



In Vitro Antimicrobial Screening of 27 Extracts from 21 Medicinal Plant Species Against Selected Clinically Relevant Bacterial and Fungal Pathogens

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Abstract

Background: Antimicrobial resistance (AMR) has emerged as a major global public health challenge because of its increasing prevalence, which is largely driven by the inappropriate and excessive use of antibiotics. This problem has renewed scientific interest in medicinal plants, which are a rich source of bioactive metabolites and have been used for centuries in traditional medicine to treat infectious diseases.

Objectives: To evaluate and compare the antimicrobial activities of ethanol extracts of different organs of selected medicinal plants against reference microorganisms and clinically isolated bacterial and fungal pathogens.

Methods: Ethanol extracts of different parts of 21 medicinal plants belonging to several botanical families were tested using the broth microdilution method. Antimicrobial activity was assessed by determining the minimum inhibitory concentration (MIC) values against the quality control strains and the corresponding clinical isolates.

Results: The tested extracts exhibited antimicrobial activity, with MIC values ranging from 8 to > 128 µg/mL against quality control strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, *Candida krusei*, and their isolated strains. Among the tested samples, the flower extract of *Alcea biennis* showed the strongest antibacterial activity, with MIC values of 8 µg/mL against the quality control strains of *E. coli*, *B. subtilis*, and *E. faecalis* and 16 µg/mL against the clinical strains. The herb extract of *Cistus creticus* also showed strong antibacterial activity against the reference strains of *E. coli* and *S. aureus*, whereas the herb extract of *Lamium purpureum* inhibited the quality control strain of *S. aureus*, with an MIC value of 8 µg/mL.

Conclusions: Among the investigated medicinal plants, *A. biennis* and *C. creticus* exhibited notable antimicrobial activity. These results support further phytochemical characterization and pharmacological evaluation of these species as promising sources of novel antimicrobial compounds.

Keywords: Antimicrobial Activity, Antimicrobial Resistance, Medicinal Plants, Plant Extracts, Minimum Inhibitory Concentration (MIC)

1. Background

The World Health Organization (WHO) has published guidelines for evaluating the safety and efficacy of traditional medicines, reflecting their long history of use.

Medicinal plants have been used as therapeutic agents since ancient times and are recognized as a rich source of bioactive secondary metabolites; they continue to attract considerable attention as potential sources of novel antimicrobial agents.

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The global emergence of antimicrobial resistance (AMR), primarily due to the inappropriate and excessive use of antibiotics, has created an urgent need for alternative therapeutic approaches (1). Consequently, natural products, particularly those derived from medicinal plants, have become an important focus of antimicrobial research because they contain structurally diverse phytochemicals with antibacterial and antifungal activities. Many studies have demonstrated the antimicrobial potential of plant extracts; however, reported activities vary widely due to differences in plant species, geographical origin, plant organ, extraction solvent, extraction procedure, test microorganisms, and antimicrobial susceptibility methods (2).

Previous studies using different methods and culture collections, such as disc diffusion, agar dilution, and microdilution, have reported variable findings for the herbal extracts included in our screening test. In one study, the antimicrobial activity of the ethanol extract of *A. biennis* was evaluated using the disk diffusion/Kirby-Bauer method; the inhibition zone diameter against Gram-positive and Gram-negative microorganisms ranged from 8 to 10 mm. In another study, MIC values against various bacteria (*S. Typhimurium*, *P. aeruginosa*, *S. aureus*, and *E. coli*) were determined using the agar-well diffusion method in an investigation of the antibacterial activity of the ethanol extract of *Arum dioscoridis* (3). In a different study, inhibition zone diameters of ethanolic extracts of *A. dioscoridis* against the tested bacteria ranged from 7.8 to 9.8 mm, as determined using the disk diffusion method (4). Although these studies confirmed the antimicrobial activity of individual medicinal plants, the results cannot be directly compared because different susceptibility testing methods and experimental conditions were used.

