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# Antimicrobial Activity of Some Medicinal Plants from the *Apiaceae* Family[A]

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**Abstract:** In this study, the antimicrobial activity of some medicinal plant seeds from the *Apiaceae* family which are anise, cumin, and fennel was investigated. Moreover, ethanol (80%) and methanol (80%) extracts of anise, cumin, and fennel seeds and essential oils of them were performed for this aim. *Escherichia coli* ATCC 25922 and *Salmonella* Enteritidis ATCC 13076, *Bacillus subtilis* ATCC 6633, *Streptecoccus mutans* ATCC 25175 and *Listeria monocytogenes* ATCC7644 were utilized as test microorganisms. Then, essential oils and ethanol extracts of cumin seeds showed the highest antimicrobial effects on almost all of the test microorganisms. While methanol extracts of fennel seeds had the highest antimicrobial effects against *St. mutans* and *B. subtilis* when compared to other methanol extracts. Additionally, methanol extracts of anise seeds indicated the highest effect on *E. coli*. However, all types of medicinal plant seeds had antimicrobial effects on test microorganisms in various severities (7.00-20.00 mm zone diameter) except *L. monocytogenes*. Thus, the results support existing scientific evidence for the evaluation of these plants as natural antimicrobial agents in several industries including food and drugs.

**Keywords:** Anise, antimicrobial activity, cumin, fennel.

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[A] This study does not require ethics committee approval. The article has been prepared in accordance with research and publication ethics.

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## *Apiaceae* Ailesine Ait Bazı Tıbbı Bitkilerin Antimikrobiyel Aktiviteleri

**Öz:** Bu çalışmada, *Apiaceae* familyasından olan anason, kimyon ve rezene gibi bazı tıbbi bitki tohumlarının antimikrobiyel aktivitesi araştırılmıştır. Ayrıca, bu amaçla tohumların etanol (%80) ve metanol (%80) ekstraktları ile uçucu yağları incelenmiştir. *Escherichia coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076, *Bacillus subtilis* ATCC 6633, *Streptecoccus mutans* ATCC 25175 ve *Listeria monocytogenes* ATCC7644 test mikroorganizmaları olarak kullanılmıştır. Kimyon tohumlarının uçucu yağları ve etanol ekstraktları, test mikroorganizmalarının hemen hemen hepsi üzerinde en yüksek etkiyi göstermiştir. Rezene tohumlarının metanol ekstraktları, diğer metanol ekstraktlarıyla karşılaştırıldığında *St. mutans* ve *B. subtilis*'e karşı en yüksek etkiye sahiptir. Ayrıca, anason tohumlarının metanol ekstraktları *E. coli* üzerinde en yüksek etkiyi göstermiştir. Yine de tüm tıbbi bitki tohumları, *L. monocytogenes* hariç test mikroorganizmaları üzerinde çeşitli düzeylerde (7,00-20,00 mm zon çapı) antimikrobiyel etki göstermiştir. Sonuç olarak; çalışmanın bulguları, bu bitkilerin gıda ve ilaç da dahil olmak üzere çeşitli endüstrilerde doğal antimikrobiyel ajanlar olarak değerlendirilmesi için mevcut bilimsel kanıtları desteklemektedir.

**Anahtar Kelimeler:** Anason, antimikrobiyel aktivite, kimyon, rezene.

## Introduction

Homemade medicines from medicinal plants have been used since the first times of human civilization. Essential oils and extracts of medicinal and aromatic plants are reported to show various biological activities like antimicrobial, anti-inflammatory, and antioxidant activities and some are classified as safe substances, and utilized to inhibit the growth of pathogenic microorganisms and contaminants for food safety and meet public health. A chemical compound of the plant extract or essential oils contains various significant antimicrobial ingredients that are secondary metabolites like terpenoids, phenols, alkaloids, and flavonoids. These components have a hydrophobicity that divides in the lipids of the cell membrane and mitochondria to increase the permeability of them. As a consequence of the rising occurrence of antibiotic-resistant pathogens, identifying and isolating new bioactive compounds from medicinal plants applying standardized modern analytical procedures has become vital. Then, compounds of medicinal plants could provide new approaches to struggle with pathogenic bacteria (Khorsandi et al., 2018; Özoğlu and Altuntaş, 2019; Tao et al., 2021; Vaou et al., 2021; Özoğlu et al., 2022; Kukhtenko et al., 2024).

