

Determination of Antimicrobial Activities of Methanolic Extracts of Some Medicinally Important Plants Found Around Çorum Province*

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Abstract

Objective: This study aimed to investigate the antimicrobial activities of methanolic extracts obtained from ten medicinal plants traditionally used in Çorum province, Türkiye.

Materials and Methods: Plant samples were collected in the spring season, dried, and extracted using 70% methanol. The antimicrobial activities of the extracts were tested by the disk diffusion method against three Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*), three Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), and two fungi (*Candida albicans*, *Aspergillus niger*). Ampicillin, Polymyxin B, and Nystatin were used as positive controls.

Results: The results showed that nine plant extracts exhibited inhibitory effects against *K. pneumoniae*, while all extracts inhibited *B. subtilis*. Seven extracts were active against *E. faecalis*, whereas acorn and oleaster extracts displayed limited antifungal activity against *A. niger*. No inhibitory activity was observed against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*.

Conclusion: The findings suggest that several plant extracts commonly used in Çorum possess notable antimicrobial potential. Extracts particularly effective against *B. subtilis* and *E. faecalis* may serve as promising sources for the development of novel natural antimicrobial agents. Further studies should focus on isolating and characterizing the bioactive compounds responsible for these effects.

Keywords: Medicinal plants, methanolic extract, antimicrobial activity, Çorum

Çorum İli Çevresinde Bulunan Tıbbi Öneme Sahip Bazı Bitkilerin Metanol Ekstraktlarının Antimikrobiyal Aktivitelerinin Belirlenmesi Öz

Amaç: Bu çalışmada, Çorum ilinde geleneksel tedavi amacıyla kullanılan on tıbbi bitkinin metanol ekstraktlarının antimikrobiyal aktivitelerinin araştırılması amaçlanmıştır.

Materyal ve Yöntem: Bitki örnekleri ilkbahar döneminde toplanmış, kurutulduktan sonra %70 metanol ile ekstraksiyon yapılmıştır. Elde edilen ekstraktlar disk difüzyon yöntemi ile üç Gram-pozitif bakteri (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*), üç Gram-negatif bakteri (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) ve iki maya-küf türü (*Candida albicans*, *Aspergillus niger*) üzerinde test edilmiştir. Pozitif kontroller olarak Ampisilin, Polimiksin B ve Nistatin kullanılmıştır.

Araştırma Bulguları: Sonuçlar, dokuz bitki ekstraktının *K. pneumoniae* üzerinde, tüm ekstraktların ise *B. subtilis* üzerinde farklı düzeylerde inhibisyon gösterdiğini ortaya koymuştur. Yedi ekstrakt *E. faecalis* üzerinde etkili olurken, yalnızca meşe palamudu ve iğde ekstraktları *A. niger* üzerinde sınırlı antifungal aktivite göstermiştir. Buna karşın, *S. aureus*, *E. coli*, *P. aeruginosa* ve *C. albicans* üzerinde herhangi bir antimikrobiyal etki gözlenmemiştir.

Sonuç: Çalışma, Çorum yöresinde kullanılan bazı bitkilerin antimikrobiyal potansiyel taşıdığını göstermektedir. Özellikle *B. subtilis* ve *E. faecalis* üzerinde etkili olan ekstraktlar, doğal ürünlerin yeni antimikrobiyal ajanlar geliştirilmesinde değerlendirilebileceğini işaret etmektedir. İlerleyen çalışmalarda bu bitkilerden elde edilen aktif bileşenlerin izolasyonu ve karakterizasyonu önerilmektedir.

Anahtar Kelimeler: Tıbbi bitkiler, metanol ekstraktı, antimikrobiyal aktivite, Çorum

Introduction

Çorum province is located in the interior of the Central Black Sea Region. It is bordered by Amasya to the east, Yozgat to the south, Çankırı to the west, Sinop to the north, Samsun to the northeast, and Kırıkkale to the southwest. Its surface area is 12,820 km². According to latitude and longitude, it is located between 34 degrees 04 minutes 28 seconds east longitude and 39 degrees 54 minutes 20 seconds north latitude. The natural vegetation of the southern region of Çorum Province is steppe. They bloom with spring rainfall and dry up in the fall. Examples include: daisy, poppy, thistle, village thistle, hawkweed, kangal grass, oxtail, yarrow, which have found a wide distribution area. Willows and poplars are found along streams (Karaman et al., 2013).