In this study, the antimicrobial activities of medicinal plant extracts from different parts (*A. biennis*, *A. dioscoridis*, *Achillea nobilis*, *C. creticus*, *Cichorium intybus*, *Doronicum orientale*, *Geranium lucidum*, *Hypericum scabrum*, *L. purpureum*, *Origanum onites*, *Phlomis grandiflora*, *Plantago lanceolata*, *Phlomis nissolii*, *Rhus coriaria*, *Ranunculus damascenus*, *Sedum acre*, *Salvia virgata*, *Silene vulgaris*, *Salvia sclarea*, *Thymus zygoides*, and *Valeriana dioscoridis*) against Gram-negative and Gram-positive bacteria (*E. coli*, *K. pneumoniae*, *B. subtilis*, *E. faecalis*, *S. aureus*), yeast-like fungi [*C. albicans*, *C. krusei* (*Pichia kudriavzevii*)], and their clinically isolated strains were determined using the microdilution method. Although the antimicrobial activities of several of these medicinal plants have been

investigated individually, previous studies have generally focused on a limited number of plant species, selected microorganisms, or standard laboratory strains and have used different experimental methods. To our knowledge, a comprehensive screening of these medicinal plant species under the same experimental conditions has not been reported. In addition, limited information is available on their antimicrobial activity against clinical isolates and on differences in activity among distinct plant parts, such as flowers and leaves. Therefore, the present study provides a comparative evaluation of ethanol extracts obtained from different parts of selected medicinal plants against both quality control strains and clinical isolates using the broth microdilution method.

2. Objectives

This study aimed to evaluate the antimicrobial activity of ethanol extracts obtained from different parts of selected medicinal plants against both quality control strains and clinical isolates of bacterial and fungal pathogens.

3. Methods

3.1. Microorganisms

One clinical isolate of each bacterial species included in the study was used for antimicrobial susceptibility testing. The clinical isolates were obtained from the routine culture collection of Gazi University Hospital (Ankara, Türkiye) and were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Among the clinical isolates, only the *S. aureus* isolate was methicillin-resistant (MRSA), and methicillin resistance was confirmed by the cefoxitin disk diffusion method according to routine laboratory procedures.

3.2. Plant Material

Different plant parts were collected from the Beyşehir-Konya and Isparta provinces and were identified by Ufuk Özbek and Galip Akaydın N. The voucher specimen was deposited in the herbarium of the Faculty of Pharmacy, Gazi University. Ten grams of powdered plant material from each sample were macerated in 200 mL of 80% ethanol for 24 h at room temperature. The extract was filtered, and the plant residue was re-extracted twice under the same conditions (three successive macerations in

