

## The Role of Flavonoids in the Antimicrobial Activity of *Peganum harmala* Extract

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### Abstract

The use of plant extracts and their active compounds for medicinal purposes, particularly for antimicrobial activity, has become increasingly prevalent in recent years. This study aimed to evaluate the antimicrobial activities of *Peganum harmala* seed extract against various microorganisms using the disc diffusion method. Ethanol (65% concentration) was used to extract the active components from the *P. harmala* plant. Eight types of flavonoids in the plant extract were analyzed through HPLC. The antimicrobial activity of the extract was assessed against 15 microorganisms, including 14 bacterial strains and 1 fungus, via disc diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) tests. The results indicated that *P. harmala* exhibited activity against *Bacillus subtilis*, *Candida albicans*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, with MIC values ranging from 6.50 µg/mL to 52.03 µg/mL. The *P. harmala* extract demonstrated both antibacterial and antifungal activity against all tested microorganisms, with varying efficacy.

**Keywords:** *Peganum harmala*, Antimicrobial activity, Flavonoid, Disc diffusion, MIC, MBC-MFC.

## Üzerlik Ekstresinin Antimikrobiyal Aktivitesinde Flavonoidlerin Rolü

### Öz

Bitki ekstraktlarının ve bunların aktif bileşiklerinin tıbbi amaçlarla, özellikle antimikrobiyal aktivite için kullanımı son yıllarda giderek yaygınlaşmaktadır. Bu çalışmada Üzerlik tohumu ekstraktının çeşitli mikroorganizmalara karşı antimikrobiyal aktivitelerinin disk difüzyon yöntemi kullanılarak değerlendirilmesi amaçlandı. Aktif bileşenlerin *P. harmala* bitkisinden ekstrakte edilmesi için etanol (%65 konsantrasyon) kullanıldı. Bitki ekstraktındaki sekiz tip flavonoid HPLC yoluyla analiz edildi. Ekstraktın antimikrobiyal aktivitesi, 14 bakteri suşu ve 1 mantar dahil 15 mikroorganizmaya karşı disk difüzyon, minimum inhibitör konsantrasyon (MIC), minimum bakterisidal konsantrasyon (MBC) ve minimum fungisidal konsantrasyon (MFC) testleri yoluyla değerlendirildi. Sonuçlar *P. harmala*'nın *Bacillus subtilis*, *Candida albicans*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium*'a karşı aktivite gösterdiğini gösterdi. MİK değerleri 6,50 µg/mL ile 52,03 µg/mL arasında değişen *Staphylococcus aureus* ve *Staphylococcus epidermidis*. *P. harmala* ekstraktı, test edilen tüm mikroorganizmalara karşı değişen etkinlikle hem antibakteriyel hem de antifungal aktivite gösterdi.

**Anahtar Kelimeler:** *Peganum harmala*, Antimikrobiyal aktivite, Flavonoid, Disk difüzyon, MİK, MBK-MFK.

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## 1. Introduction

Imagine a world where over half the population turns to ancient remedies instead of modern medicine. This is not just history; it is the reality for billions today. According to the World Health Organization (WHO), approximately 4 billion people in developing countries rely on herbal medicine and use these plants regularly. Although modern medicine is accessible in many regions, herbal remedies continue to hold a significant place due to their historical and cultural roots (Artsdall, 2004; Dönmez et al. 2016). In human physiology, reactive oxygen species (ROS), commonly known as free radicals, are produced as part of natural biochemical processes. However, excessive free radical production can lead to oxidative damage in biological systems, resulting in diseases such as cancer, diabetes, and accelerated aging. With the growing wave of industrialization, exposure to synthetic antioxidants and unnatural chemicals has further exacerbated these issues. In response, there is a global surge in demand for natural products that can neutralize free radicals and promote health. Among these, non-wood forest products and medicinal plants are gaining attention for their rich bioactive properties. Phenolic compounds, abundant in roots, leaves, shoots, and fruits of herbaceous and woody plants, stand at the forefront of this exploration. Despite substantial research, a vast number of plant species remain untapped, holding the promise of groundbreaking discoveries (Borneo et al., 2009; Loizzo et al., 2010; Dubey et al., 2020; Aburjai et al., 2007). Interestingly, previous studies have highlighted the ability of certain flavonoids, a subclass of phenolic compounds, to exhibit superior antioxidant and antimicrobial properties, making them ideal candidates for modern therapeutic applications. But what if these plants are not just traditional remedies, but vital tools in modern health systems? The accumulated knowledge about plant usage in healthcare systems worldwide holds immense value. Evaluating the relative importance of medicinal plants and the consistency of traditional knowledge using quantitative tools like use value (UV) and informant consensus factors can provide actionable insights (Gazzaneo et al., 2005; Ait Abderrahim et al., 2019). Aromatic and medicinal plants are known for their diverse secondary metabolites, many of which exhibit antioxidant, antimicrobial, and anti-inflammatory effects. Their potential is now being harnessed in innovative therapies that rival synthetic drugs. Recent studies have demonstrated their role in reducing the side effects of conventional treatments and enhancing their efficacy. The raw extracts and specific phytochemicals derived from these plants are paving the way for natural remedies with minimal adverse effects (Yuca et al., 2022; Şener et al., 2017). Moreover, the growing resistance of microorganisms to conventional antibiotics further emphasizes the need for such natural alternatives, underscoring the urgency of research into these plant-based bioactive compounds. As global health challenges intensify, the search for natural antimicrobial agents has become a race against time. The overuse of commercial antimicrobial drugs has led to an alarming rise in drug-