Many sources mention medicinal plants. Medicinal plants have been classified by Anatolian people as folk remedies or natural medicines (Kaplan, 2010; Eyüboğlu, 1977; Erdemir, 2001). Many folk remedies are plant-based, but not all of them are cultivated naturally. Plants that grow naturally are specially collected, dried, and stored for use in medicine. Some plants are collected fresh and used to make medicine. Plants that do not grow in the region are purchased from herbalists. Plants are classified according to their species and specific uses as folk remedies: This classification is generally done as trees, herbs, roots, seeds, flowers, algae, and spices (Eyüboğlu, 1977). Although traditional methods are not accepted as evidence by the scientific community, many people today prefer these methods.

The use of natural compounds instead of synthetic ones is naturally desirable, and the synthesis of new natural compounds is attracting considerable interest. In fact, natural compounds are recognized as being attractive due to their low cost, availability in large quantities from raw materials, biodegradability, and general safety for human health and the environment (Kong et al., 2010).

Since their discovery, antimicrobial drugs have proven to be quite effective in controlling bacterial infections. However, it soon became clear that bacterial pathogens were unlikely to surrender unconditionally, as some pathogens rapidly developed resistance to most of the first effective drugs discovered (Barbour et al., 2004).

New compounds that inhibit microorganisms, such as benzoin and emetine, have been isolated from plants (Cox, 1994). Antimicrobial compounds obtained from plants can inhibit bacterial growth through mechanisms different from those of currently used antimicrobials and may have significant clinical value in the treatment of resistant microbial strains (Eloff, 1998).

In this study, methanolic extracts were obtained from ten medicinal plants used in the traditional treatment of diseases prevalent in the Çorum province. The antibacterial and antifungal activities of these extracts were determined.

Materials and Methods

Collection of Plants from the Çorum Province Region

The 10 plant species used in this study are *Elaeagnus angustifolia* (*Elaeagnaceae*) (Oleaster), *Aesculus hippocastanum* (*Sapindaceae*) (Horse chestnut), *Pinus nigra* (*Pinaceae*) (Pine resin), *Brassica oleracea* var. *capitata* f. *rubra* (*Brassicaceae*) (Red cabbage, Leaf), *Pyrus elaeagnifolia* (*Rosaceae*) (Leaf, Fruit) (Wild pear), *Malva sylvestris* (*Malvaceae*) (Leaf) (Mallow), *Taraxacum officinale* (*Asteraceae*) (Dandelion), *Rosmarinus officinalis* L. (*Lamiaceae*) (Rosemary), *Equisetum arvense* (*Equisetaceae*) (Horsetail), and *Quercus robur* (*Fagaceae*) (Oak acorn), were collected in the spring season around Çorum province and dried at room temperature. The dried plant samples were stored in a cool, dark place until processing to prevent moisture absorption.

Obtaining Methanolic Plant Extracts from Plant Materials

Dried plant samples brought to the laboratory were broken into small particles using a sterile scalpel and then pulverized using a mechanical grinder. Ten grams of the powdered plant samples were soaked in 100 ml of 70% methanol and left to stand overnight in a shaking incubator at room temperature. The samples were then kept in methanol overnight, then filtered through Whatman No. 1 (Merck) filter paper, and the precipitate was left at room temperature to completely evaporate the alcohol.

The precipitate was washed with 10 ml of sterile physiological serum by centrifuging for 5 minutes (3,000 g), then the supernatant was removed, and the residue was again left in 5 ml of 20% methanol at room temperature overnight, then filtered with filter paper to complete the extraction process. The resulting residue was used in the antimicrobial activity study (Berber et al., 2013).

Determination of Antimicrobial Activities of Methanolic Plant Extracts

The Gram-positive bacteria *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, the Gram-negative bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and the fungi *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 were used in the antimicrobial activity studies.

