

Evaluation of Antimicrobial and Biofilm Inhibitory Activities of Flower, Leaf, Seed, and Pulp Extracts of *Mahonia aquifolium* (Pursh) Nutt.

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***Mahonia aquifolium* (Pursh) Nutt. Bitkisinin Çiçek, Yaprak, Tohum ve Meyve Pulpası Ekstrelerinin Antimikrobiyal ve Biyofilm İnhibitör Aktivitelerinin Değerlendirilmesi**

SUMMARY

Mahonia aquifolium (Pursh) Nutt. is a medicinal plant traditionally used for its antimicrobial and anti-inflammatory properties, largely attributed to its phytochemical contents. However, comprehensive evaluations of its antimicrobial and antibiofilm activities across different plant parts remain limited. This study investigated the antimicrobial and antibiofilm profiles of 70% ethanol and n-hexane extracts obtained from the flowers, leaves, pulp, and seeds of *M. aquifolium*. Antimicrobial activity was assessed against standard bacterial strains (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853) and yeasts (*Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258) using agar diffusion and broth microdilution methods. Leaf and flower 70% ethanol extracts exhibited the strongest antimicrobial effects, with MIC values as low as 0.625–1.25 mg/mL for Gram-positive bacteria and 1.25–2.5 mg/mL for *P. aeruginosa*, whereas antifungal activity remained weak across extracts. Agar diffusion results aligned with MIC data, showing the largest inhibition zones for leaf and flower ethanol extracts. Biofilm assays revealed significant inhibition (60–75%) of *S. aureus* and *P. aeruginosa* biofilms, especially by leaf 70% ethanol, flower 70% ethanol, and fruit pulp n-hexane extracts. These findings demonstrate that *M. aquifolium* possesses notable moderate antibacterial and antibiofilm potential, positioning it as a promising natural source for anti-infective and anti-virulence agents, particularly against biofilm-forming pathogens.

Keywords: *Mahonia aquifolium*, Berberidaceae, antimicrobial activity, antibiofilm activity

ÖZ

Mahonia aquifolium (Pursh) Nutt., antimikrobiyal ve antiinflamatuar etkileri nedeniyle geleneksel tıpta kullanılan önemli bir tıbbi bitkidir ve bu biyolojik aktivitelerin bitkinin zengin fitokimyasal içeriği ile ilişkili olduğu bilinmektedir. Bununla birlikte, bitkinin farklı organlarından elde edilen ekstrelerin antimikrobiyal ve antibiofilm potansiyelini karşılaştırmalı olarak ele alan çalışmalar sınırlıdır. Bu çalışmada, *M. aquifolium*'ün çiçek, yaprak, meyve pulpası ve tohumlarından hazırlanan %70 etanol ve n-hekzan ekstrelerinin antimikrobiyal ve antibiofilm aktiviteleri araştırılmıştır. Antimikrobiyal aktivite, agar disk difüzyon ve broth mikroseyltme yöntemleri kullanılarak *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NRRL B-3711, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028 ve *Candida krusei* ATCC 6258 suşlarına karşı değerlendirilmiştir. Yaprak ve çiçeklere ait %70 etanol ekstreleri, gram pozitif bakteriler için 0,625–1,25 mg/mL ve *P. aeruginosa* için 1,25–2,5 mg/mL aralığında değişen minimum inhibitör konsantrasyon (MIC) değerleriyle en güçlü antibakteriyel etkiyi göstermiştir. Buna karşın, ekstrelerin antifungal etkilerinin sınırlı olduğu belirlenmiştir. Disk difüzyon testlerinden elde edilen inhibisyon zonlarının, özellikle yaprak ve çiçek etanol ekstreleri için MIC sonuçlarıyla uyumlu olduğu gözlemlenmiştir. Biyofilm inhibisyon deneyleri, yaprak %70 etanol, çiçek %70 etanol ve meyve pulpası n-hekzan ekstrelerinin, *S. aureus* ve *P. aeruginosa* biyofilmleri üzerinde %60–75 oranında anlamlı inhibisyon sağladığını ortaya koymuştur. Elde edilen bulgular, *M. aquifolium*'ün belirgin antibakteriyel ve antibiofilm özelliklere sahip olduğunu göstermekte olup, özellikle biyofilm oluşturan patojenlere karşı enfeksiyon önleyici ve virülans karşıtı doğal ajanlar için umut vadeden bir kaynak olduğunu düşündürmektedir.

