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Research Article

In Vitro Evaluation of Antibacterial, Antifungal, And Antibiofilm Activities of *Polygala myrtifolia* Leaf Extract

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Abstract

Purpose: *Plants produce secondary metabolites that play key roles in adaptation and defence. Among these, saponins, xanthones, and alkaloids are notable for their antimicrobial and biomedical significance. Polygala myrtifolia is rich in these compounds and exhibits strong antimicrobial potential. However, because phytochemical characterisation was not conducted in the present study, the contributions of specific phytochemicals, such as saponins, xanthones, and triterpenoids, remain speculative.* ***Method:*** *Antimicrobial analyses were performed to evaluate the antibacterial, antifungal, and antibiofilm activities of the P. myrtifolia extract against Gram (+), Gram (-), and fungal strains.* ***Findings:*** *Antimicrobial analyses demonstrated that the extract was effective against both Gram (+) and Gram (-) bacteria strains. MIC and MBC results indicated that K. aerogenes and E. durans were inhibited at 125 ppm, suggesting strong bactericidal activity. In antifungal assays, the extract inhibited the growth of Candida albicans and Candida utilis, with the highest activity observed at 1000 µg/mL. However, the lack of a linear concentration–response relationship indicates that the antifungal effect may be influenced by factors such as compound diffusion and interactions. Antibiofilm analysis revealed high levels of inhibition (85–90%) across all tested bacterial strains at higher concentrations (500–250 µg/mL), with E. coli and B. subtilis showing the greatest sensitivity.* ***Conclusion:*** *Overall, the results indicate that P. myrtifolia has considerable potential as a natural source for the development of antimicrobial and antifungal agents. These findings also provide a basis for future molecular studies aimed at identifying the active constituents responsible for its biological activity and further support the therapeutic value of plant-derived compounds.*

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Introduction

Plants synthesize a wide range of metabolites that support both essential biochemical processes and interactions with their environment (Piasecka et al., 2014; Verma & Shukla, 2015). Primary metabolites—including carbohydrates, amino acids, fatty acids, and nucleotides—are ubiquitous and directly involved in growth and energy metabolism. In contrast, secondary metabolites such as alkaloids, terpenoids, phenolics, and saponins are often taxon-specific and play key ecological roles in defense, allelopathy, and signaling. Beyond these functions, recent evidence indicates that secondary metabolites also regulate growth and act through hormone-like signaling pathways, highlighting their integral role in plant metabolism and their potential for biotechnological applications (Sabiu, 2022; Piasecka et al., 2015).

Secondary metabolites are multifunctional compounds that mediate not only defense but also plant–plant communication, pollinator attraction, allelopathic interactions, and microbial regulation (Bocso & Butnariu, 2022; Hartmann, 2007). This functional diversity makes them central to understanding plant–environment interactions. Furthermore, due to their pharmacological relevance, particularly in traditional medicine, these compounds have gained significant attention in biotechnology and pharmaceutical research (Yadav et al., 2021).

Numerous studies have demonstrated that secondary metabolites with antimicrobial, antioxidant, and anti-inflammatory properties represent promising candidates for novel therapeutic development (Dar et al., 2017; Sabiu, 2022; Šovljanski et al., 2025; Verma & Shukla, 2015). Therefore, investigating plant chemical diversity, including biosynthetic pathways and biological activities, is essential for advancing both fundamental plant science and the discovery of new bioactive compounds.

The genus Polygala (Polygalaceae), comprising approximately 400–500 species, represents a phytochemically rich and taxonomically diverse group with broad geographic distribution and significant secondary metabolite diversity. Members of this genus produce bioactive compounds such as saponins, xanthones, phenolics, oligosaccharide esters, and flavonoids, which contribute to ecological defense and pharmaceutical applications (Johann et al., 2011). Among them, *Polygala myrtifolia*, an evergreen shrub native to South Africa, is notable for its adaptability and rich metabolite profile, particularly saponins, xanthones, and triterpenoid derivatives (Lacaille-Dubois et al., 2020). Extracts of this species have demonstrated antibacterial, antifungal, and antibiofilm activities, highlighting its potential as a source of bioactive compounds (Johann et al., 2011; Pastore et al., 2019). Similarly, *Polygala tenuifolia* exhibits diverse pharmacological effects, including anti-inflammatory, anticancer, neuroprotective, and sedative activities, further supporting the therapeutic relevance of this genus (Garg et al., 2024). Overall, the rich phytochemical composition of Polygala species—particularly phenolics, xanthone derivatives, and saponins—confers strong antimicrobial potential

(Garg et al., 2024). These compounds can disrupt bacterial cell-wall integrity or inhibit enzymatic processes, thereby suppressing the growth of clinically relevant pathogens such as *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* (Zeleke et al., 2024).

