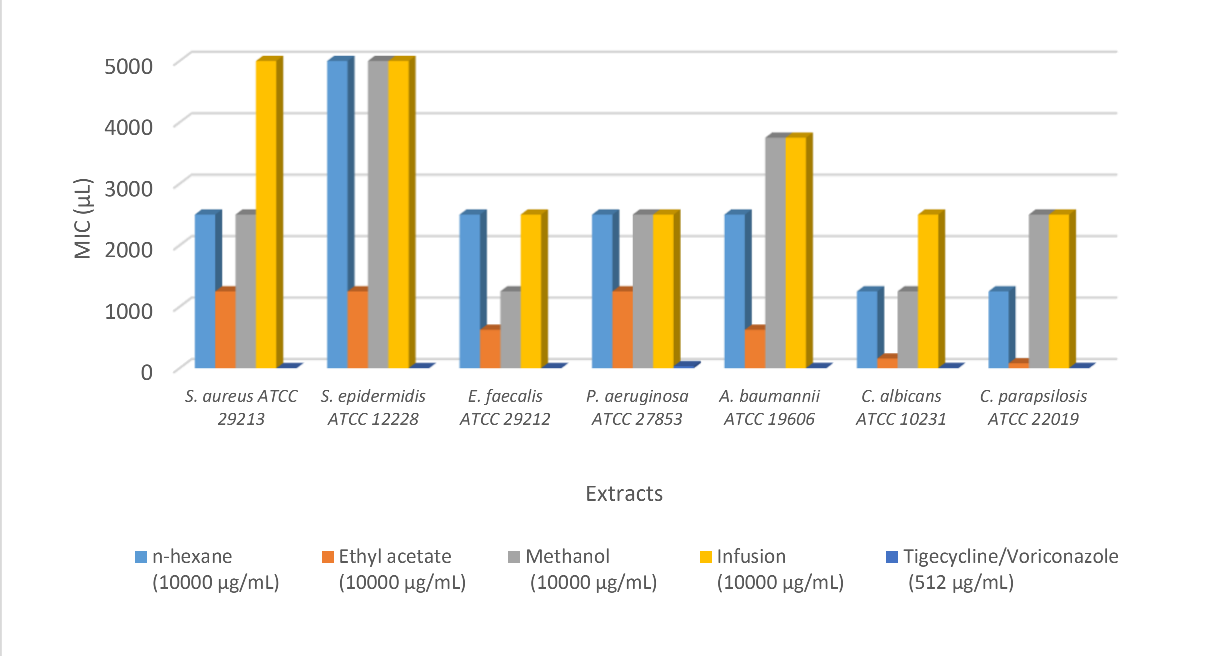

Figure 1. Comparison of antimicrobial effects of extracts prepared with different solvents