Anahtar Kelimeler: *Mahonia aquifolium*, Berberidaceae, antimikrobiyal aktivite, antibiofilm aktivite

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INTRODUCTION

The increasing resistance of pathogenic microbes to traditional antibiotics has generated heightened interest in plant-derived bioactive substances exhibiting antimicrobial and antibiofilm characteristics (Bonincontro, Scuderi, Marino, & Simonetti, 2023; Huang et al., 2022). *Mahonia aquifolium* (Pursh) Nutt., a member of the Berberidaceae family, has garnered interest for its abundant phytochemical constituents and its traditional applications in treating dermatological and gastrointestinal conditions (He & Mu, 2015; Öğüt, Kaya Tilki, Troncoso, & Özek, 2026a). The plant originates from North America but has been extensively cultivated in Europe and certain regions of Asia for its aesthetic appeal and therapeutic properties (Andreicut et al., 2018).

Prior research on *M. aquifolium* has predominantly concentrated on its alkaloid-laden root bark, especially berberine, and on its antibacterial and anti-inflammatory characteristics (Slobodníková, Košťálová, Labudová, Kotulová, & Kettmann, 2004; Slobodníková, Fialová, Rendeková, Kováč, & Mučaji, 2016). Nonetheless, the aerial parts of the plant, specifically leaves, flowers, fruits, and seeds, are inadequately investigated, particularly regarding their lipophilic and hydroalcoholic extract characteristics and biological activities (Karalija, Čavar Zeljković, Dahija, Bešta-Gajević, & Parić, 2021; Serventi et al., 2023). These anatomical components may harbor various secondary metabolites, such as phenolics, terpenoids, and fatty acids, which may demonstrate considerable antibacterial and antibiofilm properties (Öğüt et al., 2026b). In addition to their presence, the biological relevance of these secondary metabolites is increasingly associated with their ability to interfere with microbial communication systems and biofilm development (Putri, Juliaha, Kagawa, & Kurnia, 2025; Rodrigues & Černáková, 2020). Phenolic compounds and terpenoids have been reported to disrupt quorum sensing pathways, alter membrane permeability, and inhibit extracellular polymeric substance (EPS)

production, which are key processes in biofilm maturation (Mulat et al., 2025; Zhou et al., 2025). Furthermore, the combination of compounds present in crude plant extracts may exert synergistic effects, enhancing antimicrobial efficacy beyond that of isolated constituents (Kim, Zhao, Avena-Bustillos, Wang, & Nitin, 2024; Vaou et al., 2022). Therefore, evaluating whole extracts obtained from different plant organs may provide a more realistic insight into their potential therapeutic applications compared to studies focusing solely on individual compounds (Litewski, Koss-Mikołajczyk, & Kusznerewicz, 2024).

Biofilm formation by pathogenic bacteria is a critical virulence factor contributing to chronic infections and treatment failure (Öğüt et al., 2026b; Sanchez Jr. et al., 2013). Materials that are naturally able to inhibit or disrupt biofilms are consequently garnering considerable interest in the pursuit of alternative medicinal agents (Mishra et al., 2020). While some studies have reported the general antibacterial properties of *M. aquifolium*, there is a lack of comparative analyses regarding the efficacy of organ-specific extracts in inhibiting bacterial proliferation and biofilm formation. This study is to assess the antibacterial and antibiofilm properties of *n*-hexane and 70% ethanol extracts derived from the flower, leaf, fruit pulp, and seed of *M. aquifolium*. The agar diffusion and microbroth dilution methods were utilized for preliminary antimicrobial screening, and the antibiofilm potential was evaluated against specific microbial strains. This study aims to conduct a thorough comparison of extract activities by examining various plant parts and solvent polarity, thereby emphasizing the potential of underutilized plant resources for future antimicrobial applications.

MATERIALS AND METHODS

Plant material

Samples of *M. aquifolium*, including flowers, leaves, and fruits, were collected from Günalan village, Ankara, Türkiye. The flowers and leaves were

harvested during the flowering stage, whereas the fruits were gathered at full maturity during the fruiting stage. Immediately after collection, the fruits were frozen at -20°C and subsequently lyophilized. Following lyophilization, the fruit pulp and seeds were manually separated. The seeds were carefully cleaned to remove residual pulp, then air-dried in the shade at room temperature for three days, and stored at $+4^{\circ}\text{C}$ until further analysis. The plant was identified by Dr. Koray Yaylacı from Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany. A voucher specimen (ESSE 16356) was deposited in the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye.