*Apiaceae (Umbelliferae*) is a large and well-known medicinal plant family originating from the Mediterranean region, India and Iran, widely used their broad spectrum of therapeutic effects. Antimicrobial, antioxidant, therapeutic, and insecticidal effects of the family were known. Cumin (*C. cyminum* L.)*,* fennel (*Foeniculum vulgare* L.), and anise (*Pimpinella anisum* L.) are some of them that have been used as flavor enhancer, and preservatives in food products for centuries (Pecarski et al., 2017; Akbar, 2020; Demir and

Korukluoglu, 2020; Hajlaoui et al., 2021; Das et al., 2022; Thiviya et al., 2022; Hamada Saoud et al., 2023; Önder et al., 2024). Cumin *(C. cyminum* L.) is a high antioxidant capacity traditional medicinal plant. Commonly used as a spicy flavor for food due to desirable taste and also have strong carminative, painkiller, antitumor, anti-angiogenic and antibacterial characteristics (Alizadeh et al., 2019; Demir and Korukluoglu, 2020; Asgari et al., 2021; Ghasemi et al., 2022; Önder et al., 2024). Similarly, fennel (*Foeniculum vulgare* L.) has high antioxidant with intense radical quenching activity and antimicrobial properties. Essential oil of fennel is also rich in volatile compounds that provides utilization in food products or packing as safety (Ribeiro-Santos et al., 2018; Barrahi et al., 2020; Alsalman et al., 2021; Ben Abdesslem et al., 2021; Abd-Elhafeez et al., 2023; Önder et al., 2024). Also, anise (*Pimpinella anisum* L.) is other member of the *Apiaceae (Umbelliferae*) family known for antioxidant and antimicrobial activities. Besides, it is applied in the treatment of osteoarthritis, gastritis, skin irritation and flu. Essential oil content is similar to fennel and abundant with volatile compounds, especially anothole (Mahdavi et al., 2018; Mijiti et al., 2019; AlBalawi et al., 2023; Önder et al., 2024).

In light of the information, antimicrobial activity properties of ethanol and methanol extracts and essential oil of anise, cumin, coriander, and fennel seeds that belong the *Apiaceae* family were investigated in the present study.

## Material and Methods

### Medicinal Plants

Seeds of anise (*Pimpinella anisum* L.), cumin (*Cuminum cyminum* L.), and fennel (*Foeniculum vulgare* Mill. var. *dulce*) were chosen for the evaluation of antmicrobial activity in this study. Then, their seeds were supplied as a commercial product that originated from Burdur, Konya, Isparta and Antalya (Türkiye), respectively. Seeds were ground in a mill (FRITSCH Puluerisette 14) to a particle size of 1 mm for use in analyses, and stored at -18°C. Then, the extracts were prepared to follow by modifying Roby et al.'s methods (Roby et al., 2013). For this purpose, methanol (80%) and ethanol (80%) were purchased from Merck KGaA, Darmstadt, Germany. Then, extractions were performed at room temperature for 24 hours in a shaking water bath (nüve ST30). Subsequently, the extracts were filtered with Whatman paper (No 4) and the solvents were evaporated (Roby et al., 2013; Demir and Korukluoglu, 2020). The essential oils were obtained according to the method of European Pharmacopoeia using a Clevenger-type apparatus (EDQM, 2024; Ali et al., 2015).

### Antimicrobial Activity

Gram-negative; *Escherichia coli* ATCC 25922 and *Salmonella* Enteritidis ATCC 13076, Gram-positive; *Bacillus subtilis* ATCC 6633, *Streptecoccus mutans* ATCC 25175 and *Listeria monocytogenes* ATCC7644 were chosen as test microorganisms. All of the test microorganisms were obtained from Bursa Uludağ University Food Engineering Department, Türkiye, and stored in 15% glycerol at –80°C in a freezer. All of the test microorganisms were activated twice in Tryptic Soy Broth (Millipore, Germany) at 37°C.

