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# Antibacterial Activity of *Mentha pulegium* L. from Turkey

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**Abstract:** In this research, the antibacterial activity of crude extracts obtained from leaves of pennyroyal (*Mentha pulegium* L.) plant against *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228 was investigated. Acetone and methanol were used as chemical solvents to prepare the extracts of *Mentha pulegium* L. The determination of antibacterial activity was performed by using disc diffusion method. For each extract, its own solvent was utilized as negative control. Standard antibiotic discs (Clindamycin, Tetracycline and Amoxicillin-Clavulanic acid) were also used as positive control. The results of present study showed that the crude extracts of *Mentha pulegium* L. prepared in acetone and methanol had antibacterial activity against test microorganisms and the most antibacterial effect was observed against *Staphylococcus epidermidis.* It was determined that the extract of *Mentha pulegium* L. (6400 µg/disc) has more antibacterial activity against *E. faecalis* and *S. epidermidis* than Tetracycline (10 µg/disc) and has more antibacterial activity against *E. coli* than Clindamycin (10 µg/disc). However it was found that antibacterial activity of *Mentha pulegium* L. extract (6400 µg/disc) was close to Tetracycline (10 µg/disc) for *E. coli* and *S. aureus* and was close to Clindamycin (10 µg/disc) for *E. faecalis*.

**Keywords:** Antibacterial Activity, *Mentha pulegium* L., Pennyroyal

## 1. Introduction

Infectious diseases which are one of the main causes of morbidity and mortality worldwide represent a critical problem to health [1]. Pharmacological industries have produced number of new-antibiotics in the last three decades, but microbial resistance to these antibiotics has increased because of genetic ability of the bacteria to acquire and transmit the resistance against therapeutic agents [2].

Nowadays developing of new, reliable, cost-efficient and non-toxic herbal antimicrobial agent is quite important in order to minimize environmental and health problems [3]. Investigation of new antimicrobial compound with diverse chemical structures and novel mechanism of action has become a continuous and urgent necessity due to increasing in the incidence of new and re-emerging infectious diseases. In recent years development of resistance to the antibiotics has made it necessary to discover new antimicrobial substance obtained from other sources including plants. Higher plants produce diverse chemical compounds such as antimicrobial substances that are active against plant and human pathogenic microorganisms [4].

Plants which are an essential and integral part of complementary and alternative medicine, generate secondary metabolites that are used to restore health and to treat many diseases [5]. Secondary compounds play a role in defense against herbivore animals and pathogenic microorganisms. Chemical structure of these compounds is complicated. The main types of secondary compounds are phenolic, terpene, terpenoids and alkaloids [6].

National center for complementary and alternative medicine (NCCAM) is the agency for scientific researches about medical qualification of treatments that are performed with herbal medicine [6]. Nowadays, there has been a growing interest in the determination of the biological and antimicrobial properties of herb extracts derived from several

medicinal plants such as *Mentha* species. *Mentha pulegium* is considered as medicinal plant because of its pharmacological and biological properties [7].

*Mentha pulegium* L. that has been known and used since ancient times is a species of *Mentha*. This species is used in folk medicine as digestive, emmenagogue, antitussive, antiseptic and abortifaceant [8, 9, 10]. This herb is also utilized for diabetes [11]. *Mentha pulegium* L. commonly known "pennyroyal" or "poejo" has pungent odour and is spread to Europe, Mediterranean area, east to Iran [10, 12, 13]. Leaves and shoots of *Mentha pulegium* L. are used as food and tea [14]. This herb grows in humid places: on mountains at an altitude about 700 m and on plains [15]. The aim of the present study was to assess and compare the antibacterial activities of *Mentha pulegium* L. extracts prepared in acetone and methanol against pathogenic bacteria.

# 2. Material and Method

### *2.1. Material*

Pennyroyal (*Mentha pulegium* L.) used in the present study was obtained as commercially in Turkey. Test microorganisms used in experimental procedures were *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228 which were supplied from Microorganism Culture Collections Research and Application Center of Istanbul University.

### *2.2. Preparation of Extracts*

Dried leaves of *Mentha pulegium* L. were grinded by using mortar and pestle. Plant powders were kept in solvents over the night. Acetone (Merck) and methanol (Merck) were used as chemical solvents. Then, the solvents in the obtained extracts were removed by using rotary evaporator (Heidolph Laborota 4000 Efficient) under vacuum at 50°C for 15 minutes. The extract concentrations were adjusted by adding own solvent to each extract at the determined rates (6400 µg/30µl, 3200 µg/30µl, 1600 µg/30µl, 800 µg/30µl and 400 µg/30µl).