Extraction of plant material

Before extraction, the seeds, fruit pulp, flowers, and leaves of *M. aquifolium* were separately homogenized with a laboratory grinder. Each plant component underwent maceration in 70% aqueous ethanol at ambient temperatures with constant shaking for 72 hours. Following decantation and filtering, the plant materials were subjected to reextraction under identical circumstances for an additional 72 hours to optimize yield. The aggregated supernatants were filtered using Whatman No. 1 filter paper. The ethanol phase was evaporated under decreased pressure with a rotary evaporator to produce crude dry extracts. The remaining aqueous fractions were lyophilized to ensure complete solvent elimination. All desiccated extracts were preserved in amber glass vials or sealed Eppendorf tubes at 4°C until subsequent analysis.

Antimicrobial activity

Standard bacterial strains (*Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853) and yeast strains (*Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258) were employed for the antimicrobial activity assays.

Agar diffusion assay

The standard pathogenic microorganisms were

cultivated overnight on Mueller Hinton Agar (MHA) to obtain fresh cultures. The regenerated microorganisms (McFarland $0.5 - 1.5 \times 10^8$ cfu/mL) were subsequently inoculated onto fresh MHA plates by the spread-plate technique using a sterile Drigalski spatula. Following inoculation, 6 mm wells were aseptically bored into the agar, and 50 μL of each extract (stock concentration 20,000 $\mu\text{g/mL}$) was dispensed into the wells. The plates were held at refrigeration temperature for 2 hours to allow the formulations to diffuse properly and to ensure absorption into the agar matrix. As the control group, standard antimicrobial agents chloramphenicol for Gram-positive and ciprofloxacin for Gram-negative bacteria and amphotericin B for yeasts were used. Once the wells had dried, the plates were incubated at 37°C for 24 hours (de Sousa, Cortez, de Souza Carvalho Melhem, Frickmann, & de Souza, 2020; Öztürk, Aygöl, & Şenel, 2019). All experiments were conducted in triplicate to ensure reproducibility.

Broth microdilution assay

The minimum inhibitory concentrations (MICs) of *M. aquifolium* extracts were determined against a panel of reference strains. The assays were conducted according to the standardized broth microdilution procedures outlined in CLSI M07 (de Sousa et al., 2020) and CLSI M27 (O'Toole, 2011; Öztürk, Namll, & Aygöl, 2021). Twofold serial dilutions of the extract were prepared in sterile 96-well microplates using cation-adjusted Mueller Hinton broth (CAMHB) for bacterial strains and RPMI-1640 medium for yeasts. Final concentrations of extracts ranged from 5000 to 1.22 $\mu\text{g/mL}$. Each dilution was inoculated with 100 μL of standardized microbial suspensions adjusted to 0.5 McFarland, yielding approximately 5×10^5 cfu/mL for bacteria and $0.5 - 2.5 \times 10^3$ cfu/mL for yeasts. Appropriate experimental controls were incorporated, including growth controls (medium with microorganisms), sterility controls (uninoculated medium), and positive controls comprising the solvent system (10% DMSO-methanol) together with reference antimicrobial agents: erythromycin for

Gram-positive bacteria, ciprofloxacin for Gram-negative bacteria, and fluconazole for yeasts. Microplates were incubated at 37 °C for 18-20 hours under standardized conditions. Following incubation, 10 µL of sterile 0.01% (w/v) resazurin solution was added to each well. Wells that maintained a blue or violet coloration were interpreted as indicative of growth inhibition, whereas a transition to pink or colorless was considered evidence of microbial proliferation.

Antibiofilm activity

Staphylococcus aureus ATCC 6538 and *Pseudomonas aeruginosa* ATCC 27853 were selected as model biofilm-forming organisms due to their strong biofilm-forming capacity and their clinical relevance in biofilm-associated infections, and their widespread use in antibiofilm studies (Sanchez Jr. et al., 2013; Solak, Soyer, Kaya-Tilki, & Öztürk, 2025). Overnight cultures of *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 27853 were adjusted to a turbidity equivalent to 0.5 McFarland, corresponding to approximately 5×10^5 cfu/mL, using CAMHB. Subsequently, 200 µL of each standardized suspension was dispensed into the wells of sterile 96-well microplates and incubated at 37 °C for 24 hours to permit biofilm establishment on the well surfaces. Following incubation, planktonic cells were carefully removed by aspiration, and the wells were gently rinsed three times with phosphate-buffered saline (PBS) (Costa et al., 2018). Extract solutions were freshly prepared at a concentration of 20000 µg/mL and added to the pre-formed biofilms (200 µL per well). The microplates were then incubated at 37 °C for an additional 48 hours to evaluate the activity of the extracts against established biofilms. After treatment, the wells were again washed three times with PBS, air-dried at room temperature, and fixed with 200 µL of methanol for 15 min. Following methanol removal and drying, biofilms were stained with 0.1% (w/v) crystal violet for 5 mins, rinsed three times with distilled water, and allowed to dry. The adsorbed dye was subsequently solubilized with 200 µL of ethanol for 15 mins, and the absorbance of the resulting solution was quantified at 550 nm using a spectropho-