The antimicrobial activity of the extracts and essential oils of the seeds was performed by following the Kirby Bauer disk diffusion method by the Clinical and Laboratory Standards Institutes (Bauer et al., 1966; CLSI, 2018; Özkan Karabacak et al., 2021). The initial load of the microorganisms was adjusted in a sterile 0.85% NaCl (w/v) solution to obtain approximately $10^8$ CFU.mL$^{-1}$ with the McFarland. Then, the method followed spreading 100 µL of the suspensions on plates containing Tryptic Soy Agar. Then, sterilized 5 mm in diameter paper discs prepared utilizing Whatman filter paper were placed onto each plate. Subsequently, 10 µL each of the extracts was dropped in each paper disk. An equal volume of ethanol and methanol were performed as a negative control, while ampicillin (10 µg) and vancomycin (50 µg) were as a positive control. The plates were incubated at 37 °C for 18-24 hours. After incubation, the plates were evaluated for the formation of a clear inhibition zone around the disk, indicating the presence of the antimicrobial effect. The diameters of inhibition zones were measured. All experiments were performed in triplicate.

**Statistical Analysis**

Data collected during the study were statistically analyzed using Minitab. Results were verified by statistical one-way analysis of variance (ANOVA). Moreover, the statistically significant differences among the values were determined by Tukey's test ($p<0.05$).

## Results and Discussion

The antimicrobial activity of seed extracts was assessed by Disc Diffusion Assay and the results are presented in Table 1.

**Table 1.** Diameters of the inhibition zones (mm) against *S.* Enteritidis, *St. mutans*, *E. coli*, *L. monocytogenes* and *B. subtilis* of the essential oils and various extracts of test seeds from *Apiaceae* family.

| Sample | Seeds | ***Salmonella* Enteritidis** | ***Streptococcus mutans*** | ***Escherichia coli*** | ***Listeria monocytogenes*** | ***Bacillus subtilis*** |
|---|---|---|---|---|---|---|
| **Direct Essential Oil** | **Cumin** | $15.00^{A,ab}$ | $8.50^{AB,b}$ | $20.00^{A,A}$ | $13.00^{A,ab}$ | $11.00^{A,ab}$ |
| | **Anise** | $7.50^{C,ab}$ | $7.00^{C,b}$ | $7.75^{CD,a}$ | nd | $7.00^{C,b}$ |
| | **Fennel** | $7.50^{C,b}$ | $7.00^{C,c}$ | $7.75^{CD,ab}$ | nd | $8.50^{B,a}$ |
| **Methanol Extracts** | **Cumin** | $7.75^{C,a}$ | nd | $7.00^{D,b}$ | nd | nd |
| | **Anise** | nd | $7.75^{B,b}$ | $9.50^{BC,a}$ | nd | nd |
| | **Fennel** | $7.00^{D,bc}$ | $9.50^{B,a}$ | $7.00^{D,bc}$ | nd | $7.25^{C,b}$ |
| **Ethanol Extracts** | **Cumin** | $11.50^{B,a}$ | $11^{A,a}$ | $10.25^{B,b}$ | nd | $7.25^{C,c}$ |
| | **Anise** | $10^{B,a}$ | $8.00^{B,c}$ | $9.25^{BC,b}$ | nd | nd |
| | **Fennel** | $7.75^{C,a}$ | nd | $7.00^{D,b}$ | nd | $7.00^{C,b}$ |

*Data are presented as mean (n = 3)
**nd: not determined
***Values followed by the different capital superscript letters in columns and small superscript letters in rows indicate significant differences ($p<0.05$)