Plant-derived saponins exhibit significant antibacterial, antifungal, and antibiofilm activities. Their surfactant properties disrupt early microbial adhesion and destabilize mature biofilms. In vitrostudies have demonstrated that saponin-rich plant compounds act as effective antibiofilm agents against respiratory pathogens (Tatli Cankaya&Somuncuoglu, 2021). Additionally, triterpenoid saponins such as Assamsaponin A show concentration-dependent inhibition of *Candida albicans* biofilms, eradicate mature biofilms, and suppress genes associated with adhesion, hyphal development, and virulence. These findings support the evaluation of antibacterial, antifungal, and antibiofilm activities of *P. myrtifolia* extracts, given their high bioactive potential (Haddad et al., 2003; Tatli Cankaya&Somuncuoglu, 2021).

Biofilms are structured microbial communities embedded in a self-produced extracellular matrix, with formation mechanisms varying according to environmental conditions and microbial traits (Sun et al., 2013). They are associated with persistent infections involving Gram (+) bacteria (e.g., *Streptococci* and *Staphylococci*), Gram (-) bacteria (e.g., Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae), and fungi (e.g., *Candida* and *Aspergillus* spp.). Among these, *P. aeruginosa* and *C. albicans* are particularly important pathogens, with the latter frequently causing both superficial and systemic infections, especially in hospital settings (Li et al., 2022; Sun et al., 2013). The biofilm matrix acts as a barrier to antibiotic penetration, reducing treatment efficacy by delaying diffusion and by interacting with matrix components (Flemming et al., 2016). While some antibiotics penetrate biofilms effectively, others are limited by biofilm structure, contributing to antimicrobial resistance. Therefore, investigating plant-derived metabolites—particularly phenolics, flavonoids, and alkaloids—and their mechanisms of action against biofilms is of considerable importance (Sun et al., 2013).

In this paper, the antibacterial, antifungal, and antibiofilm activities of *P. myrtifolia* extracts are evaluated to explore their pharmaceutical and cosmetic potential, given the established antimicrobial relevance of Polygalaceae species.

Methodology

The Methods section should clearly present the study's scientific framework and provide a detailed explanation of the approaches and processes used to examine the research topic. In this context, the research design should be explicitly stated, and the extent to which the selected design addresses the study's purpose should be justified on theoretical grounds. The sample or study group should be clearly defined, together with its relationship to the population and the selection criteria.

Furthermore, the characteristics of the data collection instruments, their validity and reliability information, and the stages of the data collection process should be systematically presented. In addition, the theoretical and empirical foundations underlying the proposed hypotheses should be explained with reference to literature, and the relationship between these hypotheses and the research objectives should be clearly articulated.

Plant Material Collection and Preparation of Crude Extract

P. myrtifolia plants were obtained from Aykut Landscape Botanic (Antalya). To evaluate their antibacterial, antibiofilm, and antifungal activities, leaf extracts were prepared using chloroform as the extraction solvent. The leaves were air-dried under appropriate conditions and ground into a fine powder. The powdered material (60 g) was Soxhlet-extracted with chloroform (600 mL) for 8 hours. Following extraction, the solvent was removed using a rotary evaporator, and the crude extract was stored at 4°C until further use. Prior to antimicrobial, antibiofilm, and antifungal assays, the crude extract was dissolved in sterile distilled water and prepared at the desired concentrations.

Determination of Antibacterial Activity by Broth Microdilution Method (MIC and MBC)

Antibacterial activity of *P. myrtifolia* extract was evaluated using the broth microdilution method in accordance with standard guidelines (EUCAST/CLSI). Nutrient Agar (NA) and Luria Broth (LB) media were prepared and sterilized before use.

Bacterial strains (Gram (+): *Staphylococcus aureus, Bacillus subtilis, Enterococcus durans*, and Gram (-): *Escherichia coli, Klebsiella aerogenes, Salmonella enteritidis*) were reactivated on agar plates and incubated at 37 °C for 24 h. Fresh colonies were then transferred into broth medium, and bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard.

Serial dilutions of the plant extract (1000 ppm-31,25 ppm) were prepared in sterile 96-well microplates containing broth medium. Each well was inoculated with a standardized bacterial suspension to obtain the required final inoculum density. Microplates were incubated at 37 °C for 24 h under appropriate conditions. Following incubation, bacterial growth was visually assessed, and the minimum inhibitory concentration (MIC) was defined as the lowest extract concentration that prevented visible growth. To determine the minimum bactericidal concentration (MBC), aliquots from wells showing no visible growth were subcultured onto agar plates and incubated under the same conditions. The MBC was defined as the lowest concentration at which no bacterial colonies formed. All experiments were performed under aseptic conditions, and appropriate controls were included to ensure reliability of the results (Nesrin et al., 2020).