Disk Diffusion Method

The antimicrobial activity of plants was evaluated using the disk diffusion method (Bauer et al., 1966; Veyisoğlu et al., 2024 a,b). Mueller Hinton Agar (MHA) was used to activate bacterial cultures, and Sabouraud Dextrose Agar (SDA) (Difco) was used for fungi. Before the study, microorganisms were transferred to Mueller Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi (Difco) and allowed to grow overnight at 37°C (28°C for fungi). The turbidity of the suspensions prepared for the test strains was adjusted to 0.5 McFarland equivalent (1.5×10^8 cfu/ml), and pathogenic microorganisms were spread on the surface of the agar plate with 100 µl sterile swabs and allowed to dry in Laminar Flow for five minutes. Plant extracts obtained using methanol were mixed with 1 ml of 25% Dimethyl sulfoxide (DMSO). To check the antimicrobial activity of the extracts, 25 µl were impregnated onto filter paper discs (6 mm) and allowed to dry in Laminar Flow for five minutes. After drying, the bacterial-containing petri dishes were incubated overnight at 37°C, while the fungal-inoculated petri dishes were incubated at 28°C. 25% DMSO was used as a blank control, Ampicillin (AM10), Polymyxin B (PB300) for positive controls, and Nystatin (NS100) for fungi. The zone of inhibition was observed and measured in millimeters.

Results

Collecting Plants from Around Çorum Province

Ten plant species used in this study, *Elaeagnus angustifolia* (*Elaeagnaceae*) (Oleaster), *Aesculus*

hippocastanum (*Sapindaceae*) (Horse chestnut), *Pinus nigra* (*Pinaceae*) (Pine resin), *Brassica oleracea* var. *capitata* f. *rubra* (Red cabbage, Leaf), *Pyrus elaeagnifolia* (*Rosaceae*) (Leaf, Fruit) (Wild Pear), *Malva sylvestris* (*Malvaceae*) (Leaf) (Mallow), *Taraxacum officinale* (Dandelion), *Rosmarinus officinalis* L. (Rosemary), *Equisetum arvense* (Horsetail herb) and *Quercus robur* (*Fagaceae*) (Acorn), were collected in spring from around Çorum province and dried at room temperature. Images of seven of the dried plant samples are given in Figure 1.



Figure 1. Visual of seven dried plant samples

Obtaining Methanolic Plant Extracts from Plant Materials

Dried plant samples brought to the laboratory were crushed into small particles using a sterile scalpel and then pulverized with a mechanical grinder (Figure 2). Ten grams of the powdered plant samples were soaked in 100 ml of 70% methanol and left to stand overnight in a shaking incubator at room temperature. Samples soaked in methanol overnight were filtered using Whatman No. 1 (Merck) filter paper, and the precipitate was left at room temperature to completely evaporate the alcohol. The precipitate was washed with 10 ml of sterile physiological serum by centrifuging for 5 minutes (3,000 g). The supernatant was removed, and the residue was left in 5 ml of 20% methanol at room temperature overnight before being filtered with filter paper to complete the extraction process. A schematic representation of the extraction process is given in Figure 2.