tometer (Corbu et al., 2021). The percentage of biofilm inhibition was calculated using the following formula:

$$\% \text{Biofilm Inhibition} = \left(\frac{OD_{\text{control}} - OD_{\text{experiment}}}{OD_{\text{control}}} \right) \times 100$$

OD_{control}: OD of the well containing the biofilm only.

OD_{experiment}: OD of wells containing the biofilm and extract.

Statistical analysis

Statistical analysis and graphical representations were performed using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). All experiments were conducted in triplicate (n = 3). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Results are expressed as mean ± standard deviation (SD), and differences were considered statistically significant at p < 0.05.

RESULTS AND DISCUSSION

This work assessed the antimicrobial and antibiofilm properties of *M. aquifolium* extracts derived from several aerial parts, flowers, leaves, fruit pulp, and fruit seeds, utilizing antimicrobial and biofilm inhibition assays. Extracts were procured utilizing solvents of differing polarity, specifically *n*-hexane and 70% ethanol, to encompass a wider array of secondary metabolites with possible bioactivity. This study offers a comparative evaluation of lesser-explored plant sections, which may possess unique chemical and biological profiles, in contrast to prior research that primarily concentrated on the root bark and its established alkaloid content. The results are analyzed below concerning extract type, plant organ, microbial strain, and inhibitory potential, along with pertinent comparisons to existing research.

Antimicrobial activity

The agar diffusion results demonstrated that *M. aquifolium* extracts exhibited notable antibacterial activity, particularly against Gram-positive bacteria, while showing limited inhibition of *Candida* species (Table 1.).

Table 1. Antimicrobial zone inhibition diameters (mm) of *M. aquifolium* extracts

Samples	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. krusei</i>
Flower <i>n</i> -hexane	4.4 ± 0.4	3.9 ± 0.2	3.1 ± 0.1	3.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Flower 70% ethanol	8.3 ± 0.4	6.8 ± 1.0	3.2 ± 0.1	6.8 ± 1.0	2.4 ± 0.4	2.4 ± 0.4
Leaf <i>n</i> -hexane	2.1 ± 0.3	2.9 ± 0.1	2.4 ± 0.4	2.9 ± 0.1	1.1 ± 0.2	1.1 ± 0.2
Leaf 70% ethanol	8.4 ± 0.4	11.4 ± 0.5	8.0 ± 0.8	11.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Pulp <i>n</i> -hexane	8.0 ± 0.8	6.8 ± 0.4	3.7 ± 0.4	6.8 ± 0.4	1.1 ± 0.2	1.1 ± 0.2
Pulp 70% ethanol	1.5 ± 0.5	1.1 ± 0.2	1.5 ± 0.5	1.1 ± 0.2	2.1 ± 0.3	2.1 ± 0.3
Seed <i>n</i> -hexane	6.3 ± 0.3	3.7 ± 0.4	3.7 ± 0.2	3.7 ± 0.4	2.1 ± 0.3	2.1 ± 0.3
Seed 70% ethanol	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.1	3.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Chloramphenicol	18.0 ± 0.1	22.3 ± 0.2	21.1 ± 0.3	-	-	-
Ciprofloxacin	-	-	-	19 ± 0.0	-	-
Amphotericin B	-	-	-	-	13 ± 0.0	11 ± 0.0

Among the tested fractions, the leaf 70% ethanol extract produced the largest inhibition zones against *S. aureus* and *P. aeruginosa* (mean diameters ~11.4 mm for both) and the sizeable zones against *B. cereus* (~8.4 mm) and *E. faecalis* (~8 mm). The flower 70% ethanol extract of the flower also generated clear inhibition zones (e.g. ~8.3 mm for *B. cereus* and 6.8 mm for *S. aureus* and *P. aeruginosa*). These results are indicating that polar solvents effectively extracted bio-active alkaloids such as berberine, jatrorrhizine, and

palmatine. In contrast, the *n*-hexane extracts and fruit extracts tended to yield much smaller zones. For example, the leaf *n*-hexane extract only produced faint halos (~2-3 mm) for the bacteria and essentially none for the fungi, reflecting its relatively weaker activity. Likewise, the 70% ethanol extract pulp showed minimal zones (<2 mm). The *Candida* species generally showed no zones of inhibition (0-2 mm) for most extracts (Figure 1.).