Table 1. Plant Materials and Yields of Extracts

Extract Abbreviation	Plant	Part	Family	Herbarium no	Yield (%w/w)
<i>A. biennis</i> -f	<i>Alcea biennis</i> Winterl.	Flowers	Malvaceae	GUEF 3430	13.89
<i>A. biennis</i> -l	<i>Alcea biennis</i> Winterl.	Leaves	Malvaceae	GUEF 3430	11.79
<i>A. dioscoridis</i> -l	<i>Arum dioscoridis</i> Sm.	Leaves	Araceae	GUEF 3438	23.56
<i>A. nobilis</i> -h	<i>Achillea nobilis</i> L. ssp. <i>neitreichii</i> (A. Kern.) Takht.	Herb	Asteraceae	GUEF 3433	16.71
<i>C. creticus</i> -h	<i>Cistus creticus</i> L.	Herb	Cistaceae	GUEF 3449	21.55
<i>C. intybus</i> -h	<i>Cichorium intybus</i> L.	Herb	Asteraceae	GUEF 3427	10.05
<i>D. orientale</i> -h	<i>Doronicum orientale</i> Hoffm.	Herb	Asteraceae	GUEF 3441	16.78
<i>G. lucidum</i> -h	<i>Geranium lucidum</i> L.	Herb	Geraniaceae	GUEF 3440	13.29
<i>H. scabrum</i> -h	<i>Hypericum scabrum</i> L.	Herb	Clusiaceae	GUEF 3448	20.26
<i>L. purpureum</i> -h	<i>Lamium purpureum</i> L. var. <i>purpureum</i> L.	Herb	Lamiaceae	GUEF 3439	20.27
<i>O. onites</i> -h	<i>Origanum onites</i> L.	Herb	Lamiaceae	GUEF 3445	22.96
<i>P. lanceolata</i> -f	<i>Plantago lanceolata</i> L.	Flowers	Plantaginaceae	GUEF 3428	8.55
<i>P. lanceolata</i> -l	<i>Plantago lanceolata</i> L.	Leaves	Plantaginaceae	GUEF 3428	21.41
<i>P. nissolii</i> -f	<i>Phlomis nissolii</i> L.	Flowers	Lamiaceae	GUEF 3444	17.56
<i>P. nissolii</i> -l	<i>Phlomis nissolii</i> L.	Leaves	Lamiaceae	GUEF 3444	16.56
<i>P. grandiflora</i> -f	<i>Phlomis grandiflora</i> H.S. Thompson var. <i>grandiflora</i> H.S. Thompson	Flowers	Lamiaceae	GUEF 3446	20.58
<i>P. grandiflora</i> -l	<i>Phlomis grandiflora</i> H.S. Thompson var. <i>grandiflora</i> H.S. Thompson	Leaves	Lamiaceae	GUEF 3446	30.68
<i>R. coriaria</i> -fr	<i>Rhus coriaria</i> L.	Fruit	Anacardiaceae	GUEF 3434	30.34
<i>R. coriaria</i> -l	<i>Rhus coriaria</i> L.	Leaves	Anacardiaceae	GUEF 3434	38.04
<i>R. damascenus</i> -h	<i>Ranunculus damascenus</i> Boiss. and Gaill.	Herb	Ranunculaceae	GUEF 3443	24.28
<i>S. acre</i> -h	<i>Sedum acre</i> L.	Herb	Crassulaceae	GUEF 3436	4.88
<i>S. virgata</i> -h	<i>Salvia virgata</i> Jacq.	Herb	Lamiaceae	GUEF 3431	15.04
<i>S. vulgaris</i> -h	<i>Silene vulgaris</i> (Moench) Garcke.	Herb	Caryophyllaceae	GUEF 3432	14.36
<i>S. sclarea</i> -f	<i>Salvia sclarea</i> L.	Flowers	Lamiaceae	GUEF 3429	28.51
<i>S. sclarea</i> -l	<i>Salvia sclarea</i> L.	Leaves	Lamiaceae	GUEF 3429	19.15
<i>T. zygoides</i> -h	<i>Thymus zygoides</i> Griseb. var. <i>lycaonicus</i> (Čelak) Ronniger	Herb	Lamiaceae	GUEF 3447	10.53
<i>V. dioscoridis</i> -h	<i>Valeriana dioscoridis</i> Sm.	Herb	Valerianaceae	GUEF 3442	16.47

total). The combined filtrates were concentrated under reduced pressure and evaporated to dryness. Detailed information on the plant materials used in the experimental studies, the number of voucher specimens, and the yield of extracts is provided in Table 1. The medicinal plants used in this study were collected between 2018 and 2023. Each extract was prepared as a single batch, and batch-to-batch consistency was not assessed.