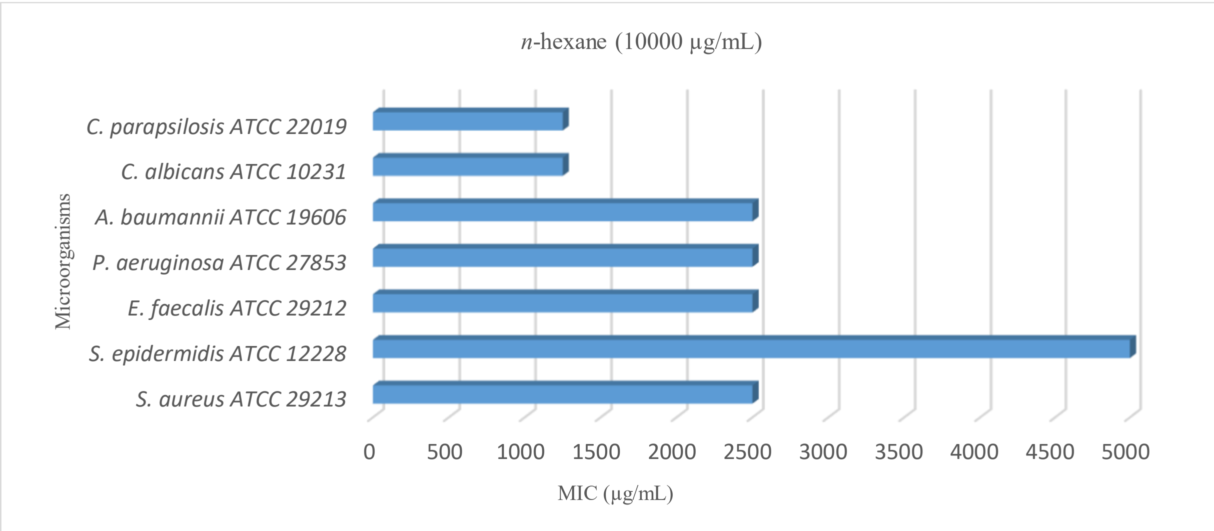

Figure 2. The antimicrobial effect of *n*-hexane extract on test microorganisms

Among the tested extracts, the ethyl acetate extract exhibited the strongest antimicrobial activity against both bacterial and yeast strains. In particular, it showed markedly high antifungal activity against *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019. According to widely accepted criteria for assessing the antimicrobial potency of plant extracts, the activity is classified as significant when MIC < 100 µg/mL, moderate when 100 < MIC ≤ 625 µg/mL, and weak when MIC > 625 µg/mL (Awouafack et al., 2013; Eloff, 2004; Kuete et al., 2011). Based on these criteria, the ethyl acetate extract demonstrated important antifungal efficacy against *C. parapsilosis* ATCC 22019, while its effects against *C. albicans* ATCC 10231, *E. faecalis* ATCC 29212 and *A. baumannii* ATCC 19606 were considered moderate (Figure 3).

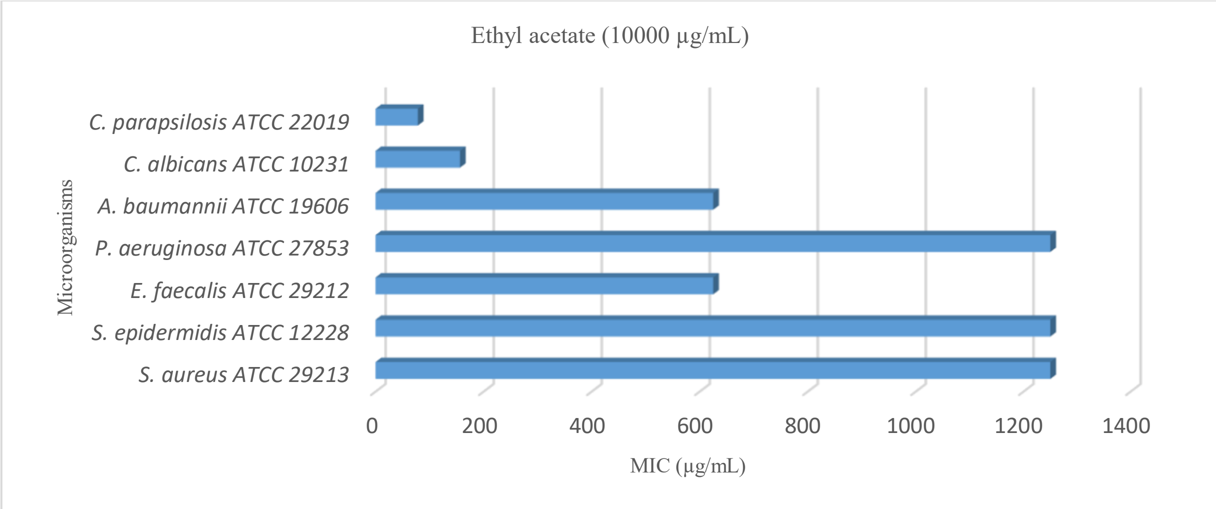

Figure 3. The antimicrobial effect of ethyl acetate extract on test microorganisms

The methanol extract exhibited weak to moderate antibacterial activity against all tested bacterial strains. However, it did not show a clearly selective effect between Gram-positive and Gram-negative bacteria. Similar to the other extracts, the methanol extract demonstrated relatively stronger antimicrobial activity against the yeast strains.