### *2.3. Determination of Antibacterial Activity*

Bacteria strains were inoculated to Tryptic Soy Broth and were incubated at 37°C for 24 hours. At the end of the incubation, bacterial density was adjusted to 0.5 McFarland by using a densitometer. Disc diffusion method was applied to determine the antibacterial activity of leaves of *Mentha pulegium* L. Sterile discs (6 mm in diameter) were saturated by the prepared 30 µl extracts and were allowed to dry. For each extract, its own solvent was used as negative control. Standard antibiotic discs (Clindamycin (10 µg), Tetracycline (10 µg) and Amoxicillin-Clavulanic acid (30 µg)) that were obtained commercially were utilized as positive control. Bacterial suspension which was adjusted to 0.5 McFarland was inoculated to Mueller Hinton Agar media by using sterile swabs. The discs slightly pressed onto the Agar. Agars which were prepared by using this protocol were kept in incubator at 37°C for 24 hours. At the end of the incubation, the diameters of the inhibition zone (IZs) were measured to determine the antibacterial activity. Experimental studies were carried out three times and the diameters of IZs were the average of three replicates.

# 3. Results and Discussion

In present study, the antibacterial activity of *Mentha pulegium* L. against *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228 were determined. The obtained results of antibacterial activity of *Mentha pulegium* L. extracts prepared in acetone and methanol were presented in (Table 1).

***Table 1.*** *Inhibition zone diameters of Mentha pulegium L. extract prepared in acetone and methanol.*

| Extracts ( µg/disc) | Test Microorganisms | | | |
|---|---|---|---|---|
| | *E. coli* | *E. faecalis* | *S. aureus* | *S. epidermidis* |
| | Inhibition zone diameters (mm) | | | |
| Acetone | | | | |
| 6400 | 15 | 13 | 17 | 22 |
| 3200 | 12 | 10 | 15 | 17 |
| 1600 | 9 | 9 | 11 | 12 |
| 800 | 9 | 0 | 10 | 10 |
| 400 | 9 | 0 | 0 | 9 |
| Control | 0 | 0 | 0 | 0 |
| Methanol | | | | |
| 6400 | 10 | 9 | 16 | 22 |
| 3200 | 9 | 9 | 14 | 18 |
| 1600 | 9 | 9 | 10 | 15 |
| 800 | 8 | 8 | 9 | 11 |
| 400 | 8 | 8 | 8 | 8 |
| Control | 0 | 0 | 0 | 0 |
| Clindamycin (10 µg) | 7 | 14 | 31 | 35 |
| Tetracycline (10 µg) | 17 | 8 | 19 | 8 |
| Amoxicillin-Clavulanic acid (30 µg) | 24 | 24 | 30 | 32 |

The inhibition zone diameter was found to increase parallel to the concentration of the extract used. The extract of *Mentha pulegium* L. prepared in acetone showed more antibacterial activity than the extract of *Mentha pulegium* L. prepared in methanol. The results showed that plant extracts which prepared acetone and methanol were determined to be most effective against *S. epidermidis*, with 22 mm inhibition zone diameter.

The inhibition zones of the extract of *Mentha pulegium* L.(6400 µg/disc) in acetone were determined to be 15 mm, 13 mm, 17 mm, 22 mm; in methanol were determined to be 10 mm, 9 mm, 16 mm, 22 mm against *E. coli*, *E. faecalis*, *S. aureus* and *S. epidermidis*, respectively. Obtained results showed that the extract of *Mentha pulegium* L. (6400 µg/disc) has more antibacterial activity against *E. faecalis* and *S. epidermidis* than Tetracycline (10 µg) and has more

antibacterial activity against *E. coli* than Clindamycin (10 µg). Also, it was determined that antibacterial activity of *Mentha pulegium* L. extract (6400 µg/disc) was close to Tetracycline (10 µg) against *E. coli* and *S. aureus* and was close to Clindamycin (10 µg) against *E. faecalis*.

Ghazghazi *et al.* have been reported that *M. pulegium* which was collected from the northwestern part of Tunisia (Ain Draham) was found to be rich in phenols and flavonoids (43.4 mg GAE/g DW and 29.3 mg CE/g, respectively). Methanolic extract of *M. pulegium* was suggested as a potential source of natural antioxidants. Pennyroyal would be center of interest in the food industry with flavoring, antioxidant and antimicrobial properties [7].