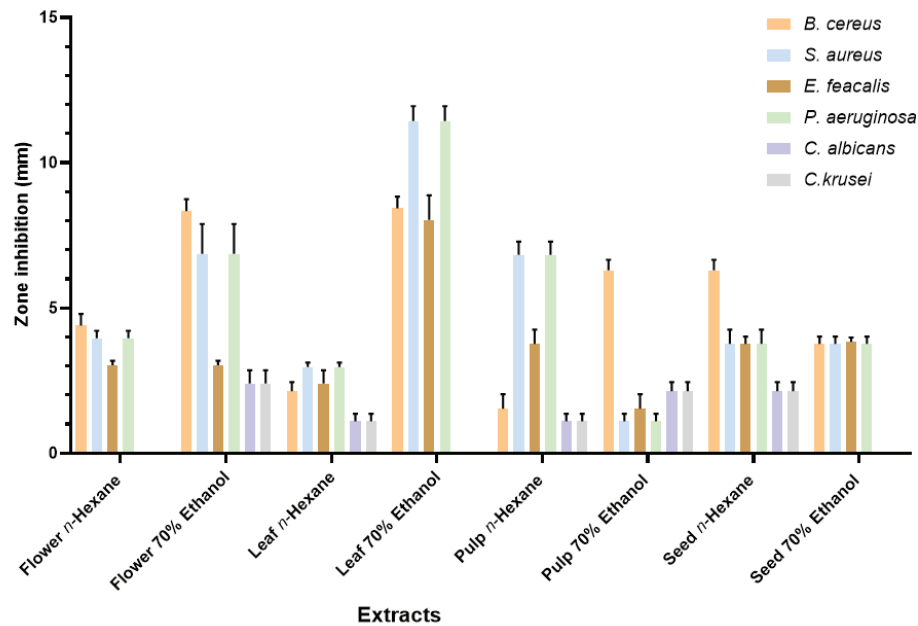


Figure 1. Antimicrobial zone inhibition values of *M. aquifolium* extracts ($p < 0.0001$). Values are expressed as mean $\pm$ SD ($n = 3$)

The MIC results demonstrated that *M. aquifolium* extracts possess broad-spectrum but variable antimicrobial activity depending on the plant part and extraction solvent. Overall, the leaf and seed 70% etha-

nol extracts exhibited the strongest inhibitory effects, with MIC values as low as 0.625-1.25 mg/mL against *S. aureus*, *E. faecalis*, and *P. aeruginosa* (Table 2.).

Table 2. MIC values (mg/mL) of *M. aquifolium* extracts. Results are reported as mean values $\pm$ SD, obtained from triplicate experiments (n = 3)

Samples	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>C. krusei</i>
Flower <i>n</i> -hexane	2.5	2.5	2.5	2.5	>5	>5
Flower 70% ethanol	2.5	2.5	1.25	1.25	2.5	2.5
Leaf <i>n</i> -hexane	2.5	2.5	2.5	1.25	2.5	2.5
Leaf 70% ethanol	1.25	0.625	0.625	1.25	5	5
Pulp <i>n</i> -hexane	1.25	1.25	1.25	1.25	5	5
Pulp 70% ethanol	5	5	1.25	2.5	2.5	2.5
Seed <i>n</i> -hexane	1.25	1.25	1.25	1.25	2.5	2.5
Seed 70% ethanol	5	5	2.5	1.25	2.5	2.5
Erythromycin	0.0019	0.0019	0.0009	-	-	-
Ciprofloxacin	-	-	-	0.0005	-	-
Fluconazole	-	-	-	-	0.0312	0.0312

Overall, the Gram-positive bacteria were the most susceptible. For example, *S. aureus* and *E. faecalis* were inhibited by several extracts at low minimum inhibitory concentrations (MICs) of 0.625-2.5 mg/mL, with the leaf 70% ethanol extract being the most potent (MIC ~0.625 mg/mL against *S. aureus* and *E. faecalis*). *B. cereus* showed similar susceptibility (MIC 1.25-2.5 mg/mL for most extracts). The Gram-negative pathogen *P. aeruginosa* was moderately susceptible, with MICs ranging from 1.25 to 2.5 mg/mL for the more active extracts (leaf and flower extracts performed best). In contrast, the yeasts *C. albicans* and *C. krusei* were far less sensitive; most plant extracts showed only weak or no inhibition up to the maximum tested concentration (MIC generally $\geq$ 2.5-5 mg/mL, with some extracts not achieving complete inhibition at 5 mg/mL). Notably, the flower 70% ethanol, pulp 70% ethanol, and leaf *n*-hexane extracts did exhibit MICs of ~2.5 mg/mL against *Candida*, but overall the fungistatic/fungicidal effect of the *M. aquifolium* extracts was limited compared to their antibacterial action. According to commonly accepted criteria for plant