resistant microorganisms. This crisis has prompted researchers to explore plant-based antimicrobial agents, including lesser-studied species, for their ability to combat resistant strains. However, many non-wood forest products with potential benefits remain unexplored. As research advances, there is a growing focus on substances with proven antioxidant and antimicrobial properties to ensure they meet the stringent requirements for therapeutic applications (Essawi and Srour, 2000; Palchykov et al., 2020). In this context, *P. harmala* emerges as a beacon of promise. Commonly known as Syrian rue, *P. harmala* is a perennial herbaceous plant widely distributed across arid regions. Blooming in May to July, it produces dark brown, pyramid-shaped seeds with remarkable therapeutic potential. Traditionally, *P. harmala* seeds have been used to stimulate the central nervous system and treat ailments such as hemorrhoids (Yuca et al., 2022; Koçak and Şahin, 2009). This plant exhibits diverse biological activities, including analgesic, anti-inflammatory, antibacterial, and anticancer properties. Notably, *P. harmala* seeds have been used in traditional treatments for skin and subcutaneous cancers. Its bioactive components demonstrate remarkable efficacy against tumor cell lines, as evidenced by both in vitro and in vivo studies, suggesting its potential as a multi-target therapeutic agent. Other traditional uses of the plant encompass treatments for digestive issues, rheumatic diseases, colic, and asthma, reflecting its broad therapeutic applications (Maksimovic et al., 2005; Shahrajabian et al., 2021). Although various parts of *P. harmala*, such as leaves and stems, have been utilized in extraction studies, the majority of research focuses on its seeds. Solvent extraction, a widely used method for isolating active compounds, has employed solvents such as water, methanol, ethanol, n-hexane, chloroform, and supercritical fluids (Hajji et al., 2020; Saeedeh et al., 2022; Arif et al., 2022; Mahmoud and Jassim, 2021; Allaq et al., 2021). These compounds have been tested for various bioactivities, including antioxidant, antibacterial, antifungal, antiviral, and antiparasitic properties (Iranshahy et al., 2019; Altuner and Canlı, 2012; Canlı et al., 2015; Krakhmalnyi et al., 2023; Tosun, 2024; Makhlof et al., 2023; Mangalagiri et al., 2021). Despite the extensive use of seed extracts, few studies have explored the bioactive potential of other aerial parts, particularly in the context of antimicrobial efficacy. More than 308 compounds have been identified in *P. harmala*, including alkaloids, flavonoids, and volatile oils. The primary bioactive compounds in its seeds, such as  $\beta$ -carboline alkaloids, exhibit potent biological activity, with harmine, harmaline, harmalol, harmane, and harmol being the most prominent. The alkaloid content of the seeds is particularly high, reaching up to 10% (Altuner et al., 2012, Estivi et al., 2022).

This study aims to unlock the untapped potential of *P. harmala* by focusing on its aerial parts. Using Soxhlet extraction with ethanol, phenolic components in the extracts were identified via standard chromatographic methods. The antimicrobial activity of these extracts was assessed against 15 different microorganisms using disk diffusion, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimal Fungicidal Concentration (MFC) tests.

This research strives to illuminate the path toward sustainable and natural alternatives in antimicrobial therapy, addressing both current and future health challenges.

## 2. Materials and Methods

### 2.1. Materials

*Peganum harmala* seeds were procured from Özşen Lokman Hekim Company, Ankara, Turkey. The plant species was identified in the laboratories of the Faculty of Forestry, Kastamonu University. Ethanol (96%) was obtained from TEKKİM Kimya. The 15 microorganisms used in the antimicrobial assays were sourced from the stock cultures at the Faculty of Sciences, Department of Biology, Kastamonu University. Additionally, Mueller Hinton Agar (ready-to-use solid media), liquid Nutrient Agar broth, and plates for antimicrobial testing were provided by Merck Company. Also, GCMS and HPLC analyses were carried out at Kastamonu University Central Research (MERLAB) laboratory.

### 2.2. Microbial Strains

The following 15 microbial strains were utilized to evaluate the antimicrobial activity of *P. harmala* seed extract: *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* DSMZ 50071, *Salmonella typhimurium* SL 1344, *Pseudomonas fluorescens* P1, *Salmonella kentucky*, *Salmonella enteritidis* ATCC 13075, *Enterococcus faecalis* ATCC 29212, *Salmonella infantis*, *Bacillus subtilis* DSMZ 1971, *Enterobacter aerogenes* ATCC 13048, *Candida albicans* DSMZ 1386, *Enterococcus faecium*, *Klebsiella pneumoniae*, and *Escherichia coli* ATCC 25922. These strains are microorganisms that generally cause disruption of the intestinal flora and stomach disorders. In this study, a large number of microorganisms were studied in order to identify alternative antimicrobial agents in response to multidrug resistance in bacteria that reduces the effectiveness of commonly used antimicrobials.

### 2.3. Extraction method

The *P. harmala* seeds were washed with distilled water for 2-3 minutes to remove dust and then air-dried at room temperature. After drying, the seeds were ground into a fine powder using a large chamber grinder. A 30 g dry weight sample was extracted with 250 mL of 65% ethanol using a Soxhlet apparatus for 24 hours. This extraction process was performed in triplicate. The resulting

extracts were filtered through Whatman No.1 filter paper. The solvents were evaporated using a rotary evaporator to obtain the final extracts. The extracts were stored in amber bottles at 4°C to prevent exposure to light. Consider adding the specific duration and temperature for the drying process, if applicable, as this may influence extract yield.