The chemical composition of *Mentha pulegium* L. oil was determined by number of studies which have shown a difference in its composition depending on the geographical origin and the specific ecological sites from which plant material was collected. Marzouk *et al.* reported that the major components of the leaves collected from Monastir were menthol (46.60-49.86%), 1.8 cineole (13.53-17.31%), menthone (11.13-12.34%) and pulegone (3.76-4.78%) However, in another research presented by Boukhebti *et al.* the major component of *Mentha pulegium* L. oil was pulegone (38.815 %), other components were: menthone (19.240%), pipériténone (16.528%), pipéritone (6.348%) and isomenthone (6.096%), Limonène (4.293%), Octaan-3-ol (1.854%) [16].

In the study of Shirazi *et al.*, methanolic extract of *Mentha pulegium* L. has shown no cytotoxic effects, but the essential oil of this plant proved to be a potent cytotoxic agent on human ovary adenocarcinoma SK-OV-3, human malignant cervix carcinoma Hela, and human lung carcinoma A549 cell lines [17].

Sivropoulou *et al.* determined that the *Mentha pulegium* L. essential oil has shown antibacterial activity against *Escherichia coli* (NCIMB 8879 and NCIMB 12210), *Pseudomonas aeruginosa* (NCIMB 12469), *Salmonella typhimurium* (NCIMB 10248), *Staphylococcus aureus* (NCIMB 9518 and NCIMB 8625), *Rhizobium leguminosarum* (NCIMB 11478), and *Bacillus subtilis* (NCIMB 3610) [18]. Marzouk *et al.* also reported that *Mentha pulegium* L. essential oil from leaves has exhibited antibacterial effect against *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIMB 8166, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, *Aeromonas hydrophila, Citrobacter freundii, Enterobacter cloaceae, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enteritidis, Serratia marcescens, Shigella flexneri, Vibrio cholerae* non-O1. A study of the antimicrobial effect of *Mentha pulegium* L. essential oil noticed that EO has exhibited a significant effect against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Candida albicans* and *Vibrio cholerae* with IZ = 13–21 mm [19]. Antimicrobial activity and major constituents of *Mentha pulegium* L. leaves essential oil reported in different studies and countries were summarized in the literature [7].

In the study of Stanojkovic *et al.*, antibacterial activity of water, ethanol and ethyl acetate extracts of *Mentha pulegium* aerial parts (stem with flowers) was determined by the filter disc diffusion method against *Rhizobium radiobacter*, *Bacillus subtilis*, *Pectobacterium carotovorum*, *Escherichia coli*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Micrococcus luteus* and *Staphylococcus aureus*. It was reported that the water and ethanol extracts of *M. pulegium* were more active than its ethyl acetate extract and the most susceptible bacteria were *R. radiobacter*, *P. fluorescens* and *M. luteus* [20].

The results of the present study showed that *Mentha pulegium* L. leaves extracts prepared in acetone and methanol had antibacterial activity against *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis* and the most antibacterial effect was observed against *Staphylococcus epidermidis.* The diameters of IZs for bacterial strains, which were sensitive to *M. pulegium* extracts (6400 µg/disc) were in the range of 9-22 mm.

It was determined that the extract of *Mentha pulegium* L. (6400 µg/disc) has more antibacterial activity against *E. faecalis* and *S. epidermidis* than Tetracycline (10 µg) and has more antibacterial activity against *E. coli* than Clindamycin (10 µg). However it was found that antibacterial activity of *Mentha pulegium* L. extract (6400 µg/disc) was close to Tetracycline (10 µg) for *E. coli* and *S. aureus* and was close to Clindamycin (10 µg) for *E. faecalis*. It is remarkable that the effect of the *Mentha pulegium* L. extract against some bacterial strains is close to or more than standard antibiotics. It was observed that antibacterial effect of Pennyroyal extracts were dose-dependent.

# 4. Conclusion

It was determined that *Mentha pulegium* L. leaves extracts prepared in acetone and methanol had antibacterial activity against *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis*. The most antibacterial effect was observed against *Staphylococcus epidermidis.* The dose dependent antibacterial activity of *Mentha pulegium* L. methanol and acetone extract against *E. coli*, *E. faecalis*, *S. aureus* and *S. epidermidis* are reported in this study for the first time.

This species might be a good candidate for developing new antimicrobials and may be used in important applications in food and pharmaceuticals industry. Further research is required to determine the efficacy and side effects of this plant before using it in human.

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