3.3. Preparation of Test Samples and Antimicrobial Activity

The study was conducted between 2018 and 2023, during which medicinal plants were collected. Plant extracts were prepared at the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, and antimicrobial susceptibility assays were performed at the Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, Gazi University. Microbial suspensions were prepared from fresh cultures. Mueller Hinton Broth (MHB; Himedia) and Mueller Hinton Agar (MHA; Himedia) were used to culture and dilute the

bacterial suspensions. Before testing, all fungal strains were cultured on Sabouraud Dextrose Agar (SDA; Himedia) and passaged at least twice to ensure purity and viability at 35°C for 24 - 48 h. The synthetic medium RPMI-1640 with L-glutamine was buffered to pH 7 with 3-[N-morpholino]-propanesulfonic acid, and culture suspensions were prepared. The turbidity of the bacterial and fungal suspensions was adjusted to the 0.5 McFarland standard (about 1×10^8 CFU/mL) and subsequently diluted to obtain a final inoculum concentration of 1×10^5 CFU/mL. Stock solutions (256 µg/mL) were prepared by dissolving the extracts in distilled water with 5% (v/v) of an 80:20 (v/v) DMSO/ethanol blend, followed by twofold serial dilutions to achieve final concentrations of 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL. Broth microdilution assays were performed in sterile 96-well microplates containing the appropriate culture medium. Ten microliters of the standardized microbial suspension were added to each well containing the diluted extract solutions. Amoxicillin-clavulanate,

meropenem, ketoconazole, and fluconazole were used as positive controls and were tested at concentrations ranging from 16 to 0.12 µg/mL, 16 to 0.12 µg/mL, 32 to 0.25 µg/mL, and 128 to 1 µg/mL, respectively. Wells containing the solvent mixture (80% DMSO/20% ethanol) served as solvent controls, wells containing culture medium with inoculum but without extract served as growth controls, and wells containing culture medium alone served as sterility controls. The microplates were sealed and incubated under aerobic conditions at 35°C for 24 h for bacteria and 48 h for fungi. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that completely inhibited visible microbial growth. All assays were performed in triplicate (5,6).

4. Results

In the present study, ethanol extracts obtained from different parts of 21 medicinal plant species exhibited antimicrobial activity, with MIC values ranging from 8 to > 128 µg/mL against the tested quality control strains and clinical isolates of bacterial and fungal pathogens (Table 2). To facilitate comparison among the investigated extracts, antimicrobial activity was categorized as high (MIC ≤ 16 µg/mL), moderate (MIC 32 - 64 µg/mL), or low (MIC ≥ 128 µg/mL). Among the tested quality control strains, *S. aureus* generally exhibited the lowest MIC values, followed by *E. coli*, *B. subtilis*, and *E. faecalis*, whereas *K. pneumoniae* and the corresponding clinical isolates were generally less susceptible to the extracts. Among the investigated extracts, *A. biennis* flower extract demonstrated the broadest antibacterial activity, with MIC values of 8 µg/mL against the quality control strains of *E. coli*, *B. subtilis*, and *E. faecalis*, whereas the corresponding leaf extract showed MIC values of 8 - 16 µg/mL against the same organisms. Similarly, *C. creticus* herb extract showed high activity against the quality control strain of *E. coli* (MIC = 8 µg/mL).

The quality control strain of *S. aureus* was the most susceptible microorganism. Most investigated extracts exhibited MIC values of 8 - 16 µg/mL against this strain. MIC values of 8 µg/mL were observed for the extracts of *A. dioscoridis*, *A. nobilis*, *C. creticus*, *C. intybus*, *D. orientale*, *G. lucidum*, *H. scabrum*, *L. purpureum*, *O. onites*, the flower extract of *P. grandiflora*, the fruit and leaf extracts of *R. coriaria*, *R. damascenus*, *S. acre*, *S. virgata*, *S. vulgaris*, *S. sclarea*, *T. zygioides*, and *V. dioscoridis*. Moderate antibacterial activity (MIC 32 - 64 µg/mL) was observed for several extracts, particularly against the quality control strain and clinical

isolate of *K. pneumoniae*, indicating that this microorganism was less susceptible than the remaining bacterial species. Low antibacterial activity was mainly observed against the *S. aureus* isolate (MRSA). Most investigated extracts exhibited an MIC value of 128 µg/mL against MRSA, whereas the flower extract of *P. lanceolata* and the leaf extract of *P. grandiflora* showed MIC values of > 128 µg/mL. In contrast, *A. biennis* flower and leaf extracts showed MIC values of 16 µg/mL against the quality control strain of *S. aureus* but 128 µg/mL against MRSA. Overall, MRSA was the least susceptible bacterial isolate included in the study.

