

Antibacterial activity of *Phlomis fruticosa* L. plant extracts

Phlomis fruticosa L. bitki ekstrelerinin antibakteriyel aktivitesi

Abstract

Aim: This study aimed to determine the antibacterial activities of extracts obtained from the *Phlomis fruticosa* plant.

Materials and methods: Petroleum ether, ethyl acetate, methanol, and methanol:water (70:30) extracts were obtained from *Phlomis fruticosa* plant by the Soxhlet method. The antibacterial activity of the extracts was determined using the disc diffusion method. Antibacterial activity was started at a concentration of 50 mg/disc. *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* were used in the antibacterial activity tests.

Results: It was determined that the methanol extract showed the highest activity on *Pseudomonas aeruginosa* bacteria among the four extracts by forming a zone diameter of 25 mm. Other extracts were found to have inhibition zone diameters in the 6-12 mm range against the tested bacteria.

Conclusion: In this study, the antibacterial activity of petroleum ether, ethyl acetate, and methanol:water extracts of *P. fruticosa* was studied for the first time. Since the methanol extract has a high inhibition zone diameter against *P. aeruginosa* bacteria, the bioactive compounds responsible for this activity should be investigated in future studies.

Keywords: *Phlomis fruticosa*, extract, antibacterial activity, the disc diffusion method, Soxhlet extraction

Özet

Amaç: Bu çalışmanın amacı, *Phlomis fruticosa* bitkisinden elde edilen ekstrelerin antibakteriyel aktivitelerini belirlemektir.

Gereç ve Yöntem: *Phlomis fruticosa* bitkisinden Soxhlet yöntemi ile petrol eteri, etil asetat, metanol ve metanol:su (70:30) ekstreleri elde edilmiştir. Ekstrelerin antibakteriyel aktiviteleri disk difüzyon yöntemi ile belirlenmiştir. Antibakteriyel aktivite testi 50 mg/disk konsantrasyonda başlatılmıştır. *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Escherichia coli* ve *Pseudomonas aeruginosa* bakterileri antibakteriyel aktivite testlerinde kullanılmıştır.

Bulgular: Dört ekstre içinde metanol ekstresinin 25 mm zon çapı oluşturarak *Pseudomonas aeruginosa* bakterisi üzerinde en yüksek aktivite gösterdiği belirlenmiştir. Diğer ekstrelerin test edilen bakterilere karşı 6-12 mm aralığında inhibisyon zon çaplarına sahip olduğu tespit edilmiştir.

Sonuç: Bu çalışmada, *P. fruticosa* bitkisinin petrol eteri, etil asetat ve metanol:su ekstrelerinin antibakteriyel aktivitesi ilk defa çalışılmıştır. Metanol ekstresinin *P. aeruginosa* bakterisine karşı yüksek inhibisyon zon çapına sahip olması nedeniyle aktiviteden sorumlu biyoaktif bileşiklerin ileriki çalışmalarda araştırılması gerekmektedir.

Anahtar kelimeler: *Phlomis fruticosa*, ekstre, antibakteriyel aktivite, disk difüzyon yöntemi, Soxhlet ekstraksiyonu

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1. Introduction

Plants are a promising option for discovering new antimicrobial agents due to their use in traditional medicine to treat bacterial diseases and their potential to provide an endless variety of chemical compounds for research [1]. Plants have great potential to develop effective solutions against existing drug resistance and to create safer and more effective treatment methods. Bioactive compounds of plant origin attract attention with their broad-spectrum antimicrobial properties and are evaluated in scientific studies [2].

Phlomis fruticosa L. belongs to Lamiaceae family. *P. fruticosa* is known as "Parşamba" in Türkiye [3]. *P. fruticosa* is an ornamental plant widely grown in many gardens worldwide. The plant's natural habitat is the Mediterranean region, where it grows wild from Sardinia in Italy to Anatolia in Türkiye. *Phlomis* species are used to treat various conditions, including diabetes, stomach ulcers, haemorrhoids, inflammation, and wounds. *P. fruticosa* is traditionally used in Italy to prepare pasta sauces and as an antitussive agent, while in Türkiye and Greece, it is used to treat stomach ulcers [4-6].