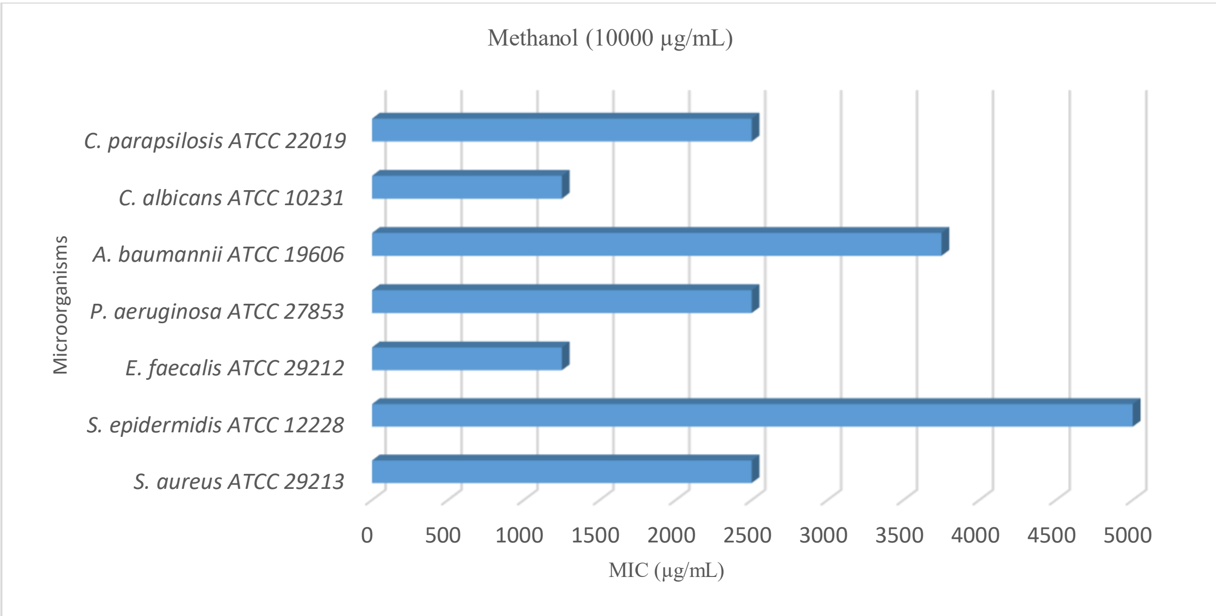

Figure 4. The antimicrobial effect of methanol extract on test microorganisms

The infusion exhibited the weakest antimicrobial activity, showing the highest MIC values among all tested extracts. However, similar to the others, it demonstrated relatively stronger antifungal activity than antibacterial activity. These results suggest that medium-polarity solvents such as ethyl acetate may extract more active antimicrobial constituents compared to non-polar or aqueous extraction.