In contrast to the antibacterial results, antifungal activity showed relatively little variation among the investigated extracts. All tested extracts exhibited comparable antifungal activity against the quality control strain of *C. albicans*, with MIC values of 16 µg/mL. Moderate antifungal activity was observed against the clinical isolate of *C. albicans* and the quality control strain of *C. krusei*, with MIC values of 32 and 64 µg/mL, respectively (Table 2). Overall, antifungal activity was more uniform than antibacterial activity, with only minor differences in MIC values among the investigated extracts.

5. Discussion

In the present study, ethanol extracts of various organs from 21 species of medicinal plants were compared for their antimicrobial activity against quality control strains and clinical isolates of bacteria and fungi. Many studies evaluating the antimicrobial activity of crude plant extracts have proposed interpretative criteria for MIC values. MIC values equal to or lower than 100 µg/mL are considered to indicate promising antimicrobial activity, whereas MIC values higher than 1000 µg/mL generally indicate low antimicrobial activity (7). The MIC values obtained in the present study were interpreted according to these criteria. Most of the active extracts evaluated herein demonstrated promising antimicrobial activity, particularly the extracts of *A. biennis* and *C. creticus*, which exhibited the lowest MIC values against several tested microorganisms. Among the investigated medicinal plants, *A. biennis* and *C. creticus* exhibited greater antimicrobial activity. *A. biennis* showed higher antibacterial activity, while the herb extract of *C. creticus* also exhibited inhibitory activity against several bacterial reference strains. Another important finding of this study was the higher susceptibility of Gram-positive bacteria, compared with Gram-negative bacteria, to the evaluated

Table 2. The MIC Values ($\mu\text{g/mL}$) of the Screened Extracts Tested Against Quality Control Strains as Well as Isolates ^a

Extracts	Gram-negative Microorganisms						Gram-positive Microorganism				Fungi		
	<i>E. coli</i>		<i>K. pneumoniae</i>		<i>B. subtilis</i>		<i>E. faecalis</i>		<i>S. aureus</i>		<i>C. albicans</i>		<i>C. krusei</i>
	ATCC 35218	Clinical isolate	RSKK 574	Clinical isolate	ATCC 6633	Clinical isolate	ATCC 29212	Clinical isolate	ATCC 29213	Clinical isolate (MRSA)	ATCC 10231	Clinical isolate	ATCC 14243
<i>A. biennis</i> -f	8	16	32	32	8	16	8	16	16	128	16	32	64
<i>A. biennis</i> -l	16	32	32	32	8	16	8	16	16	128	16	32	64
<i>A. dioscoridis</i> -l	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>A. nobilis</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>C. creticus</i> -h	8	16	32	32	16	32	16	32	8	128	16	32	64
<i>C. intybus</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>D. orientale</i> -h	32	64	32	64	64	64	64	64	8	128	16	32	64
<i>G. lucidum</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>H. scabrum</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>L. purpureum</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>O. onites</i> -h	16	32	64	64	16	32	16	32	8	128	16	32	64
<i>P. lanceolata</i> -f	32	64	16	64	16	32	16	32	64	> 128	16	32	64
<i>P. lanceolata</i> -l	32	64	16	64	64	64	64	64	16	128	16	32	64
<i>P. nissolii</i> -f	32	64	32	64	16	32	16	32	16	128	16	32	64
<i>P. nissolii</i> -l	32	64	32	64	16	32	64	64	16	128	16	32	64
<i>P. grandiflora</i> -f	16	32	64	64	16	32	16	32	8	128	16	32	64
<i>P. grandiflora</i> -l	16	32	16	64	16	32	16	32	64	> 128	16	32	64
<i>R. coriaria</i> -fr	32	64	16	64	16	32	16	32	8	128	16	32	64
<i>R. coriaria</i> -l	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>R. damascenus</i> -h	32	64	32	32	16	32	16	32	8	128	16	32	64
<i>S. acre</i> -h	16	32	64	32	16	32	16	32	8	128	16	32	64
<i>S. virgata</i> -h	16	32	32	64	16	32	16	32	8	128	16	32	64
<i>S. vulgaris</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>S. sclarea</i> -f	32	64	16	64	16	32	16	32	8	128	16	32	64
<i>S. sclarea</i> -l	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>T. zygoides</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>V. dioscoridis</i> -h	32	64	32	64	16	32	16	32	8	128	16	32	64
<i>Amoxicillin-clavulanate</i>	2	NT	< 0.12	NT	0.12	NT	0.5	NT	< 0.12	NT	NT	NT	NT
<i>Meropenem</i>	NT	NT	NT	NT	NT	NT	NT	NT	0.25	NT	NT	NT	NT
<i>Ketoconazole</i>	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.5	NT	4
<i>Fluconazole</i>	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2	NT	64