Antimicrobial activity of the samples was classified as weak (inhibition zone < 12 mm), moderate (inhibition zone between 12 - 20 mm) and strong (inhibition zone ≥20 mm) (Rota *et al.*, 2008; Denev *et al.*, 2014; Altuntas and Korukluoglu, 2024). Antibiotic zones are observed as ranking 13-17 mm and 13-19 mm (except *E. coli*) for Ampicilin and Vancomycin respectively. According to the results presented in Table 1, cumin seeds exhibited the highest antimicrobial activity with inhibition zone diameters ranging from 8.50 to 20.00 mm against the test microorganisms. Moreover, the essential oil extracted directly from the cumin seeds is more effective on all test microorganisms. Then, the only strong antimicrobial activity was obtained from cumin essential oil against *E. coli* with 20 mm zone diameter. The effect is higher than the antibiotic discs. So, the studied cumin essential oil has an advantageous as a natural antimicrobial agent instead of drugs against *E. coli*. This is a significant result considering antibiotic resistance among microorganisms that a vital issue for public health in recently (Özoğlu *et al.*, 2022; Thakur *et al.*, 2024; Wirtu *et al.*, 2024).Also, moderate antimicrobial activities observed against *S.* Enteritidis and *L. monocytogenes* by cumin essential oil. Apart from these, all the seed extracts demonstrated antimicrobial activity even weak level against all test microorganisms except *L. monocytogenes*. Then, the effect was altered on the microorganisms and the alteration was statistically significant ($p<0.05$). For example, essential oils and ethanol extracts from cumin seeds had the highest effect against all test microorganisms while methanol extracts from anise seeds had on *E. coli*. Similarly, methanol extracts of fennel seeds showed the most intense effect on *St. mutans* when, cumin seeds showed on *S.* Enteritidis. Hence, the antimicrobial effects of the seeds were not directly associated with the type of the performed plants and extraction solvents. Then, the reason of this could be that the antimicrobial activity is connected not solely to the overall phenol content but also linked to the varieties, structures, and synergistic effect between the active compounds of the extracts. In other words, the effect of the solvent is related to the type of the plant, the extracted part of the plant, features of the bioactive compounds (Vaou et al., 2021; Önder et al., 2024). Moreover, these issues could be effective in different ways according to test microorganisms in the current study.

Overall, the inhibition effect of the medicinal plant seeds was generally decreased by essential oil, ethanol extract and methanol extract, respectively. It is known that ethanol and methanol are most commonly used extract solvents (Vaou et al., 2021; Archana and Geetha Bose, 2022; Nwozo et al., 2023). Commonly, methanol extract is a more strong solvent for the dissolubility of bioactive compounds when compared to other solvents like ethanol, ethyl acetate, water etc. (Vaou et al., 2021; Archana and Geetha Bose, 2022; Nwozo et al., 2023). However, mostly ethanol extracts of the seeds indicated more considerable antimicrobial effects on test microorganisms compared to methanol ones in the current study. This could be related with the compound of interest that has inhibition effects on test microorganisms (da Silva Martins et al., 2022; Tarfaoui et al., 2022). Also, strong effects of the ethanol extracts ones are a promising result due to more safety of the ethanol in terms of human health and the environment especially considering using in food matrixes (da Silva Martins et al., 2022; Turkmen et al., 2022). Apart from these, essential oils have outstanding antimicrobial effect because of the synergistic effects of some active compounds rather than single compounds (Vaou et al., 2021; Archana and Geetha Bose, 2022; Nwozo et al., 2023). Similar results were obtained in the study that investigated the antibacterial effects of *Zingiber officinale* Roscoe and *Elettaria cardamomum* (L.) Maton essential oils and ethanol, methanol extracts on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus Mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Candida tropicalis* and *Candida albicans*. Essential oils, ethanol extracts and methanol extracts of the plants showed antimicrobial effects with decreased 24-7 mm zone diameters on test microorganisms, respectively. Also, some of the plants did not show any inhibition effects on some test microorganisms contrary to findings from many studies in the literature (Tarfaoui et al., 2022; Mihai et al., 2023; Önder et al., 2024). Additionally, the extraction method is a significant parameter to determine the antimicrobial and antioxidant characteristics of medicinal aromatic plants (da Silva Martins et al., 2022). As an example the study about the effect of solvents on aniseed aerial plant extraction using soxhlet and ultrasound methods indicated that organic solvent extracts showed higher antimicrobial activity compared to aqueous extracts. Besides, the highes antimicrobial activity of the ethanolic extract obtained from ultrasound extraction when compared soxhlet (Bontzolis *et al.*, 2024). Therefore, this may explain the variability in antimicrobial effectiveness observed with different extraction solvents, which often lacks consistency.

In a study evaluating the antimicrobial activity of thymus and cumin extracts (ethanol and water) and their nanoparticle against *S.* Enteritidis, the cumin ethanol extract showed a higher effect than thyme oil, thyme and cumin mixture and water extract ones, similar to the findings in the current study(Sh.AL.siraj et al., 2022). Another study performed antimicrobial activity of cumin essential oil against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Listeria innocua* and *Candida albicans*. Then, essential oil of the cumin in different concentrations has various ranged antimicrobial effects up to type of the test microorganisms like the present study (Alizadeh et al., 2019). Similarly, a study carried out with zein electrospun fibers including different concentrations of cumin essential oil indicated antimicrobial activity with different ratios of cumin on *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, and *Salmonella enterica* (Ghasemi et al., 2022).