#### **2.4. High-Performance Liquid Chromatography (HPLC) Analysis**

HPLC analysis was conducted using an Agilent Eclipse XDB device with a C18 5 µm, 4.6 x 250 mm column at a column temperature of 30°C in the Central Research Laboratory of Giresun University. Flavonoid content was measured at 280 nm with an injection volume of 20 µL. Standard solutions were prepared using a 35% distilled water and 65% ethanol mixture. The mobile phases used were: mobile phase A (5% formic acid and 95% water) and mobile phase B (5% acetonitrile and 95% formic acid). The flow rate of the mobile phases was maintained for 39 minutes.

#### **2.5. Preparation of Extract Stock**

Extract stocks for antimicrobial assays were prepared by dissolving 1 mg of the extract in 3 mL of ethanol. Ethanol, which was used as a solvent in the extraction of the extract, was also preferred in the preparation of the solution for the antimicrobial study. For the Minimum Inhibitory Concentration (MIC) tests, distilled water was used as the solvent. The prepared extract stocks were filtered using 0.45 µm Whatman No.1 filter paper to remove any particulate matter. It may be beneficial to clarify the rationale for using ethanol as the solvent, especially in comparison with other solvents, as it can impact the solubility of bioactive compounds.

#### **2.6. Preparation of Inocula**

Each microorganism was spread on solid medium before the study and the bacteria were incubated at 37 °C for 24 hours and *C. albicans* was incubated at 27 °C for 48 hours. The inoculum preparation followed the protocols of Altuner and Canlı (2012), and Canlı et al. (2015). Colonies exhibiting morphologically similar characteristics to the organisms of interest were selected and transferred to physiological saline to prepare the inocula, as outlined in the study by Canlı et al. (2016). Using the 0.5 McFarland standard, the microbial suspensions were adjusted to approximately 10<sup>8</sup> cfu/mL for bacterial strains and 10<sup>7</sup> cfu/mL for *C. albicans* (Canlı et al., 2016).

## 2.7. Disk Diffusion Test

The disk diffusion test was performed as described by Canlı et al. (2014). Mueller Hinton Agar plates were prepared, and 20, 50, and 100  $\mu$ L of the extract stock were loaded onto sterile antibiotic discs. In order to compare the efficacy of antibiotic discs impregnated from the extract stock on microorganisms, impregnation process was carried out according to 20, 50 and 100 mL ratios. The inoculum was spread evenly on the surface of the agar plates, and the extract-impregnated discs were applied to the plates under aseptic conditions for 2-3 minutes. The inhibition zones were measured according to the method established by Altuner et al. (2014).

## 2.8. Minimum Inhibitory Concentration (MIC) Test

The MIC test was carried out according to the microdilution method outlined by Balouiri et al. (2016). Sterilized 96-well microtiter plates were used for the dilution series, ranging from 6.50  $\mu$ g/mL to 33 mg/mL of the extract. After the dilutions were prepared, the dilution was completed by diluting in microtiter plate wells. Bacterial strains and *C. albicans* were incubated at 37°C for 24 hours and 27°C for 48 hours, respectively, with the plates in a fixed position.

## 2.9. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) Test

In the MBC and MFC tests performed according to the results of the MIC test, samples taken from the most dilute dilution that had a suppressive or lethal effect against microorganisms were planted on solid medium. In this way, the effectiveness of the dilution at the lowest concentration was determined. MBC and MFC tests were performed on samples where microbial growth was visibly inhibited in the MIC test wells. The samples from the last diluted well, which showed inhibition, were streaked on partitioned nutrient agar plates. The plates were incubated at 37°C for 24 hours for bacterial strains and at 27°C for 48 hours for *C. albicans* (Altuner, 2011).

## 2.10. Controls

For the disk diffusion test, empty disks were used as controls in each solid medium, in addition to the extract-impregnated discs. In the MIC test, liquid medium without extract was included as a negative control, and inoculated wells ensured positive control for the microorganisms (Altuner, 2011).

### 2.11. Statistical Analysis

All tests were performed in triplicate, and the results were averaged across the three repetitions. Statistical analysis was conducted using the Kruskal-Wallis one-way analysis of variance (ANOVA), a non-parametric test. The significance level was set at  $p < 0.05$ . Statistical analysis was performed using the SPSS 23 package program.

