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# Antimicrobial Activity of *Pulicaria* Species from Turkey

## Türkiye'deki *Pulicaria* Türlerinin Antimikrobiyal Aktivitesi

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## ABSTRACT

**Objective:** In traditional medicine, the *Pulicaria* species are used for various disorders such as inflamed wounds, skin diseases, and bronchitis. This study investigated the antibacterial effect of five *Pulicaria* species in Turkey; (*Pulicaria (P) arabica* (L.) Cass., *P. dysenterica* (L.) Bernh., *P. odora* (L.) Reichb., *P. sicula* (L.) Moris, *P. vulgaris* (L.) Gaertn.) against certain significant pathogenic gram-negative and gram-positive reference bacteria.

**Material and Method:** Four extracts (decoction, infusion, aqueous, and ethanol (EtOH) extracts) were prepared from the *Pulicaria* species. The antimicrobial activity of the samples was examined against reference organisms; *Bacillus (B) subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Methicillin-resistant Staphylococcus (S) aureus (MRSA), Proteus mirabilis, S. aureus* and *Pseudomonas aeruginosa*. The minimal bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) ranges of the extract samples were demonstrated based on a microbroth dilution method.

**Results:** The EtOH extracts of the studied four *Pulicaria* species were found to be weakly active against gram-positive bacteria such as *B. subtilis*, MRSA and *S. aureus* but the EtOH extract of *P. dysenterica* showed exceptionally good activity against the reference strains of *S. aureus*, MRSA. No antimicrobial activity was detected in the infusion, decoction, and aqueous extracts.

**Conclusion:** The *Pulicaria* species, especially *P. dysenterica* could be evaluated as antimicrobial agents. Further studies with the extracts and essential oils from *Pulicaria* sp. on other bacteria and pathogenic fungi should be performed.

**Keywords:** *Pulicaria*, antimicrobial activity, extracts, Turkey

## ÖZ

**Amaç:** Geleneksel tıpta *Pulicaria* türleri iltihaplı yaralar, cilt hastalıkları, bronşit gibi çeşitli rahatsızlıklarda kullanılmaktadır. Bu çalışmada Türkiye'deki beş *Pulicaria* türünün (*Pulicaria (P) arabica* (L.) Cass., *P. dysenterica* (L.) Bernh., *P. odora* (L.) Reichb., *P. sicula* (L.) Moris, *P. vulgaris* (L.) Gaertn.), bazı önemli patojenik gram-negatif ve gram-pozitif referans bakterilere karşı etkisi araştırılmıştır.

**Gereç ve Yöntem:** *Pulicaria* türlerinden dört ekstre (kaynatma, infüzyon, sulu ve etanol (EtOH) ekstreleri) hazırlandı. Örneklerin, antimikrobiyal aktivitesi referans organizmalara karşı incelendi; *Bacillus (B) subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae,* Metisiline dirençli *Staphylococcus (S) aureus* (MRSA), *Proteus mirabilis, S. aureus* ve *Pseudomonas aeruginosa*. Ekstre numunelerinin minimum bakterisidal konsantrasyon (MBC) ve minimum inhibitör konsantrasyon (MIC ) aralıkları, mikrobroth seyreltme yöntemi temel alınarak gösterilmiştir.

**Bulgular:** İncelenen dört *Pulicaria* türünün EtOH ekstrelerinin; *B. subtilis,* MRSA ve *S. aureus* gibi gram pozitif bakterilere karşı genellikle zayıf aktif olduğu bulunmuştur, ancak *P. dysenterica*'nın EtOH ekstresi, *S. aureus*, MRSA referans suşlarına karşı çok iyi aktivite göstermiştir. İnfüzyon, dekoksiyon ve sulu ekstrelerde antimikrobiyal aktivite tespit edilememiştir.

**Sonuç:** *Pulicaria* türleri, özellikle *P. dysenterica* antimikrobiyal ajan olarak değerlendirilebilir. *Pulicaria* sp.'den elde edilen ekstreler ve uçucu yağlar ile diğer bakteri ve bazı patojenik mantarlar üzerinde daha ileri çalışmalar yapılmalıdır.