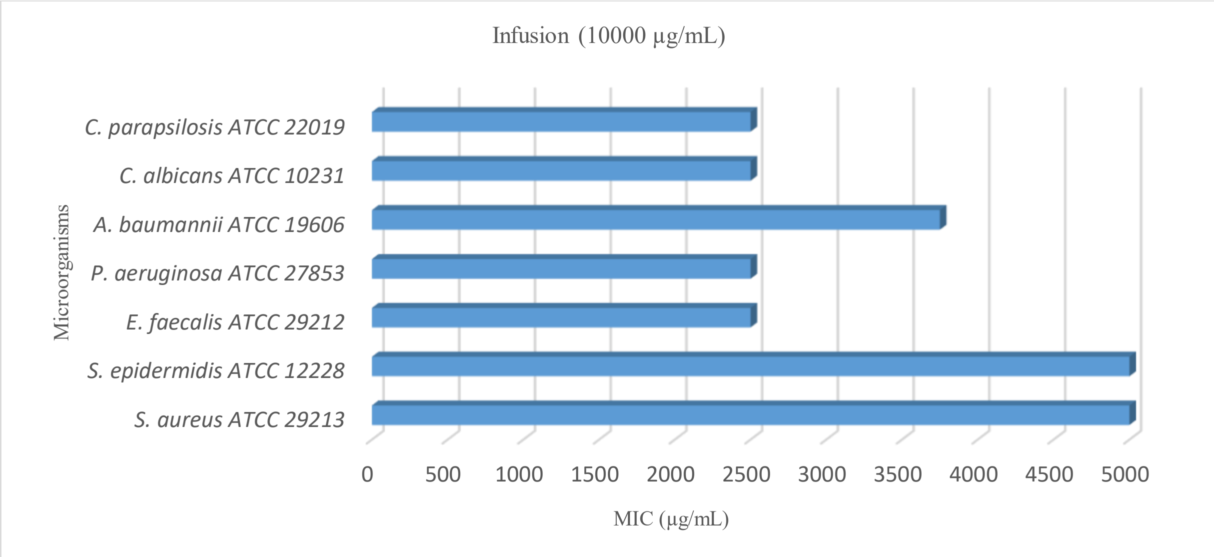

Figure 5. The antimicrobial effect of the infusion on test microorganisms

According to previous reports, the different solvent extracts obtained by *Centaurea* species exhibit notable antimicrobial properties, which showed variety depending on solvent polarity, chemical composition, and environmental conditions influencing plant growth (Bibi et al., 2023; Skliar et al., 2005). In a 2013 study, methanol extracts of five different *Centaurea* species were tested against bacteria and yeasts. Four extracts were moderately effective against *P. aeruginosa* (MIC: 312 µg/mL), and similarly, six extracts showed a moderate antifungal response against *C. albicans* with MIC values of 312 µg/mL. Among the studied extracts, one of them is belonging to *C. iberica* collected from Şile (East part of İstanbul) only showed antifungal acitivity against *C. albicans* at 312 µg/mL while no antibacterial effects were detected against either Gram-positive or Gram-negative bacteria (Şen et al., 2013). The *n*-hexane and methanolic extracts of *C. iberica* collected in Pakistan have been evaluated for their biological properties, including antimicrobial potential. In antibacterial assays, the methanolic extract exhibited the strongest activity, producing the largest inhibition zone (20 ± 1.5 mm) against *Klebsiella pneumoniae* at 33.33 µg/mL. Similarly, in antifungal testing, the highest inhibitory effect was again observed with the methanolic extract, which generated an inhibition zone of 18 ± 2.4 mm against *Aspergillus niger*. Overall, the methanolic extract demonstrated superior antimicrobial efficacy compared to the *n*-hexane extract (Bibi et al., 2023). In another study, the methanol extracts from twelve *Centaurea* species from Türkiye, were studied for antibacterial activity against four bacteria (*Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*). Among them eight species namely *C. calolepis*, *C. balsamita*, *C. cariensis* subsp. *maculiceps, C. kotschyi* var. *kotschyi*, *C. cariensis* subsp. *microlepis*, C. *solstitialis* subsp. *solstitialis*, *C. virgata* and *C. urvillei* subsp. *urvillei* were effective against various bacterial strains included in the study (Tekeli et al., 2011). Albayrak et al. were investigated the phenolic composition, antioxidant and antimicrobial activity of the methanol extracts prepared by using diffrerent extraction methods obtained from *Centaurea amaena* and *Centaurea aksoyi*. According to their results, the macerated (ME) and Soxhlet (SE) extracts of *C. aksoyi* showed no antibacterial activity, but both inhibited *Aspergillus parasiticus* with noticeable zones of inhibition (13–14 mm). The ultrasonicated extract (UE) displayed limited antibacterial potency and was only active against *Escherichia coli* O157:H7 (7 mm; MIC = 3.75 mg/mL). In contrast, *C. amaena* displayed a broader antimicrobial profile. While ME and SE extracts inhibited only *Pseudomonas aeruginosa* and *Yersinia enterocolitica*, the UE extract showed comparatively strong antibacterial effects studied Gram-positive and Gram-negative strains except against *Klebsiella pneumoniae* and *Morganella morganii* within the Gram-negative group. Regarding antifungal activity, only *Candida albicans* was inhibited, with inhibition zones ranging from 7 to 8 mm (MIC ≈ 30 mg/mL) by all *C. amaena* extracts (Albayrak et al., 2017). The antimicrobial activity of the ethyl acetate, acetone, chloroform, and ethanol extracts from the *Centaurea* species namely *C. odyssei*, *C. ptosomipappoides*, *C. ptosomipappa*, *C. kurdica*, and *C. amonicola* were investigated using agar-well diffusion method by Güven et al. According to their results, ethyl acetate extracts of *C.*

odyssei and *C. kurdica* showed remarkable antimicrobial properties (Güven et al., 2005). Our findings on *Centaurea iberica* largely corroborate previous reports on the antimicrobial potential of *Centaurea* species, especially with respect to the consistently greater antifungal activity observed compared to antibacterial effects. Differences in reported antimicrobial activities among previous studies, particularly those related to solvent-dependent effects, are likely associated with changes in extraction efficiency and phytochemical composition. Previous phytochemical studies on species of the genus *Centaurea* have revealed the presence of bioactive secondary metabolites, including sesquiterpene lactones, flavonoids, phenolic acids, and polyacetylenes, which are known to contribute to antimicrobial activity (Aktumsek et al., 2013; Arif et al., 2004). In particular, sesquiterpene lactones, commonly detected in medium-polarity extracts, have been reported to possess antibacterial and antifungal effects. As a result, it can be suggested that the phytochemical composition and extraction solvent play a significant role in determining biological efficacy.

Conclusion

In the present study, the antifungal, and antibacterial properties of different *C. iberica* extracts were investigated. In conclusion, all extracts obtained from the plant exhibited stronger activity against yeast strains compared to bacteria. As to MIC values, the ethyl acetate extract of *C. iberica* showed significant antifungal activity against *C.* albicans and *C. parapsilosis* 156.25 μg/mL and 78.125 μg/mL respectively. Given these findings, the ethyl acetate extract may be worth further exploration as a source of natural antifungal agents. Despite these promising findings, *C. iberica* remains an under-explored medicinal plant. More detailed research is needed to characterize its secondary metabolite profile comprehensively, identify the primary antimicrobial constituents, and clarify their mechanisms of action. Given the growing global challenge of drug-resistant pathogens, exploration of *C. iberica* as a source of novel antimicrobial agents represents a valuable direction for natural product research. As a result, further research into this species could provide valuable information for the development of alternative medicinal techniques based on plant-derived bioactive chemicals.

Author contributions

DD: Project administration, antimicrobial activity; EM: Antimicrobial activity; BG: Collection of plant material and identification; GGT: Supervision, project administration, extraction.

Declaration of interests

The authors declare no conflict of interest.

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