### 3. Results and Discussions

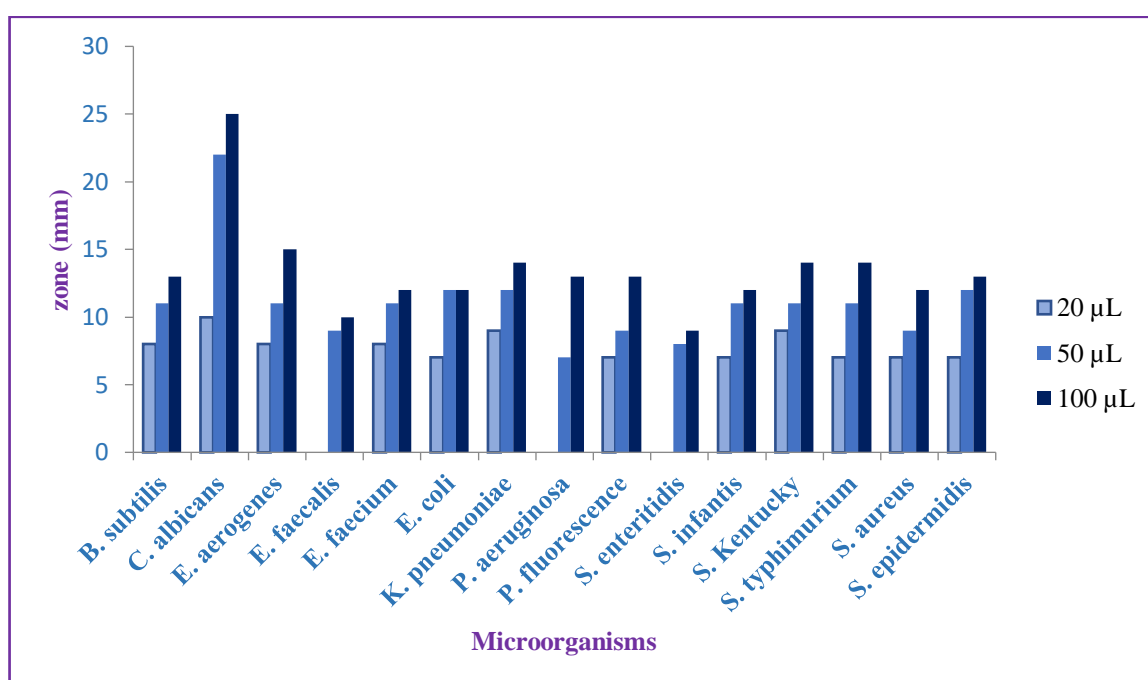
The quantitative results of HPLC, obtained using the available standard flavonoid samples, are presented in Table 1. According to the data shown in the table, rutin, luteolin, and apigenin were detected in *P. harmala* extracts at concentrations of 3.41  $\mu\text{g/g}$ , 2.73  $\mu\text{g/g}$ , and 1.69  $\mu\text{g/g}$ , respectively. Rutin, one of the most abundant flavonoids, is a strong antioxidant, offering advantages such as being non-oxidizing and non-toxic (Satari et al., 2021). Luteolin, a flavonoid commonly found in plant structures, has significant antioxidant and anti-inflammatory properties (Mizuno and Nishitani, 2013). Additionally, apigenin, commonly found in fruits, vegetables, nuts, onions, oranges, and tea, is a flavone with various beneficial health effects, including antioxidant, anti-inflammatory, and chemopreventive activities (Shukla et al., 2019). These findings suggest that the presence of these flavonoids may contribute to the therapeutic potential of *P. harmala* seeds.

**Table 1.** The results of HPLC analysis for some flavonoids ( $\mu\text{g/g}$  plant)

	Catechin	Epicatechin	Rutin	Naringin	Myricetin	Luteolin	Naringenin	Apigenin
<i>P. harmala</i>	-	-	3.41	-	-	2.73	-	1.69

Figure 1 shows the antimicrobial activity results of the *P. harmala* seed extract against 15 microorganisms using the disc diffusion method. Impregnated discs with a 100  $\mu\text{L}$  extract concentration showed high antimicrobial activity across all tested microorganisms. However, no activity was observed for *P. harmala* extract against *E. faecalis*, *P. aeruginosa*, and *S. enteritidis* strains at a dosage of 20  $\mu\text{L}$ . The highest antimicrobial activity was recorded against *C. albicans*, with zones of inhibition measuring 10 mm, 22 mm, and 25 mm for dosages of 20  $\mu\text{L}$ , 50  $\mu\text{L}$ , and 100  $\mu\text{L}$ , respectively. Activity against the other microorganisms was similar and appeared relatively consistent across the tested dosages.

Iranshahy et al. (2019) reported similar findings, stating that *P. harmala* extract was most effective against *C. albicans* and showed less sensitivity to *P. aeruginosa*. Additionally, Mahmoud and Jassim (2021) found the best antimicrobial effect of *P. harmala* extract was against *S. aureus* and *K. pneumoniae*, with inhibition zones of 19 mm and 20 mm, respectively. Abdulridha et al. (2019) observed that *P. harmala* extract formed inhibition zones ranging from 10 mm to 18 mm against *Streptococcus*, *E. coli*, *Staphylococcus*, and *Acinetobacter* but showed no effect on *Aeromonas* and *Klebsiella*. Similarly, Kaya and Akbaş (2023) conducted studies on the antioxidant and antimicrobial activity of methanol extract from *P. harmala* seeds, which exhibited inhibition zones ranging from 22 mm to 30 mm. Shaheen et al. (2022) also reported strong antifungal activity of ethanol extracts of *P. harmala* fruit against *A. flavus* and *C. albicans*, with inhibition zones of  $5 \pm 1$  mm and  $4 \pm 1$  mm, respectively.