^a Abbreviations: f, flowers; h, herb; l, leaves; fr, fruit; ATCC, American type culture collection; RSKK, Culture collection of the Turkish public health institution; MRSA, Methicillin-resistant *Staphylococcus aureus*; NT, not tested.

extracts. Moreover, the tested clinical isolates were less susceptible than the reference strains, with this difference being most evident in the methicillin-resistant *Staphylococcus aureus* (MRSA) isolate. The MIC values obtained for the reference antimicrobial agents were included as positive controls and confirmed the validity and reliability of the assay conditions. Amoxicillin-clavulanate demonstrated antimicrobial activity against *E. coli*, *K. pneumoniae*, *B. subtilis*, *E. faecalis*, and *S. aureus*, with

MIC values of 2, < 0.12, 0.12, 0.5, and < 0.12 $\mu\text{g/mL}$, respectively. Meropenem demonstrated antimicrobial activity against *S. aureus*, with an MIC value of 0.25 $\mu\text{g/mL}$. Ketoconazole and fluconazole demonstrated antifungal activity against *C. albicans* and *C. krusei*, with MIC values of 0.5 and 2 $\mu\text{g/mL}$ against *C. albicans* and 4 and 64 $\mu\text{g/mL}$ against *C. krusei*, respectively. These results confirmed the expected antimicrobial activity of the reference agents under the tested conditions. Although few studies have

directly evaluated the antimicrobial activity of *A. biennis*, similar results have been observed in other species of the *Alcea* genus. Ethanolic extracts of *Alcea rosea* have been reported to exhibit antibacterial activity against different Gram-positive and Gram-negative bacteria, including *S. aureus*, *E. coli*, and *K. pneumoniae*, suggesting that antimicrobial activity could be a common characteristic of the genus (8). In the study conducted by Keser et al., various solvent extracts of *Alcea calvertii* were identified as having antimicrobial activity and being rich in phenolic acids, flavonoids, sterols, and vitamins such as gallic acid, vanillic acid, and catechin, which might have contributed to the biological activities (9). A study by Şener et al. (10) found that methanolic extracts of *A. calvertii* had antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, *Candida tropicalis*, and *Mycobacterium smegmatis*. GC-MS analysis showed the presence of volatile terpenoid components, such as o-cymene and sesquicineole. Nevertheless, phytochemical characterization of the *A. biennis* extracts was beyond the scope of the present study. Further studies should aim to identify the compounds responsible for the observed antimicrobial activity. Previous studies have demonstrated that phenolic compounds, flavonoids, and terpenoids can enhance the antimicrobial activity of plant extracts through several mechanisms. However, the relevance of these findings to the present study is unknown, as no phytochemical analysis was performed to identify or quantify these components in the examined extracts. Therefore, phytochemical characterization of the extracts examined in the present study is necessary to establish a direct relationship between extract composition and antimicrobial activity.