**Anahtar Kelimeler:** *Pulicaria*, antimikrobiyal aktivite, ekstreler, Türkiye

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## INTRODUCTION

The genus *Pulicaria* Gaertn. is represented with about 149 taxa in the world (1). The *Pulicaria* species are widely used in traditional Turkish medicine. For instance: the species are used for bronchitis, colds, and inflamed wounds in Turkey (2), for diabetes, ankle sprains, headaches, and flatulent colic in Yemen (3), as cicatrizant, anti-inflammatory, for muscular-skeletal diseases, and skin diseases in Spain (4,5), and for abdominal pain in Mauritania (6). These species are also used in folk veterinary medicine in Italy as antiparasitics, repellents, and for respiratory ailments (7).

Distinct types of *Pulicaria* phytochemicals have been reported, including essential oils, flavonoids, phenolic derivatives, monoterpene derivatives, sesquiterpenes, diterpenes, triterpenes, steroids, and others (8). Mohamed et al. (2020) demonstrated that methanolic crude extracts of *Pulicaria (P) undulata and P. crispa* were rich in coumarins, saponins, sterols, tannins, and terpenes based on preliminary phytochemical screening (9). Until that time no flavonoids, alkaloids, and anthraquinones were found. According to identification of the essential oils of *P. crispa*; 1.4-ditert-butylbenzene, carvone, caryophyllene, and neryl (s)- 2-methylbutanoate were determined as the main compounds. The percentages of active ingredients are given respectively; 22.81%, 11.80%, 13.19%, and 10.33%. In the same study the main compounds of *P. undulata* essential oil were determined as camphor, thymyl acetate, bicycle, and azulenol. The percentages of active ingredients are given respectively 44.48%, 10.31%, 3.46%, and 3.40%. In the literature, there are several studies on essential oil constituents of *Pulicaria* species (10-16).

In the literature, there are several biological activity studies on *Pulicaria* species such as antimicrobial, antioxidant, anticholinesterase, analgesic, antipyretic, anti-inflammatory, cytotoxicity (HL-60, MCF-7, Hep-G2 cells), and hepatoprotective (11,14,17-22). The number of studies on the antimicrobial activities of the medicinal plant as a potential antimicrobial drug which functions as new antibacterial agents have increased in recent years (23). The antimicrobial activity of essential oils, plant extracts or isolated compounds used in traditional medicines is the subject of several studies. This study was conducted with the idea of determining the antimicrobial activities of *Pulicaria* species to illuminate the traditional uses of wound healing, bronchitis, colds, skin diseases, and abdominal pain.

The main goals of this research were to find out how effective five *Pulicaria* species (*P. arabica* (L.) Cass., *P. dysenterica* (L.) Bernh., *P. odora* (L.) Reichb., *P. sicula* (L.) Moris, *P. vulgaris* (L.) Gaertn.) were at fighting bacteria. Aqueous extracts, ethanol (EtOH) extracts, infusion, and decoction of these five *Pulicaria* species were evaluated against the reference strains of forementioned microorganisms.

## MATERIAL AND METHOD

### Plant Materials

*Pulicaria* species were gathered between 2018-2019. The studied species, herbarium numbers, and localities are given in Table 1. The plants were identified by Dr. Bahar Gürdal and Dr. Ebru Özdemir Nath. The *Pulicaria* species are stored in Istanbul University Herbarium of the Faculty of Pharmacy (ISTE).