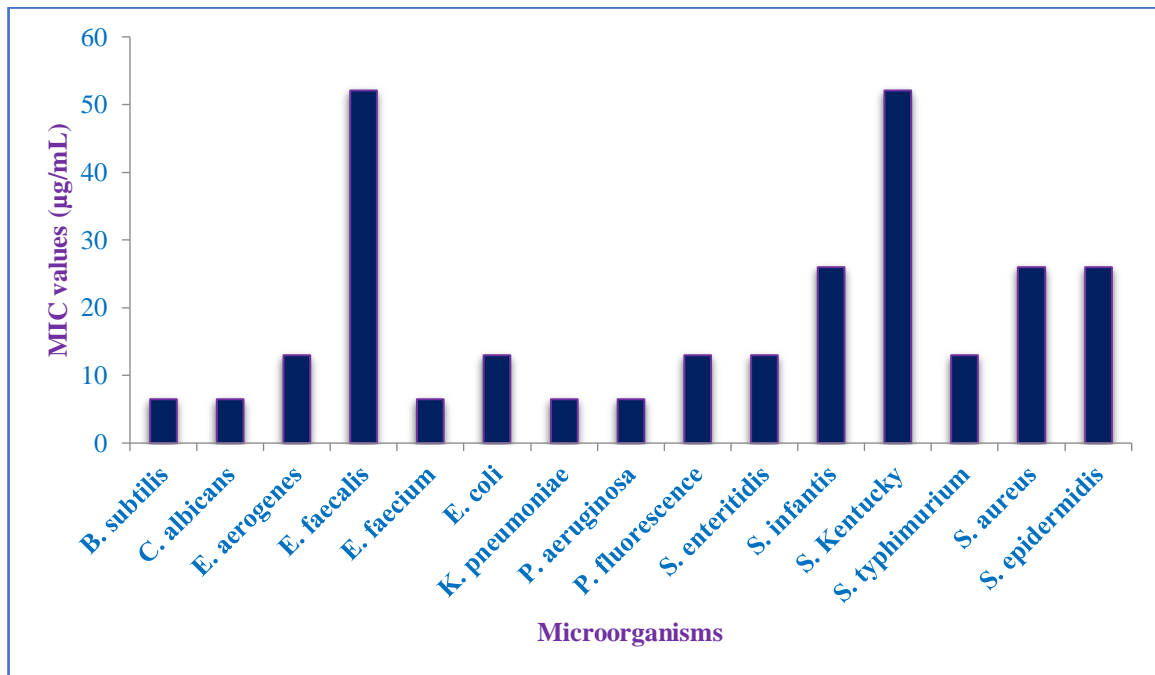


**Figure 1.** Disk diffusion test results of *P. harmala* seed extracts

Building upon the disk diffusion results, a Minimum Inhibitory Concentration (MIC) test was performed to further investigate the suppressive or lethal effects of the *P. harmala* seed extract. As shown in Figure 2, the seed extract demonstrated an inhibitory effect against all tested microorganisms. The MIC values for *B. subtilis*, *C. albicans*, *E. faecium*, *K. pneumoniae*, and *P. aeruginosa* were found to be  $6.50 \mu\text{g/mL}$ , indicating the most diluted concentration with antimicrobial activity. The MIC for *E. faecalis* and *S. kentucky* was found to be  $52.03 \mu\text{g/mL}$ , the highest MIC value among all tested microorganisms. These findings highlight the variable susceptibility of microorganisms to *P. harmala* seed extract, with certain bacteria requiring higher concentrations for inhibition.

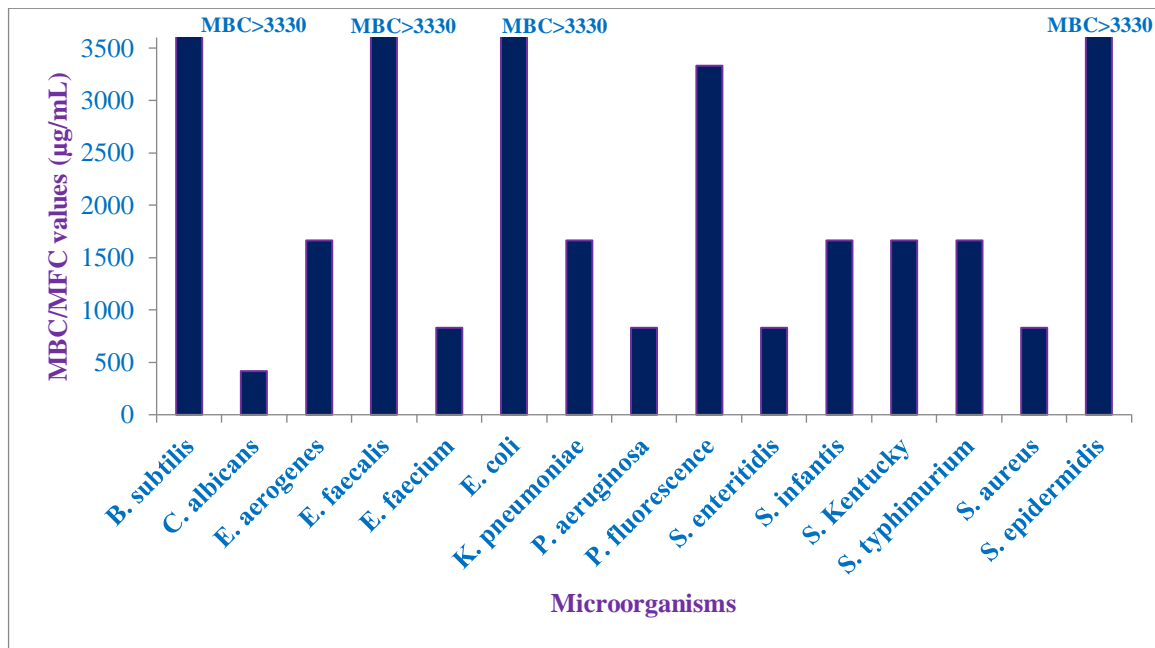


A. Abderrahim et al. (2019) reported similar MIC values for *P. harmala* seed extracts, with MIC values of 0.6 mg/mL against *C. albicans* and 0.5 mg/mL against *S. aureus*. In another study, Hadadi et al. (2020) found that methanol extracts of *P. harmala* fruit achieved a MIC value of 1.56  $\mu\text{g/mL}$  against *S. aureus* and *E. coli*, while chloroform fruit extracts achieved a MIC of 1.56  $\mu\text{g/mL}$  against *P. aeruginosa*.