extracts, MIC values in the range of 0.625–2.5 mg/mL indicate moderate antimicrobial activity. Despite this disparity in absolute potency, some *M. aquifolium* extracts (especially polar extracts from leaves and flowers) showed antibacterial effects that are notably moderate for natural products and thus warranted further examination.

Antibiofilm activity

One of the most significant outcomes of this study is the antibiofilm activity exhibited by the *Mahonia* species extracts, especially against *S. aureus* and *P. aeruginosa*. Biofilms are structured bacterial communities that are notoriously resistant to antibiotics and host defenses, often underpinning chronic and device-related infections. The anti-biofilm results revealed that *M. aquifolium* extracts possess substantial inhibitory activity against both *S. aureus* and *P. aeruginosa*, with the highest biofilm suppression observed for the leaf 70% ethanol (73.26% and 72.4%, respectively) and flower 70% ethanol extracts (63.17% and 74.7%) (Table 3.).

Table 3. % Biofilm inhibition values of *M. aquifolium* extracts. Data are presented as mean $\pm$ standard deviation (SD) based on three independent replicates (n = 3)

Samples	<i>S. aureus</i>	<i>P. aeruginosa</i>
Flower <i>n</i> -hexane	51.90 $\pm$ 1.70	46.69 $\pm$ 0.90
Flower 70% ethanol	63.17 $\pm$ 1.81	74.72 $\pm$ 0.78
Leaf <i>n</i> -hexane	43.36 $\pm$ 1.00	42.93 $\pm$ 1.52
Leaf 70% ethanol	73.26 $\pm$ 0.62	72.42 $\pm$ 1.03
Pulp <i>n</i> -hexane	66.03 $\pm$ 1.01	65.21 $\pm$ 0.62
Pulp 70% ethanol	56.73 $\pm$ 0.92	56.32 $\pm$ 0.31
Seed <i>n</i> -hexane	37.33 $\pm$ 0.44	40.04 $\pm$ 0.72
Seed 70% ethanol	62.10 $\pm$ 0.31	62.17 $\pm$ 0.77

These results indicate that the two most antimicrobial extracts also possessed the strongest anti-biofilm activity, effectively suppressing the establishment of bacterial biofilms. The pulp *n*-hexane and seed 70% ethanol extracts were also quite effective, blocking roughly 56–66% of biofilm mass in both organisms. In contrast, the 70% ethanol extract of the pulp was the

least active, achieving only about 37% inhibition of *S. aureus* biofilm and 40% of *P. aeruginosa* biofilm, a result consistent with its relatively weak antimicrobial profile. The remaining extracts (flower *n*-hexane, seed *n*-hexane, leaf *n*-hexane) showed intermediate biofilm suppression (approximately 42–57% inhibition) (Figure 2.).