### Extraction of Plant Materials

For each species, four extracts (infusion, decoction, aqueous, and EtOH extracts) were prepared from aerial parts. The diverse techniques of extract preparation were chosen to compare the biological activity of traditionally made decoctions and infusions to water and EtOH extracts prepared under controlled laboratory circumstances. At room temperature, the plant materials were air-dried and powdered. For EtOH extracts, 50 grams (g) of each species were macerated with EtOH (250 mL, Merck) for 24 hours at room temperature and filtered. The solvents were evaporated to dryness under vacuum (Buchi Rotavapor R-210). For aqueous extracts, 5 g of each species were macerated with distilled water (25 mL) for 24 hours. Then it was filtered. Infusions were prepared by adding 25 mL of boiling distilled water to each species (5 g) and allowed to stand at room temperature for 20 min. For decoction, 5 g of each species were added to 25 mL of distilled water and heated for 20 minutes, and then filtered.

### Determination of Antimicrobial Activity

The antibacterial effect of the plant extracts was evaluated against some of the reference bacteria using a standard microbroth dilution technique as defined in the guideline of Clinical Laboratory Standards Institute (24). The test organisms were provided from the Microorganism Culture Collection of Istan-

**Table 1.** Studied species information.

| Species | Locality | Herbarium number |
|---|---|---|
| *P. arabica* | Muğla: Bodrum, Göltürkbükü, 19.vii.2019, B. Gürdal, E. Özdemir Nath | ISTE 116891 |
| *P. dysenterica* | İstanbul: Çatalca, Subaşı, 14.viii.2018, B. Gürdal, E. Özdemir Nath | ISTE 116730 |
| *P. odora* | Yalova: Armutlu, 06.vi.2019, B. Gürdal, E. Abamor | ISTE 116747 |
| *P. sicula* | Bursa: Nilüfer, Gölyazı, 19.x.2019, B. Gürdal, E. Özdemir Nath | ISTE 116910 |
| *P. vulgaris* | Edirne: Enez, Sultaniçe, 20.viii.2019, B. Gürdal, E. Özdemir Nath | ISTE 116895 |

bul University, Department of Pharmaceutical Microbiology. The reference antimicrobials, Ciprofloxacin (Bayer Türk Kimya San. Istanbul/Turkey) as antibiotic and dimethyl sulfoxide (Merck, Darmstadt, Germany) as a solvent and growth mediums Mueller Hinton Broth (MHB) and tryptic soy agar (TSA) were obtained from BD Difco (Fisher Scientific, Göteborg, Sweden).

DMSO was used as a solvent for EtOH extracts with a concentration of 10,000µg/mL and as a vehicle control to detect the possible inhibitory activity of the extract dilution. Ciprofloxacin was used as a reference antibiotic. The minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) were determined for each extract.

### Examination of MIC and MBC Assays

Inoculums were prepared from overnight cultures from each microorganism in MHB medium. The density was adjusted using McFarland densitometer (Biosan, Riga, Latvia) equivalent to 0.5 McFarland and diluted 1/100 to the final concentration $10^6$ CFU/m.

After the preparation of inoculums, the microtiter plate wells were inoculated with 50 µg/mL MHB starting from the second until the last well. 10,000 µg/mL from EtOH extracts and 100,000 µg/mL concentration from infusion, decoction, and maceration extracts were inoculated to the first two wells. Two-fold serial dilutions were made (starting concentration of 10,000 µg/m L and 100,000 µg/mL in the first well to the minimum concentration 19.52 µg/mL and 195.2 µg/mL in the tenth well respectively). Finally, bacterial suspensions and 25 µl of resazurin solution (0.001%) were inoculated to each well, except the negative control well, and the range of 5000-9.76 µg/mL and 50.000-97.6 µg/mL were achieved, respectively.

The standard antibiotic ciprofloxacin's 2-fold serial dilutions were prepared in cation-adjusted MHB (CAMHB), in the ranges 5,120 to 64 mg/L. 50 µL from 32 to 0.03 µg/mL concentrations of the antibiotic solution as well as DMSO were directly inoculated to each corresponding microtiter well by serial dilutions and the microtiter plates were incubated at 37°C for 24 hours. Microbial growth in the wells was determined as positive which turned from lilac to pink by resazurin dye. The MIC was defined as the lowest concentration of the samples that completely inhibits the growth of microorganisms. To determine the MBC, broth samples were removed from the wells with no growth and placed on the TSA plates overnight at 37°C. MBCs were detected with samples that did not show any bacterial growth.