**Figure 2.** Minimum Inhibitory Concentration (MIC) test results of extracts

The Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) results for *P. harmala* seed extract are shown in Figure 3. These tests demonstrated that the extract had a "cidal" effect against all tested microorganisms. The lowest MBC/MFC values were observed for *C. albicans*, with an MBC/MFC value of 416.25  $\mu\text{g/mL}$ , while the highest MBC/MFC values (MBC/MFC > 3330  $\mu\text{g/mL}$ ) were achieved for *B. subtilis*, *E. faecalis*, and *E. coli* strains. These findings suggest that while *P. harmala* seed extract may exhibit a bactericidal and fungicidal effect, the required concentrations for complete eradication of certain microorganisms are quite high.



**Figure 3.** Minimum Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC) test results of extracts

In the similar studies, close results were reported to our findings; Mahmoud and Jassim (2021) was found the minimum inhibitory concentration (MIC) of *P. Harmala* extract as 4 mg/mL for *S. aureus* and 8 mg/mL for *K. pneumonia*, and the minimum bactericidal concentration (MBC) was 8 mg/mL for *S. aureus* and 16 mg/mL for *K. pneumonia*. Allaq et al. (2021) observed that the crude ethanol extract of *P. harmala* seeds had significant antioxidant activity with an  $IC_{50}$  value of  $179.62 \pm 7.32$  µg/mL. The minimum inhibitory concentration (MIC) of the extract was 31.25 mg/mL for *E. coli*, 15.62 mg/mL for *S. aureus* and 1.95 mg/mL for *B. subtilis*, and the minimum bactericidal concentration (MBC) was 62.50 mg/mL for *E. coli*, 31.25 mg/mL for *S. aureus* and 7.98 mg/mL for *B. subtilis*. Ansari (2020) investigated the antispasmodic and antidiarrhoeal effects of the extract of *P. harmala* L. obtained with methanol as a solvent. The antimicrobial activity of *P. harmala* extract against *B. subtilis*, *S. aureus*, *K. pneumonia*, *E. coli*, *Aspergillus niger* and *C. albicans* microorganisms was investigated by disc diffusion method. *P. harmala* was found to form a zone in the range of 28-40 mm against *B. subtilis*, *S. aureus*, *E. coli*, *A. niger* and *C. albicans* microorganisms. However, it was not determined any effect against *K. pneumonia*.

Bin-Masalam et al. (2021) investigated the antimicrobial activity of extracts obtained from *P. harmala* by disc diffusion and minimum inhibitory concentration (MIC). *P. harmala* extracts gave effective MIC values against *K. pneumonia*, *B. cereus* and *S. aureus* microorganisms.

#### 4. Conclusion

The results of our study, in conjunction with similar research, highlight the growing potential of plant-based extracts to meet the increasing demand for natural products. These extracts, particularly those rich in flavonoids, offer valuable antimicrobial properties that warrant further exploration for their diverse applications. In particular, the broad-spectrum antimicrobial effects observed in this study underscore the need for deeper investigation into the underlying mechanisms of these natural compounds.

HPLC analysis, adhering to current standards, identified three flavonoid types-rutin, luteolin, and apigenin-in the ethanol extract of *Peganum harmala*. Out of the eight flavonoids tested from the studied sample library, these compounds were found to contribute to the antimicrobial activity of the extract. The study revealed that *P. harmala* demonstrated broad-spectrum antimicrobial activity against a wide range of microorganisms, including *B. subtilis*, *C. albicans*, *E. aerogenes*, *E. faecalis*, *E. faecium*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. fluorescens*, *S.a enteritidis*, *S. infantis*, *S. kentucky*, *S. typhimurium*, *S. aureus*, and *S. epidermidis*. These findings suggest that *P. harmala* seed extract has significant potential as an antimicrobial agent. Notably, the extract's effectiveness against both Gram-positive and Gram-negative bacteria, as well as fungi, positions it as a promising candidate for further development. The identification of active flavonoids and the extract's strong antimicrobial properties open avenues for future in vivo studies to assess the efficacy of these compounds in living organisms. Furthermore, this study encourages further investigation into the therapeutic potential of other plants from the *P. harmala* family, which have been used in folk medicine for centuries, and their relevance in modern medical practices. The pharmacological potential of *P. harmala* could expand the scope of natural medicine, particularly in combating infections that are resistant to conventional antibiotics. Future research should focus on isolating and identifying the specific compounds responsible for the antioxidant and antimicrobial activities of *P. harmala* seed extract. Advanced techniques such as bioassay-guided fractionation could aid in this pursuit. Additionally, a deeper understanding of the extract's mechanism of action against bacterial pathogens and its potential toxicity in human cells is necessary to establish its safety and therapeutic efficacy. Moreover, further studies could explore the potential industrial and medicinal applications of this extract, particularly in formulation development for pharmaceutical or natural product industries. Investigating synergistic effects with existing antibiotics could also present a promising approach to enhance therapeutic outcomes.

## Authors' Contributions

All authors contributed equally to the study.