The structure of the outer membrane of Gram-negative bacteria acts as a natural permeability barrier and prevents many phytochemical compounds from entering the cell; consequently, the susceptibility of these bacteria to plant extracts is reduced. In addition, lipopolysaccharides, selective porins, multidrug efflux pumps, or other intrinsic resistance mechanisms further decrease the intracellular accumulation of antimicrobial compounds (11, 12). Therefore, the higher MIC values observed against Gram-negative bacteria in the present study are biologically plausible and consistent with previous reports describing the lower susceptibility of Gram-negative pathogens to plant-derived antimicrobial agents. These results highlight bacterial cell envelope architecture as a major factor determining antimicrobial susceptibility.

C. creticus also showed one of the strongest antimicrobial activities. This finding is consistent with the results of previous studies. A previous study reported antibacterial and antibiofilm activities of aqueous and ethanolic extracts of *C. creticus*, especially against Gram-positive bacteria (13). In another study, MIC values of hydroethanolic extracts of *Cistus* species were reported to range from 32 to 128 µg/mL (14). In the study by Gedikoglu et al., antimicrobial activity was associated with high levels of phenolic compounds, including quercetin, chlorogenic acid, benzoic acid, and gallic acid, although the inhibitory activity of the extracts was relatively weak (15). In the present study, the MIC values obtained for *C. creticus* were lower than those in previous studies. These differences may be related to plant origin, extraction methods, phytochemical composition, or the susceptibility of the microorganisms studied. The general agreement between previous studies and the present results supports these findings.

This research demonstrated that the antimicrobial activity of plant extracts differed among plant organs, indicating that antimicrobial activity may vary depending on the plant part used for extraction. In the present study, flower extracts of *A. biennis* and *P. lanceolata* showed higher antimicrobial activity than leaf extracts of the same species. However, the chemical composition of the different plant organs was not characterized in the present study; therefore, the basis for these differences cannot be determined from the current data. In the present study, a pattern of greater antimicrobial activity was not observed in flower extracts across all plant species studied. In addition, geographical origin, environmental conditions, harvesting season, developmental stage, extraction solvent, and extraction protocol may contribute to differences in antimicrobial activity reported among different studies.

Another observation in the present study was that the specific clinical isolates tested generally showed higher MIC values than their corresponding quality control strains. However, each bacterial species was represented by only one clinical isolate. Therefore, these observations reflect the susceptibility patterns of the specific isolates included in this study and should not be generalized to the broader clinical population. The observed differences may be influenced by isolate-specific characteristics, including resistance mechanisms and other phenotypic properties; however, these factors were not investigated in the present study. Future research should incorporate larger, diverse

collections of clinically varied isolates from various sources and resistance profiles to provide a more thorough evaluation of the antimicrobial potential of the investigated extracts. In addition to acquired resistance genes, clinical isolates often show increased biofilm-forming capacity, decreased membrane permeability, increased expression of multidrug efflux pumps, and other adaptive responses that together reduce susceptibility to antimicrobial compounds, including phytochemicals (16, 17). Our data suggest that medicinal plant extracts retain antimicrobial activity against clinically relevant pathogens; however, the lower susceptibility among the tested clinical isolates suggests that resistance-associated traits may limit their antimicrobial potency. The antimicrobial activities reported in these studies are generally in agreement with our findings; however, MIC values should be interpreted with caution, because antimicrobial activity may be affected by differences in plant material, extraction methods, phytochemical constituents, and experimental conditions. Recent evaluations have emphasized the importance of these factors and the need for improved standardization in studies investigating plant-derived antimicrobial agents (18, 19). Nevertheless, despite methodological differences, the overall consistency of the current research with other studies further strengthens the evidence that medicinal plants remain important sources of bioactive compounds with notable antimicrobial potential. In conclusion, the current investigation revealed that various medicinal plant extracts exhibited promising in vitro antimicrobial activity against the tested bacterial and fungal microorganisms. Among the plant species examined in this study, *A. biennis* and *C. creticus* showed the highest antimicrobial activity. The results also showed that antimicrobial activity is related not only to plant species but also to the plant organ used for extraction, the properties of the bacterial cell membrane, and the resistance profile of the studied microorganisms. The present results are consistent with previous findings showing the antimicrobial potential of medicinal plants, while also expanding knowledge by comparing several medicinal plant species, different plant organs, reference strains, and tested clinically relevant bacterial and fungal isolates under the same experimental conditions (8, 15). This standardized comparative approach minimized methodological variability and enabled a more reliable assessment of the relative antibacterial activity of the investigated extracts than is usually possible when comparing individual experiments.