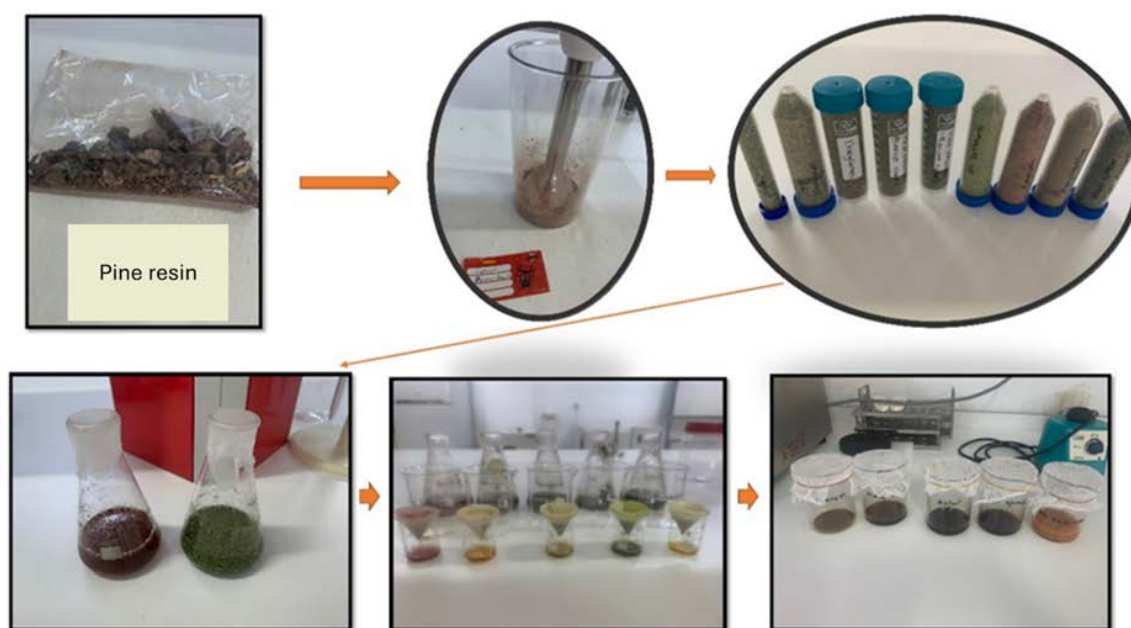


Figure 2. Schematic view of the preparation of plant extracts

Determination of Antimicrobial Activities of Methanolic Plant Extracts-Disk Diffusion Method

The antimicrobial activities of methanol extracts of plants against 3 Gram-positive, 3 Gram-negative, and 2 pathogenic fungi are listed in Table 1. When

Table 1. Antimicrobial activities of different plant extracts on Gram-positive, Gram-negative and Fungal microorganisms

| Microorganisms | Inhibition zones (mm) of different plant extracts on Gram-positive, Gram-negative and Fungal microorganisms | | | | | | | | | | | | |
|-------------------------------|---|----------|------------|-----------|--------|-----------|-------------|----------|----------------|----------------|-------------------|---------------------|------------------|
| | Acorn | Oleaster | Pine resin | Wild Pear | Mallow | Dandelion | Red cabbage | Rosemary | Horsetail herb | Horse chestnut | Ampicillin (AM10) | Polymyxin B (PB300) | Nystatin (NS100) |
| <i>Bacillus subtilis</i> | 10 | 28 | 10 | 22 | 8 | 8 | 8 | 22 | 22 | 12 | 30 | 22 | - |
| <i>Staphylococcus aureus</i> | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Enterococcus faecalis</i> | - | - | - | 12 | 8 | 8 | 8 | 20 | 20 | 10 | 30 | 12 | - |
| <i>Escherichia coli</i> | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Klebsiella pneumoniae</i> | 10 | 20 | 12 | 15 | 8 | 8 | - | 20 | 18 | 16 | 48 | 20 | - |
| <i>Pseudomonas aeruginosa</i> | - | - | - | - | - | - | - | - | - | - | - | - | - |
| <i>Candida albicans</i> | - | - | - | - | - | - | - | - | - | - | - | - | 20 |
| <i>Aspergillus niger</i> | 10 | 20 | - | - | - | - | - | - | - | - | - | - | 30 |

Seven plant extracts produced inhibition zones against *E. faecalis*, and two plant extracts produced inhibition zones against the fungus *A. niger*. No plant extract produced inhibition zones against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*.

Discussion

In this study, the antimicrobial activities of ten different plant extracts were investigated and the results were comparatively evaluated. The findings showed that pine resin, rosemary, horsetail herb, and oleaster extracts exhibited significant inhibition, particularly against strains of *B. subtilis* and *E.*

evaluated overall, nine plant extracts produced inhibition zones against the bacterium *K. pneumoniae*, while all extracts obtained from all plants produced inhibition zones against *B. subtilis* (Figures 3-4).

faecalis. However, some extracts (oleaster, rosemary, horsetail, and horse chestnut) were moderately effective against *K. pneumoniae*. No antimicrobial activity was observed against *S. aureus*, *E. coli*, or *P. aeruginosa*.

When evaluated with respect to fungal species, acorn and oleaster extracts were found to exhibit limited antifungal activity against *A. niger* but didn't affect *C. albicans*. In general, plant extracts exhibited partial and species-specific effects on selected microorganisms, while antibiotic and antifungal agents used as positive controls provided more potent inhibition.