## Statement of Conflicts of Interest

There is no conflict of interest between the authors

## Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

## References

- Abdulridha, M.M., Abdulhusein, H.S., Alyaseen, F.F., and Hassan, B.A. (2019). Phytochemical and Antibacterial Activity of the Peganum harmala Seeds and Its Alkaloids. *Plant Archives*. 19:1439-1444.
- Aburjai, T., Hudaib, M., Tayyem, R., Yousef, M., and Qishawi, M. (2007). Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. *Journal of Ethnopharmacology*. 110:294–304.
- Ait Abderrahim, L.A., Taibi, K., and Abderrahim, C.A. (2019). Assessment of the Antimicrobial and Antioxidant Activities of ziziphus lotus and Peganum harmala. *Iran J. Sci. Technol. Trans Sci*. 43:409-414.
- Allaq, A.A., Sidik, N.J., Abdul-Aziz, A., and Ahmed, I.A. (2021). Antioxidant, antibacterial and phytochemical screening of ethanolic crude extracts of Libyan Peganum harmala seeds. *Journal of Pharmaceutical Research International*. 33(13):74-82.
- Altuner, E.M. (2011). Investigation of antimicrobial activity of Punica granatum L. fruit peel ash used for protective against skin infections as folk remedies especially after male circumcision. *African Journal of Microbiology Research*. 5(20):3339-3342.
- Altuner, E.M., and Canlı, K. (2012). In vitro antimicrobial screening of Hypnum andoi A.J.E. Sm. *Kastamonu U. J. For. Fac.* 12:97-101.
- Altuner, E.M., Akata, I., and Canlı, K. (2012). In vitro antimicrobial screening of Bovista nigrescens (Pers.). *Kastamonu U. J. For. Fac.* 12:90-96.
- Altuner, E.M., Canlı, K., and Akata, I. (2014). Antimicrobial screening of Calliergonella cuspidata, Dicranum polysetum and Hypnum cupressiforme. *Journal of Pure and Applied Microbiology*. 8(1):539-545.
- Ansari, M.N. (2020). Assessment of Antidiarrheal, Antispasmodic and Antimicrobial Activities of Mmethanolic Seeds Extract of Peganum harmala L. (Nitrariaceae). *Journal of Pharmaceutical Research International*. 32(24):74-82.
- Arif, M., Wang, X., Khan Kazi, M.S., Khan, S.U., Saeed, S., Khan, A.M., et al. (2022). Antimicrobial Activities of different solvent extracts from stem and seeds of Peganum harmala L. *Plos One*. 17:4.
- Arzdall, V.A. (2023). *Medieval Herbal Remedies: The Old English Herbarium and Early-medieval Medicine*. Routledge.
- Balouiri, M., Sadiki, M., Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 6(2):71-79.
- Borneo, R., Le´on, A.E., Aguirre, A., Ribotta, P., and Cantero, J.J. (2009). Antioxidant capacity of medicinal plants from the Province of C´ordoba (Argentina) and their in vitro testing in a model food system. *Food Chem*. 70:112:664.
- Canlı, K., Altuner, E.M., and Akata, I. (2015). Antimicrobial screening of Mnium stellare. *Bangladesh Journal of Pharmacology*. 10:321-325.