Determination of Antifungal Activity by Agar Well Diffusion Method

Antifungal activity of *P. myrtifolia* extract was evaluated using the well diffusion method. Potato Dextrose Agar (PDA) medium was prepared, sterilized, and poured into sterile Petri dishes. Candida albicans and Candida utilis strains were reactivated on PDA plates and incubated at 27 °C for 48 h. Fungal suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard.

The standardized suspensions were inoculated onto agar surfaces, and wells were aseptically created. Different concentrations of the plant extract (2000, 1000, 500, and 250 µg/mL) were added to the wells, and the plates were incubated at 27 °C for 48 h. Antifungal activity was determined by measuring the inhibition zone diameters, and the results were recorded accordingly (Tozyilmaz et al., 2020).

Determination of Antibiofilm Activity by Crystal Violet Assay

Antibiofilm activity of *P. myrtifolia* extract was evaluated using the crystal violet staining method, with the plant extract added at the time of bacterial inoculation. Bacterial strains (*S. aureus, B. subtilis, E. durans, E. coli, K. aerogenes, S. enteritidis*) were incubated in 96-well microplates at 37 °C for 48 h to allow biofilm formation. After incubation, the wells were gently washed 2–3 times with distilled water to remove planktonic cells and then air-dried.

For fixation, 130 µL of 95% methanol was added to each well, and the mixture was incubated for 15 min. The methanol was removed, and the plates were air-dried. Subsequently, 125 µL of 0.1% crystal violet solution was added to each well, and the mixture was incubated at room temperature for 10 min. Excess stain was removed by washing the plates 2–3 times with distilled water, followed by drying.

To solubilize the bound dye, 200 µL of 33% glacial acetic acid was added to wells containing Gram (+) bacteria, while 200 µL of 95% ethanol was added to wells containing Gram (-) bacteria. After 15 min incubation at room temperature, the optical density (OD) was measured at 600 nm using a spectrophotometer. All assays were performed alongside positive control.

Antibiofilm activity was determined by comparing absorbance values with the control, and the percentage reduction in biofilm formation was calculated to quantify inhibition (Tozyilmaz et al., 2020).

Results and Discussion

Determination of Antibacterial Activity by Broth Microdilution Method (MIC and MBC)

The antimicrobial activity of *P. myrtifolia* extract was assessed using MIC and MBC values, as illustrated in Figure 1. The findings indicated that the extract was active against all six tested bacterial strains, encompassing both Gram (+) and Gram (–) strains.

Evaluation of MIC values showed that *K. aerogenes* and *E. durans* were the most sensitive strains, with inhibition observed at the lowest concentration tested (125 ppm). In contrast, the other strains required 250 ppm for inhibition. Analysis of MBC values further demonstrated that bactericidal activity generally occurred at concentrations approximately twice the MIC. Notably, *K. aerogenes* displayed a comparatively low MBC value (250 ppm), indicating a strong bactericidal effect of the extract against this strain. Overall, these results suggest that the chloroform extract of *P. myrtifolia* effectively suppresses bacterial growth and exhibits bactericidal activity at higher concentrations.