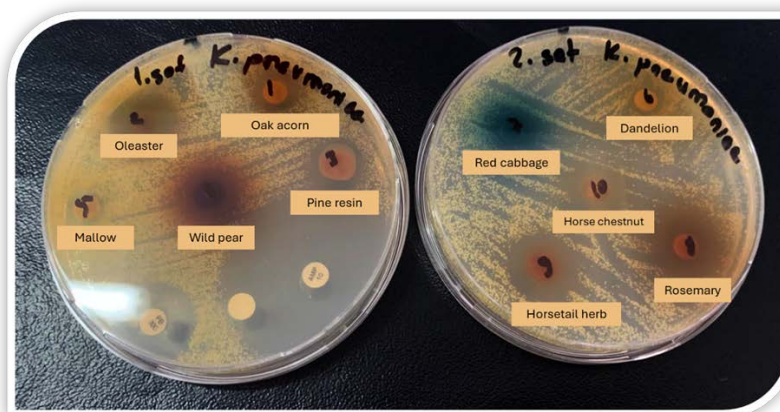


Figure 3. Inhibition zones created by plant extracts against the *K. pneumoniae* pathogen

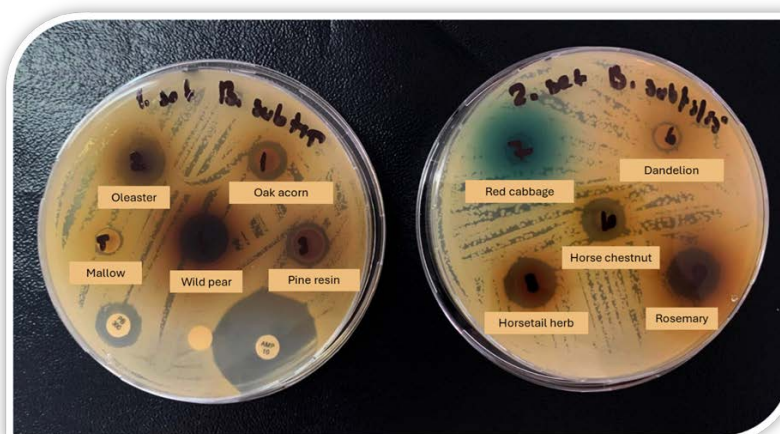


Figure 4. Inhibition zones created by plant extracts against the *B. subtilis* pathogen

These results are consistent with the literature. Phenolic compounds, flavonoids, and essential oils contained in plant extracts have been reported to exert antimicrobial effects by disrupting bacterial cell wall and membrane integrity (Cowan, 1999; Daglia, 2012). However, it is emphasized that these effects are generally weaker than those of antibiotics and may be specific to the target microorganism. Furthermore, the higher efficacy of secondary metabolites derived from different plant species against Gram-positive bacteria is explained by the outer membrane structures of Gram-negative bacteria providing a natural resistance barrier against these compounds (Nazzaro et al., 2013).

In their study, Berber et al. (2013) tested the antimicrobial activities of extracts obtained from 15 plant species growing in Sinop against eight different microorganisms using the disk diffusion method. They determined that the extracts obtained from the

different plant species had significant antimicrobial activity against the tested microorganisms.

In a study conducted in Lebanon, Barbour et al. (2004) tested the in vitro antimicrobial activity of water and methanol extracts obtained from the endemic species *Centaurea ainetensis*. The methanol extract of the plant was found to have better activity than the water extract, demonstrating 88.8% antimicrobial activity.

Akbulut et al. (2017) conducted a study in Çorum to determine the purposes and methods of use of certain plants used in traditional treatment of diseases by local people in Çorum. The study found that plants are mostly used in the Çorum region as infusions and externally. Local people use different parts of the plant species, with the leaves and fruits being the most frequently used parts.

Conclusions

Although numerous synthetic drugs are produced today, it has been reported that these drugs can exhibit not only toxic properties against human health but also some side effects. Natural compounds are becoming increasingly important due to their beneficial effects on both human health and nature.

In conclusion, this study examined the antimicrobial activities of methanol extracts of plants against 3 Gram-positive, 3 Gram-negative, and 2 pathogenic fungi. Plant extracts exhibiting antimicrobial activity against various pathogens have the potential for use in healthcare. In future studies, more in-depth studies should be conducted to evaluate these plants to isolate the active components that contribute to their antimicrobial activity.

Supplementary Information: There are no supplementary tables and figures related to the manuscript. A table and numerous pictures are given in the manuscript.

Contributing authors: Demet Tatar designed the study. Demet Tatar, Aysel Veyisoğlu, Hünkar Avni Duyar and Rukiye Yılmaz performed the laboratory experiments. Demet Tatar and Aysel Veyisoğlu wrote the paper. All authors read and approved the manuscript.

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Declarations

Competing Interests The authors declare no competing interests.

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants and/or animals performed by any of the authors. Formal consent is not required in this study.

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