- Canlı, K., Altuner, E.M., Akata, I., Türkmen, Y., and Üzek, U. (2016). In vitro antimicrobial screening of *Lycoperdon lividium* and determination of the ethanol extract composition by gas chromatography/mass spectrometry. *Bangladesh Journal of Pharmacology*. 11(2):389-394.
- Dönmez, İ.E., Hemming, J., and Willför, S. (2016). Bark Extractives and Suberin Monomers from *Arbutus Andrachne* and *Platanus Orientalis*. *BioRes*. 11(1):2809-2819.
- Dubey, R.K., Upadhyay, G., Singh, V., and Pandey, S. (2020). Antioxidant potential and free radical scavenging activity of *Parkia roxburghii*, G. Don, a lesser known leguminous tree from North East India, *South African Journal of Botany*. 131:454-461.
- Essawi, T., and Srour, M. (2000). Screening of Some Palestinian Medicinal Plants for Antibacterial Activity. *Journal of Ethnopharmacology*. 70:343-349.
- Estivi, L., Buratti, S., Fusi, D., Benedetti, S., Rodríguez, G., Brandolini, A., & Hidalgo, A. (2022). Alkaloid content and taste profile assessed by electronic tongue of *Lupinus albus* seeds debittered by different methods. *Journal of Food Composition and Analysis*, 114, 104810.
- Gazzaneo, L.R., de Lucena, R.F.P., and de Albuquerque, U.P. (2005). Knowledge and use of medicinal plants by local specialists in a region of Atlantic Forest in the state of Pernambuco (Northeastern Brazil). *Journal of Ethnobiology and Ethnomedicine*. 1:9.
- Hadadi, Z., Nematzadeh, G.A., and Ghahari, S. (2020). A study on the antioxidant and antimicrobial activities in the chloroformic and methanolic extracts of 6 important medicinal plants collected from North of Iran. *BMC Chemistry*. 14:33.
- Hajji, A., Bnejdi, F., Saadoun, M., Ben Salem, I., Nehdi, I., Sbihi, H., Alharthi, F. A., El Bok, S., and Boughalleb-M'Hamdi, N. (2020). High reserve in  $\delta$ -tocopherol of *Peganum harmala* seeds oil and antifungal activity of oil against ten plant Pathogenic fungi. *Molecules*. 25:4569.
- Iranshahy, M., Bazzaz, S.F., Haririzadeh, G., Abootorabi, B.Z., Mohamadi, A.M., and Khashyarmanesh, Z. (2019). Chemical composition and antibacterial Properties of *Peganum harmala* L.. *Avicenna Journal of Phytomedicine*. 9(6):530-537.
- Kaya, E., and Akbaş, P. (2023). Determination of the Antimicrobial, Antioxidant Activities and Fatty Acid Composition of *Peganum harmala* Seed. *Brazilian Archives of Biology and Technology*. 66:23220237.
- Koçak, Y., and Şahin, A. (2009). *Peganum harmala* L. (üzelik) tohum ekstresinin analjezik aktivitesi ve akut toksisitesinin fareler üzerinde belirlenmesi. *Y.Y.U. Veteriner Fakültesi Dergisi*. 20:27-30.
- Krakhmalnyi, M., Isikhuemhen, O. S., Jeppson, M., Wasser, S. P., & Nevo, E. (2023). Species Diversity of *Lycoperdaceae* (Agaricales) in Israel, with Some Insights into the Phylogenetic Structure of the Family. *Journal of Fungi*, 9(10), 1038.
- Loizzo, M.R., Tundis, R., Chandrika, U.G., Abeysekera, A.M., Menichini, F., and Frega, N.G. (2010). Antioxidant and antibacterial activities on foodborne pathogens of *Artocarpus heterophyllus* Lam. (Moraceae) leaves extracts. *J Food Sci*. 75:291-5.
- Mahmoud, S.S., and Jassim, E.H. (2021). Antibacterial activity of alkaloids extract from *Peganum harmala* L. seeds against *Staphylococcus aureus* and *Klebsilla pneumonia* isolated from burns. *Plant Archives*. 21:951-957.
- Makhlof, M. E., El-Sheekh, M. M., & El-Sayed, A. I. (2024). In vitro antibiofilm, antibacterial, antioxidant, and antitumor activities of the brown alga *Padina pavonica* biomass extract. *International Journal of Environmental Health Research*, 34(4), 1861-1878.
- Maksimovic, Z.A., Dordeviv, S., and Mraovic, M. (2005). Antimicrobial activity of *Chenopodium botrys* essential oil. *Fitoterapia*. 76:112-114.
- Mangalagiri, N. P., Panditi, S. K., & Jeevigunta, N. L. L. (2021). Antimicrobial activity of essential plant oils and their major components. *Heliyon*, 7(4).
- Mizuno, M., and Nishitani, Y. (2013). Chapter Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease, 14 - Antioxidant, Luteolin Exhibits Anti-inflammatory Effect in In Vitro Gut Inflammation Model, ISSN: 978-0-12-397154-8 Pages 227-234, <https://doi.org/10.1016/C2011-0-07464-1>
- Palchykov, V.A., Zazharskyi, V.V., Brygadyrenko, V.V., Davydenko, P.O., Kulishenko, O.M., and Borovik, I.V. (2020). Chemical composition and antibacterial effect of ethanolic extract of *Buxus sempervirens* on cryogenic strains of microorganisms in vitro. *Chemical Data Collections*. 25:100323.
- Saeedeh, F., Oryan, S., Ahmadi, R., and Eidi, A. (2022). Evaluation of chemical components, Antioxidant Properties and lethal toxicity of alkaloids extracted from *Peganum harmala*. *Journal of Applied Biological Sciences*. 16(2):257-265.

- Satari, A., Ghasemi, S., Habtemariam, S., Asgharian, S., & Lorigooini, Z. (2021). Rutin: A flavonoid as an effective sensitizer for anticancer therapy; insights into multifaceted mechanisms and applicability for combination therapy. *Evidence-Based Complementary and Alternative Medicine*, 2021(1), 9913179.
- Shaheen, G., Ashfad, A., Shamim, T., Asif, H.M., Ali, A., Rehman, S., and Sumreen, L. (2022). Antioxidant, Antimicrobial, Phytochemical and FTIR Analysis of Peganum harmala (Fruit) Ethanolic Extract from Cholistan Desert, Pakistan. *Dose-Response: An International Journal*. 1-7.
- Shahrajabian, M.H., Sun, W., and Cheng, Q. (2021). Improving health benefits with considering traditional and modern health benefits of Peganum harmala. *Clinical Phytoscience*. 7:18.
- Shukla, R., Pandey, V., and Lodhi, S. (2019) Chapter 18 - Role of Flavonoids in Management of Inflammatory Disorders, Pages 293-322, Book: Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases, ISSN:978-0-12-813820-5 Elsevier Inc. All.
- Şener, İ., Gür, M., Verep, D., Güney, K., and Altuner, E. M. (2017). Antimicrobial Activities and Some Flavonoids in Extracts of Some Medicinal Plants *Indian Journal of Pharmaceutical Education and Research*. 51:234-238.
- Tosun, B. (2024). Introduction to cultivation *Angelica sylvestris* var. *sylvestris* L.: Chemical composition of essential oil and antimicrobial activity. *Journal of Essential Oil Bearing Plants*, 27(4), 1175-1189.
- Yuca, H., Aydın, B., Tekman, E., Göger, G., Karakaya, S., Güvenalp, Z., and Ayşe Mine, G. Ö. (2022). Peganum harmala L. (Nitrariaceae) bitkisinin  $\alpha$ -amilaz ve  $\alpha$ -glukozidaz enzim inhibisyon, antioksidan ve antimikrobiyal aktivitelerinin değerlendirilmesi. *Türk Tarım ve Doğa Bilimleri Dergisi*. 9(4):925-932.