## RESULTS

The EtOH extract of *P. sicula* showed activity against *Enterococcus (E) faecalis, Methicillin-resistant Staphylococcus (S) aureus (MRSA),* and *S. aureus* and the EtOH extracts of *P. arabica*, *P. vulgaris* and *P. odora* showed almost the same activity against *S. aureus*, MRSA, and *E. faecalis*. The EtOH extract of *P. odora* was also active against *Bacillus (B) subtilis*. Especially the EtOH extract of *P. dysenterica* was found active against most of the reference organisms. The MIC/ MBC results of the EtOH extract of *P. dysenterica* against MRSA, *S. aureus*, *E. faecalis*, *Proteus mirabilis* and *Klebsiella (K) pneumoniae* are 39/156 µg/mL, 156 µg/mL, 1250 µg/mL, 625/1250 µg/mL, and 625/1250 µg/mL, respectively as shown in Table 2.

The findings show that the EtOH extracts of *Pulicaria* species in Turkey are especially active against gram-positive pathogens such as *MRSA, S. aureus* and *E. faecalis* with a decrease up to 2-64 times in MIC values compared to DMSO, as shown in Table 2. In each case, the activities of the EtOH extracts of *Pulicaria* species were lower than those of the standard antibiotic ciprofloxacin. No antimicrobial activity was detected in the infusion, decoction, and aqueous extracts. It has been also determined that the *Pulicaria* extracts have not shown any antibacterial activity against some of the clinically important pathogenic bacteria such as *Pseudomonas aeruginosa* and *Escherichia (E) coli.*

**Table 2.** The results of antimicrobial assays (*Pulicaria* EtOH extracts, MICs/MBCs, µg/mL).

| ***Pulicaria* sp.** | ***E. coli*** | ***S. aureus*** | ***MRSA*** | ***E. faecalis*** | ***K. pneumoniae*** | ***B. subtilis*** | ***Pseudomonas aeruginosa*** | ***Proteus mirabilis*** |
|---|---|---|---|---|---|---|---|---|
| *P. sicula* | 1250 | 625 | 312.5 | 2500 | 1250 | 1250 | 1250 | 1250 |
| *P. arabica* | 1250 | 625/1250 | 625 | 1250/2500 | 1250 | 1250 | 1250 | 1250 |
| *P. vulgaris* | 1250 | 1250 | 625 | 1250/2500 | 1250 | 1250 | 1250 | 1250 |
| *P. odora* | 1250 | 625/1250 | 625 | 1250/2500 | 1250 | 625/1250 | 1250 | 1250 |
| *P. dysenterica* | 1250 | 156 | 39/156 | 1250/1250 | 625/1250 | 1250 | 1250 | 625/1250 |
| DMSO | 1250 | 2500 | 2500 | 2500 | 1250 | 1250 | 1250 | 2500 |
| Ciprofloxacin | 0.125 | 1 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

*Reference strains: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, MRSA (methicillin-resistant S. aureus) ATCC 43300, Enterococcus faecalis ATCC 29212, Klebsiella pneumoniae ATCC 4352, Proteus mirabilis ATCC 14153, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633*

## DISCUSSION

In the studies conducted to ascertain the antimicrobial activities of the *Pulicaria* species, successful results on gram-positive and gram-negative, clinically important bacteria were obtained in different extracts. Nickavar and Mojab, (25) assessed the methanolic, aqueous, and chloroform extracts of *P. dysenterica* aerial parts from Tehran, Iran for their antibacterial effect using the disc-diffusion technique. The six bacteria - *E. coli, Bacillus cereus, Shigella dysenteriae*, *Salmonella typhi*, *Vibrio cholera* and *S. aureus* - were tested and the most powerful extract against *V. cholera, B. cereus, S. aureus* and was the methanolic extract. All the extracts against *Vibrio cholera* were successfully active. Similar to this study, the EtOH extract of *P. dysenterica* has good activity against tested gram-positive bacteria. In both studies, there were not any activity against one of the significant pathogenic gram-negative bacteria such as *E. coli*.