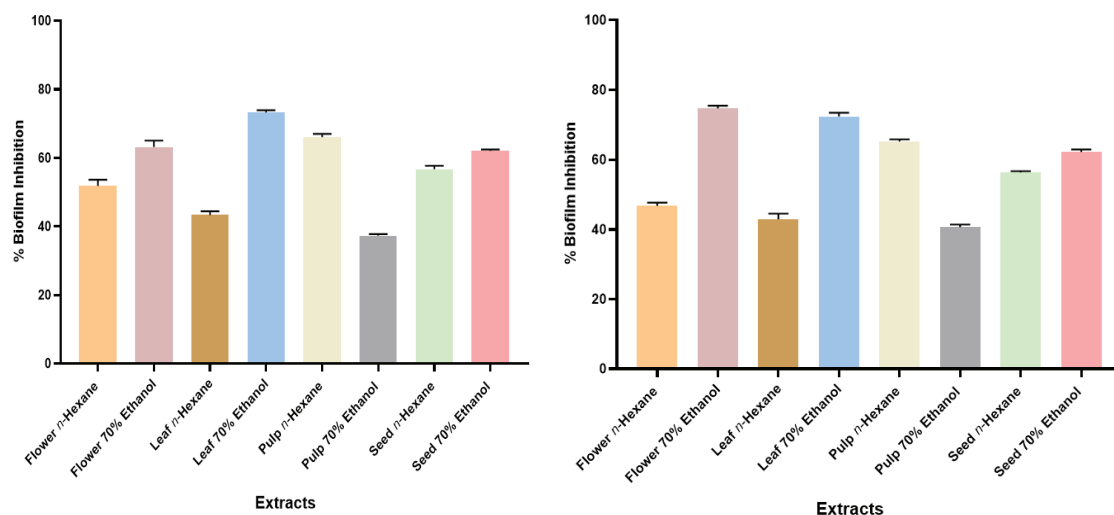


Figure 2. a) *S. aureus* and b) *P. aeruginosa* % biofilm values ($p < 0.0001$) of *M. aquifolium* extracts

These findings are generally consistent with previous studies reporting the presence of protoberberine alkaloids, such as berberine, jatrorrhizine, and palmatine, in polar extracts of *Mahonia* species. However, as no phytochemical characterization was performed in the present study, this relationship should be interpreted with caution (Černáková & Košťálová, 2002;

Stermitz, Lorenz, Tawara, Zenewicz, & Lewis, 2000). In contrast, *n*-hexane extracts, particularly those derived from seeds and pulp, exhibited lower antimicrobial activity (MIC 2.5–5 mg/mL), which may be attributed to the reduced extraction of polar bioactive compounds. Gram-positive bacteria were found to be more susceptible than Gram-negative strains, likely

due to the permeability barrier associated with the outer membrane of Gram-negative bacteria (Volková, Košťálová, Kettmann, & Tóth, 2003). Interestingly, *P. aeruginosa*, typically highly resistant to plant metabolites, was inhibited at MICs of 1.25–2.5 mg/mL by leaf 70% ethanol, flower 70% ethanol, and pulp 70% ethanol extracts. This observation aligns with reports that berberine interferes with quorum sensing and virulence pathways in *P. aeruginosa* even when growth inhibition is modest. Conversely, *C. albicans* and *C. krusei* were inhibited only at high concentrations (≥ 2.5 –5 mg/mL), consistent with previous findings that *Mahonia* extracts exert poor antifungal activity unless enriched in jatrorrhizine (Paudel et al., 2020). Overall, the MIC data confirm that ethanol extracts, particularly from leaves and seeds, are the most effective antimicrobial fractions and support existing literature describing *M. aquifolium* as a promising natural source of antibacterial compounds, with limited but noteworthy activity against Gram-negative pathogens and weak antifungal effects.

Previous studies on *Mahonia* species have also reported antimicrobial effects against various bacterial strains, although activity levels vary depending on the plant part, extraction method, and tested microorganism. These studies generally support the notion that *Mahonia*-derived extracts may exhibit moderate antibacterial activity, particularly against Gram-positive bacteria. The minimum inhibitory concentration (MIC) values for *S. epidermidis* and *S. hominis* were rather high, between 100 and 500 μ g/mL. The extract exhibited the most significant antibacterial activity against *P. acnes* strains, with MIC values between 25 and 50 μ g/mL. It was also reported that there was moderate efficacy against additional strains of *Staphylococcus*, such as *S. warneri*, *S. lentus*, and *S. hyicus* (MIC $\approx$ 100 μ g/mL). The extract had the strongest antifungal impact against *Candida tropicalis*, with an MIC value of 31.3 μ g/mL (Slobodníková et al., 2004). The methanol extract of *M. napaulensis* (Jamanemandro) bark was assessed for antibacterial efficacy against five different human pathogens. The disk diffusion meth-