In a study investigating chromatographic profile, acute toxicity, antioxidant, and antimicrobial characteristics of Algerian wild fennel essential oils, antimicrobial and strong antifungal activity was observed on *E. coli*, *Pseudomonas aeruginosa*, *B. subtilis*, *Candida albicans* and *Saccaromyces cerevisiae*. Moreover, the essential oil was more effective on *B. subtilis* than *E.coli*, consistent with the findings of the current study(Dahmani et al., 2022). Likely, antimicrobial activity of fennel essential oils was supported by the study performed for identification of chemical composition, antioxidant, and antimicrobial activities of wild fennel essential oils from southeast Algeria. The study indicated that test bacteria which were *Pseudomonas aeruginosa*, *B. subtilis*, *Micrococcus luteus*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* more sensitive to seeds and flowers of the fennel than leafs (Hamada Saoud et al., 2023).

The antimicrobial effects of anise on multidrug-resistant bacteria were evaluated in a study aimed at determining the chemical constituents, antioxidant potential, and antimicrobial efficacy of *Pimpinella anisum*. *Salmonella typhi*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecalis* and *Staphylococcus aureus* were chosen as test microorganisms and methanol extracts were used in the study. Then, antimicrobial activity observed was between 17.56-22.86 mm zone diameter (AlBalawi et al., 2023). The higher values observed in the study could be related origin and part of the plant and extraction methods (Vaou et al., 2021; da Silva Martins et al., 2022; Mihai et al., 2023). Antimicrobial activity of anise and fennel essential oils were determined against *Bacillus cereus, B. subtilis*, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *S.* Typhimurium, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium sphaerospermum*, *Mucor racemosus*, *Penicillium chrysogenum*, *Rhizopus arrhizus*, *Candida albicans*, *Debaryomyces hansenii* and *Pichia membranifaciens*. Afterward, antimicrobial activities were observed with 8-26 mm and 9-33 mm zone diameters for anise and fennel, respectively (Abd-Elhafeez et al., 2023). Thus, the results of the study supported the findings of the present study.

The study investigating antimicrobial activity of *Ficifolius A. Rich* root which is an Ethiopian indigenous wart curing medicinal plant, *S. aureus* and *E. coli* were chosen as test microorganisms. To the results, the crude extracts (oil) of the medicinal plant showed antimicrobial activity with zone diameters at 6.67 and 10.67 mm while, they are 28.67 and 30 mm for positive control antibiotics against *S. aureus* and *E. coli*, respectively (Adem *et al.*, 2024). Therefore, the results are pretty similar to the current study. Then, the current study has higher antimicrobial activity results. While both of the studies have lower antimicrobial activity results compared to antibiotic discs, the results proved that the medicinal plants exhibit remarkable inhibition effects on the pathogen microorganisms. Besides, more detailed antimicrobial studies like MIC (minimum inhibitory concentration) /MBC (minimum bactericidal concentration) are required suitable comparison for antibiotic drugs (Rota *et al.*, 2008; Adem *et al.*, 2024; Altuntas and Korukluoglu, 2024; Wirtu *et al.*, 2024).

## Conclusion

Medicinal aromatic plants and their essential oils and extracts have been extensively studied due to their antimicrobial, antioxidant, and antiinflammatory properties. However, there is lack of adequate literature information on the antimicrobial effects of these plants due to the effects could be changed based on type, origin, and part of the plant and also test microorganisms or obtaining/performing methods. Hence, three seeds of medicinal plants from the *Apiaceae* family that are anise, cumin, fennel and their essential oils, methanol, and ethanol extracts were evaluated for antimicrobial activity characteristics against *S.* Enteritidis, *St. mutans*, *E. coli*, *L. monocytogenes* and *B. subtilis*. Then, all of the samples exhibited antimicrobial effects at varying levels depending on test microorganisms. With this, the study promoted the antimicrobial effects of some *Apiaceae* family members to be considered utilizing as natural antimicrobial agents. Therefore, much more studies are required in this area.

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the study was planned by Mihriban Korukluoglu. Ozum Ozoglu and Mihriban Korukluoglu contributed to the design of the study. All the authors have read and approved the final manuscript. There was no conflict of interest between the authors.

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