Touati et al. (26), researched the antibacterial activity of leaves and roots of *P. odora* from northern Algeria. The findings showed that *S. aureus* was the most sensitive to the acetonic root extract, while *Pseudomonas aeruginosa* was the most resistant to the chloroformic leaf extract. According to Naqvi et al. (27), the findings of the analysis revealed that EtOH extract of *P. gnaphalode* from Quetta, Pakistan demonstrated a maximum inhibition zone for *B. subtilis* from all others. The methanol extract (ME) demonstrated a maximum inhibition zone for *S. aureus*. Zhanzhaxina et al. (28), analyzed the structure of the EtOH and $CHCl_3$ extracts of *P. vulgaris* from Akmola, Kazakhstan, and determined the biological function of the extracted compounds. All isolated compounds have been tested against *E. coli, S. aureus, B. cereus, Salmonella enteritidis,* and *Candida albicans*. The percent inhibition showed negative values in some cases, indicating that the compound did not decrease the number of bacteria. The antimicrobial activity of the $CH_2Cl_2$, MeOH, EtOAc extracts of the *P. undulata* aerial parts from Egypt was investigated by Abdel Bar et al. (29). The antimicrobial activity was measured against *S. aureus*, *K. pneumoniae*, *E. coli,* and *Pseudomonas aeruginosa*, and *C. albicans*. The phenolic rather than the terpenoidal compounds exhibited remarkable antimicrobial activity. The research of Foudah et al. (30), was intended to question the antimicrobial activity of the ME of *P. crispa* from Alkharj- the, Saudi Arabia. The antimicrobial activity was tested against *S.aureus, B. subtilis, E. coli, K. pneumonaie, Aspergillus niger*, *Proteus vulgaris,* and *C. albicans*. Antimicrobial activities were observed which may be due to the presence of phenols, tannins, and flavonoids in the ME. The study of El-Shahaby et al. (31) aimed to determine the antimicrobial potential of ethyl acetate and diethyl ether extracts of *P. incisa* (Lam.) DC from Egypt. The antimicrobial activities of extracts against *K. pneumoniae, B. subtilis, S. epidermidis, S. aureus, E. coli,* and *C. albicans* were determined by the disc diffusion technique. As a result of this study, the ethyl acetate extract has antimicrobial effect on *S. epidermidis, S. aureus* and *C. albicans* while the diethyl ether has activity against *B. subtillis* and *C. albicans.* Nair et al. (32) screened for antibacterial activity of *P. wightiana* from Rajkot Gujarat, India. Agar disc diffusion assay was used for aqueous extract and Agar disc diffusion assay used for ME against *Staphylococcus epidermidis, Pseudomonas testosteroni, B. subtilis, Proteus morganii, Micrococcus flavus,* and *K. pneumoniae.* The ME showed greater activity than the aqueous extract. The methanolic extracts are active against *Micrococcus flavus, B. subtilis* and *Proteus morganii.* The aqueous extract revealed a negligible amount of action. In this study, the aqueous extracts of studied *Pulicaria* species also showed the same results.

## CONCLUSION

In this research, the antimicrobial activity of five species of *Pulicaria* in Turkey was investigated by the microdilution method modified with resazurin. The EtOH extracts obtained from *Pulicaria* species were found to be weakly active against the gram-positive bacteria such as *B. subtilis,* MRSA, *S. aureus*, but the EtOH extract of *P. dysenterica* showed particularly good activity against *S. aureus*, MRSA. These results suggest that the EtOH extract of *P. dysenterica* would be a good therapeutic agent against these bacteria. Further studies such as *in vitro* antimicrobial activity testing are suggested to evaluate antifungal and antibacterial activities of essential oils obtained from active *Pulicaria* species in Turkey.

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