Studies have shown that extracts obtained from *P. fruticosa* have antibacterial and antifungal activities and play a potential role in relieving the clinical symptoms associated with ulcerative colitis [7,8]. Flavonoids, iridoids, and phenylethyl alcohol were the major secondary metabolite groups of *Phlomis* extracts. Some *Phlomis* extracts have been reported to have antidiabetic, antinociceptive, anti-ulcerogenic, anti-inflammatory, anti-allergic, and antioxidant activities [9-17]. Methanol extracts of *P. bruguieri*, *P. herba-venti*, and *P. olivieri* showed antibacterial and antifungal activities against some bacteria and fungi [18]. The ethanol extract of *P. fruticosa* showed antifungal activity against *Aspergillus ochraceus*, *Cladosporium cladosporioides*, and *Phomopsis helianthi* [8]. Methanol extract of *P. fruticosa* and *P. lanata* showed antioxidant activity by inhibiting bleomycin-Fe(II)-catalyzed arachidonic acid superoxidation [19].

Based on the information obtained from previous research, this study aims to investigate further the antibacterial properties of extracts obtained from *P. fruticosa* using the Soxhlet method. Extracts with four different polarities (petroleum ether, ethyl acetate, methanol, and methanol:water (70:30)) were obtained from the aerial parts of *P. fruticosa*. Additionally, the antibacterial effect of extracts was determined by using the disc diffusion assay.

2. Materials and methods

2.1 Plant material

The aerial part of *P. fruticosa* was collected from Denizli-Türkiye (37° 51' 40"N; 29° 09' 28"E). The dry aerial part of the plant was ground in a grinding mill and powdered.

2.2 Extraction

The dry aerial parts of *P. fruticosa* (123 g) were extracted with petroleum ether, ethyl acetate (EtOAc), methanol (MeOH), and methanol:water (70:30) by the Soxhlet method, respectively. In Soxhlet extraction, 400 mL of each solvent was used, and the heater temperature was set at 60°C. The extraction process continued for 4 hours. After Soxhlet extraction, each extract was filtered with filter paper. The extract was concentrated at 45 °C under reduced pressure using a rotary evaporator (Heidolph) to obtain crude petroleum ether, ethyl acetate, methanol, and methanol:water (70:30) extracts. The extracts were stored at 4 °C in the refrigerator for further analysis [20].

2.4 Antibacterial activity

Antibacterial activity of the extracts was evaluated against two strains: Gram-positive (+) *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*, and Gram-negative (-) *Escherichia coli* and *Pseudomonas aeruginosa* by using the disc diffusion method. Chloramphenicol was used as a positive control. 10% Dimethyl sulfoxide (DMSO) was used as a negative control. All the experiments were performed in triplicate.

3. Results

The disc diffusion method was performed using 50 mg/disc. The petroleum ether extract showed antibacterial activity against only *E. coli*, the inhibition zone diameter was 11 mm. The methanol extract showed the highest activity on *P. aeruginosa* bacteria among the four extracts by forming a zone diameter of 25 mm. The inhibition zone diameters of methanol extract were 6-7 mm for other bacteria except *B. subtilis*. The inhibition zone diameters of ethyl acetate extract were detected between 8-10 mm against *B. subtilis*, *P. aeruginosa*, and *E. coli*. The methanol:water extract was effective against *E. coli*, *B. cereus*, and *P. aeruginosa* (Table 1).

4. Discussion

There are limited studies on the antibacterial activity of *Phlomis fruticosa*. According to a study, ethanol (70%)

**Table 1** The disc diffusion method and inhibitory zone diameters (mm) of *P. fruticosa* extracts

Bacteria Plant Materials	Gram Negative (-)		Gram Positive (+)		
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>
Petroleum ether extract	11	-	-	-	-
Ethyl acetate extract	10	8	-	8	-
Methanol extract	7	25	7	-	6
Methanol:water (70:30)	12	10	-	-	10
Chloramphenicol (positive control)	25	26	30	32	28
DMSO (10%) (negative control)	-	-	-	-	-

extract of *P. fruticosa* leaves investigated antibacterial and antifungal activity against seven bacterial and seven fungal species. The inhibition zone diameters of ethanol extract were 16 mm for *S. aureus* and 17 mm for *B. subtilis* at 100 µg/mL concentration and 12 mm for both bacteria at 20 µg/mL concentration, respectively. The ethanol extract did not form inhibition zones against *E. coli* and *P. aeruginosa* at both concentrations [8].

The antibacterial activity of ethanol, ethanol:water (1:1), and water extracts of *P. fruticosa* and *P. herba-venti* aerial parts were studied against *E. coli*, *P. aeruginosa*, and *S. aureus* using the broth microdilution assay. The ethanol extracts of both plants displayed the highest antibacterial activity against these three bacteria. The ethanol:water extracts showed moderate activity. The water extracts were not effective against test bacteria [7].