od revealed the extract's most significant antibacterial action against *S. aureus*, resulting in an inhibition zone of 27.3 mm. This effect closely resembles that of chloramphenicol, a conventional antibiotic. The extract demonstrated suppression of *P. aeruginosa*, *Shigella spp.*, *E. coli*, and *S. typhi*; however, the inhibition zones for these bacteria were smaller than those for *S. aureus*. The methanol extract of *M. napaulensis* bark showed considerable antibacterial efficacy against many pathogenic bacteria, especially *S. aureus* (Kakar et al., 2022). In addition, polysaccharides extracted from *M. bealei* have exhibited significant antibacterial properties. In particular, they showed that they were able to inhibit *E. coli* and *B. subtilis* at a concentration of 20 μ g/mL (Li et al., 2008). In another study, extracts from *M. fortunei* and *M. bealei* exhibited significant antibacterial properties, especially against multi-drug-resistant *S. aureus* strains and *B. thuringiensis* (Vishnu, Kingsley, Dharani, & Narayanan, 2023). The ethanol extract of *M. leschenaultii* (Wallich ex Wight & Arnott) Takeda demonstrated significant antibacterial efficacy against *Klebsiella pneumoniae* (21.33 mm) and *S. aureus* (20.29 mm). Additionally, the ethyl acetate extract of the fruit exhibited antibacterial activity against *E. coli* (23.23 mm), *S. typhimurium* (22.27 mm), and *B. subtilis* (22.63 mm) at a concentration of 20 mg/mL (Vishnu et al., 2023). The methanol extract derived from the stem of *M. nepalensis* exhibited a significant antibacterial activity, with an inhibition zone measuring 18 mm against *S. aureus* (Pfoze, Kumar, Myrboh, Bhagobaty, & Joshi, 2011). A study on *M. manipurensis* revealed that crude alkaloid extracts from the stem bark had significant antibacterial activity against both Gram-positive and Gram-negative clinical pathogens. The agar diffusion method revealed growth inhibition in four of the five tested bacteria at concentrations of 2.5 and 5 mg/mL, with *Bacillus cereus* exhibiting the lowest minimum inhibitory concentration (MIC) value (Wang & Chu, 2016).

The pronounced accumulation of protoberberine alkaloids in the ethanol extract—mainly berberine, palmatine, and jatrorrhizine—reflects the phy-

tochemical profile expected from *Mahonia* species. These alkaloids are known to interact with microbial regulatory pathways, which may contribute to the antimicrobial tendencies observed in this study. The strong inhibition observed against *S. aureus* aligns with evidence showing that berberine disrupts the assembly of phenol-soluble modulins (PSMs), key amyloid-forming peptides that stabilize the *S. aureus* biofilm scaffold (Aswathanarayan & Vittal, 2018). Similarly, the high inhibition rates against *P. aeruginosa* correspond with previous reports indicating that berberine significantly reduces biofilm biomass by down-regulating quorum sensing circuits (Las and Rhl systems) and suppressing genes involved in exopolysaccharide synthesis (*pslA* and *pelA*) (Weinstein et al., 2018). The moderate inhibitory effects of pulp *n*-hexane (66% and 65.2%) and seed 70% ethanol extracts (62.1% and 62.2%) further indicate that biofilm inhibition is not strictly correlated with bactericidal potency, suggesting that *M. aquifolium* metabolites act primarily by targeting biofilm regulatory pathways rather than killing cells directly. Interestingly, even extracts with relatively weak planktonic antimicrobial activity, such as *n*-hexane extracts, still reduced biofilm formation (seed *n*-hexane: 37.33% and 40%), supporting the hypothesis that non-alkaloid constituents, possibly lipophilic phenolics or terpenoids, may also contribute to anti-biofilm effects. These results confirm that *M. aquifolium* extracts, particularly ethanol-based fractions from leaves and flowers, possess potent anti-biofilm activity against two clinically significant pathogens. Taken together, these findings suggest that *M. aquifolium* extracts may exert both antimicrobial and antibiofilm effects; however, further studies are required to clarify the underlying mechanisms and to identify the specific active compounds responsible for these activities.

CONCLUSION

In conclusion, the present findings indicate that extracts obtained from different parts of *M. aquifolium* exhibit antimicrobial and antibiofilm activities,

with 70% ethanol extracts of the leaves and flowers showing comparatively higher effectiveness against the tested bacterial strains (*B. cereus*, *S. aureus*, *E. faecalis*, and *P. aeruginosa*) and demonstrating a notable capacity to inhibit biofilm formation. These biological activities may be associated with the presence of protoberberine alkaloids and other phytochemical constituents, which are known to exhibit bacteriostatic or bactericidal effects as well as potential anti-quorum-sensing and anti-adhesive properties. However, as no phytochemical characterization was performed in the present study, this relationship should be interpreted with caution. Further studies are needed to isolate and characterize the active compounds, evaluate their mechanisms of action, and assess their efficacy under *in vivo* conditions, as well as to explore possible synergistic interactions with conventional antimicrobial agents. Overall, *M. aquifolium* extracts may represent a promising natural source for the development of alternative or complementary strategies against biofilm-associated and antimicrobial-resistant pathogens.

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AUTHOR CONTRIBUTION STATEMENT

The conceptualization, methodology, investigation, literature review, data curation, formal analysis, visualization, writing – original draft, and writing – review & editing (KO), the conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing – original draft, writing – review & editing (PS).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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