Unlike many previous studies (8, 15) that focused on a limited number of plant species or tested only reference microorganisms, the present study simultaneously examined ethanol extracts of different parts of 21 species of medicinal plants against quality control strains and clinically important bacterial and fungal isolates under the same susceptibility-testing conditions. From a broader perspective, more detailed chemical characterization of these extracts and their bioactive components is needed to elucidate the reasons for the observed differences in antimicrobial activity. The current research did not perform chromatographic fingerprinting or quantitative phytochemical analysis, and because each extract was prepared as a single batch, consistency across batches could not be evaluated.

Future studies should include complete phytochemical screening and assessment of biological activity in relation to chemical composition. While the present data show promising in vitro antimicrobial activity, they should be considered preliminary screening results and not evidence of therapeutic efficacy or safety. Importantly, MIC values determined in vitro are not sufficient to predict therapeutic efficacy. Clinical translation depends on many factors, such as achievable drug concentrations at the site of infection, pharmacokinetic and pharmacodynamic properties, bioavailability, host-cell toxicity, tissue distribution, and the stability and reproducibility of complex plant extracts. To improve the assessment of the pharmaceutical potential of these extracts, further research should include cytotoxicity assessment, determination of the selectivity index, minimum bactericidal/fungicidal concentration (MBC/MFC) measurements, time-kill studies, synergy testing, comparison with clinically achievable concentrations, comprehensive phytochemical profiling, and in vivo validation. Several limitations should be noted. The present study was limited to in vitro antimicrobial susceptibility testing and did not include identification of active compounds, toxicity testing, mechanistic studies, or in vivo validation. MBC/MFC determinations were not performed in this study; thus, the bactericidal or fungicidal efficacy of the tested extracts is unknown. The observed antimicrobial activities are promising, but the compounds responsible for the activities and the mechanisms of action need to be determined. Future studies should evaluate the cytotoxicity of the investigated extracts, especially those with promising MIC values (8 - 16 µg/mL). In addition, the safety and therapeutic utility of

these extracts need to be properly evaluated by determining host-cell toxicity and selectivity index values. Therefore, future research should focus on bioactivity-guided fractionation, isolation, and structural elucidation of active phytochemicals using analytical approaches such as LC-MS/MS and metabolomic profiling, elucidation of their antimicrobial mechanisms, evaluation of their synergistic interactions with conventional antimicrobial agents, and assessment of their efficacy and safety in suitable in vivo infection models.

5.1. Conclusions

These findings provide preliminary insights from in vitro screening of the antimicrobial potential of the studied medicinal plants. Nevertheless, further phytochemical characterization, pharmacological evaluation, toxicity assessment, and in vivo validation are needed to establish their therapeutic potential. Such studies may facilitate the future development of novel plant-derived antimicrobial agents to combat the increasing global challenge of antimicrobial resistance.

Footnotes

AI Use Disclosure: Quillbot for grammar check was used for text editing in the abstract section at a Moderate level.

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