In another study, the antimicrobial activity of the methanol extract of the aerial part of *P. fruticosa* was studied against eight bacteria and eleven fungi species using the broth microdilution assay. The methanol extract showed moderate antibacterial activity. The extract had the highest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) value (1.25 mg/mL) against *S. aureus* and methicillin-resistant *S. aureus* bacteria [6].

The methanol extracts of *P. bruguieri*, *P. herba-venti*, and *P. olivieri* were obtained by the maceration method. The antimicrobial activity of the extracts was studied against *S. aureus*, *Streptococcus sanguis*, *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*, and *Aspergillus niger* at 10-1000 µg/disc concentrations by using disc diffusion method. It was determined that the methanol extract of *P. bruguieri* had inhibition zone diameters in the 10.1-16.7 mm and 13.8-18.6 mm

range against *S. aureus* and *S. sanguis* at all tested concentrations, respectively. The inhibition zone diameters of the methanol extract of *P. bruguieri* were 9.8-15.8 mm against *K. pneumoniae* at concentrations of 100-1000 µg/disc, 9.2-11.1 mm against *E. coli* bacteria and 9.9-12.3 mm against *P. aeruginosa* at concentrations of 500-1000 µg/disc. The inhibition zone diameters of methanol extract of *P. herba-venti* were 8.7-14.6 mm against *K. pneumoniae* at 50-1000 µg/disc, 8.3-13.3 mm against *S. sanguis* at 100-1000 µg/disc, 8.7-12.2 mm against *S. aureus* and 8.4-11.2 mm against *E. coli* at 250-1000 µg/disc, 8.5-10.9 mm against *P. aeruginosa* at 500-1000 µg/disc. The methanol extract of *P. olivieri* was effective on *K. pneumoniae* (8.9-15.9 mm) at all tested concentrations. The inhibition zone diameters of methanol extract of *P. olivieri* were 8.9-13.1 mm against *S. aureus* and 9.0-13.6 mm against *E. coli* at 250-1000 µg/disc, 8.9-11.5 mm against *P. aeruginosa* and 8.9-12.0 mm against *S. sanguis* at 500-1000 µg/disc [18]. When the results were compared, it was determined that the methanol extract of the current study had a higher inhibition zone against *P. aureoginosa* bacteria. The reasons for these differences may be the chemical compositions of the plants, extraction methods, and geographical origins.

The antibacterial, antiviral, and antifungal activities of petroleum ether and methanol extracts of seven *Phlomis* species growing in Türkiye were examined. The petroleum ether and methanol extracts showed similar antibacterial activity against the tested bacteria. Methanol extracts were found to have the highest MIC value against *S. aureus* (4 µg/mL) and *Enterococcus faecalis* (2 µg/mL) bacteria. Petroleum ether extract had the highest MIC values against *E. faecalis* (4 µg/mL) and *S. aureus* (8 µg/mL). Both extracts had lower MIC values against *E. coli* (128 µg/mL), *P. aureoginosa* (128 µg/mL), and *B. subtilis* (64 µg/mL and 128 µg/mL) [21]. The petroleum extract of the current study didn't show antibacterial activity against *E. coli*, *S. aureus*, *P. aureoginosa*, and *B. subtilis*. The methanol extract of the current study had better antibacterial activity against *P. aureoginosa* than the previous study. Plant species and extraction methods were different between current and previous studies. These factors may affect the chemical composition of the extracts. For this reason, current and previous studies may show different antibacterial activity.

There are differences between the activity results in the current study and the literature. In the literature, it has been determined that *Phlomis* species are generally

effective against *S. aureus* bacteria. However, the current study determined that only the methanol extract created a very low inhibition zone against *S. aureus*, and the other extracts were ineffective. Contrary to the results in the literature, it was observed that the methanol extract in the current study created a significant inhibition zone against *P. aeruginosa*. The reason for this difference in activity results may be related to the different extraction methods, extract concentrations, extract contents, storage conditions of the extracts, and geographical regions. Additionally, the use of solvents of different polarity may affect the chemical composition of the extracts and, therefore, exhibit different synergistic effects against bacteria.

In this study, the antibacterial activity of petroleum ether, ethyl acetate, and methanol:water extracts of *P. fruticosa* was studied for the first time. Since the methanol extract has a high inhibition zone diameter against *P. aeruginosa* bacteria, the bioactive compounds responsible for the activity should be investigated in future studies.

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