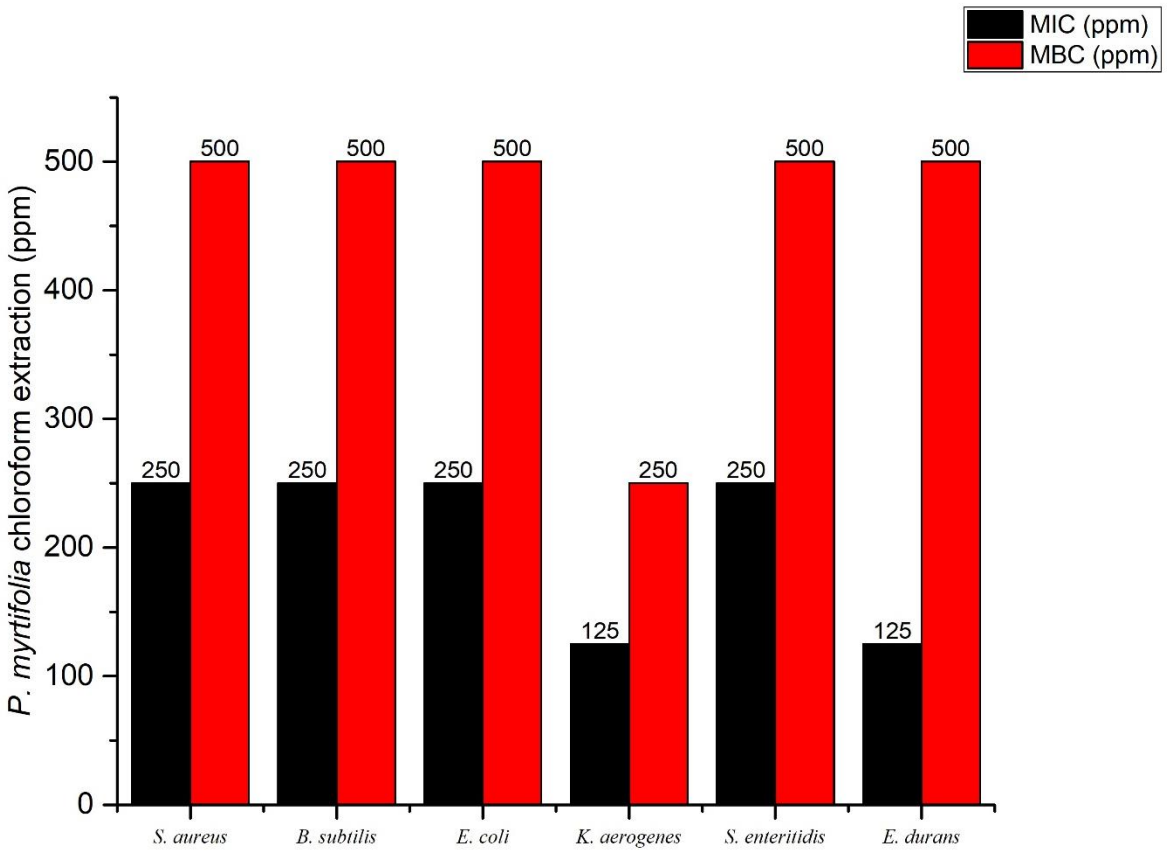

Figure 1 *MIC and MBC values of P. myrtifolia chloroform extract against selected bacterial strains.*

Extensive phytochemical and pharmacological studies have been conducted on several *Polygala* species, including *Polygala tenuifolia, Polygala japonica*, and *Polygala sibirica*. These species are known to contain a wide range of bioactive secondary metabolites, such as triterpenoid saponins (e.g., tenuigenin, tenuifolin, and polygalasaponins), terpenoids,

xanthones, flavonoids, coumarins, oligosaccharide esters, benzophenones, styryl-pyrones, and polysaccharides. These compounds have been reported to exhibit diverse biological activities, including anti-inflammatory, neuroprotective, antidepressant, sedative, analgesic, antitumor, and enzyme inhibitory effects, highlighting the therapeutic potential of *Polygala* species (Dar et al., 2017; Lacaille-Dubois et al., 2020).

Previous studies have demonstrated the antimicrobial activity of *Polygala* species. For example, the methanolic extract of *Polygala sadebeckiana* exhibited MIC and MBC values of 1.67 mg/mL and 10 mg/mL against *S. aureus*, and 0.98 mg/mL and 4 mg/mL against *Streptococcus pyogenes*, respectively (Zeleke et al., 2024). In comparison, the present study's findings indicate that the chloroform extract of *P. myrtifolia*showed antibacterial activity against *S. aureus* at lower MIC and MBC concentrations, suggesting enhanced antimicrobial efficacy. These results emphasize the importance of solvent selection and phytochemical composition in determining antibacterial activity and further support the potential of this plant as a source of bioactive compounds.

According to EUCAST data, the broad-spectrum antibiotic levofloxacin exhibits MIC ranges of 0.25–1.00 mg/mL for *S. aureus*, 0.03–0.25 mg/mL for *E. coli*, 0.03–0.5 mg/mL for *K. aerogenes*, and 0.06–0.25 mg/mL for *S. enteritidis* (EUCAST, 2025). Compared with these reference values, the results of this study suggest that *P. myrtifolia* extract exhibits considerable antibacterial activity against several tested strains. Furthermore, the extract showed comparable activity against both Gram (+) and Gram (-) bacteria, despite structural differences in their cell walls. This pattern indicates that the antibacterial effects of the extract may involve mechanisms that are not strictly dependent on cell wall composition, suggesting a broader, potentially multifaceted mode of action.

Plant-derived secondary metabolites exert antibacterial effects through diverse mechanisms, including disruption of membrane integrity, inhibition of essential enzymatic pathways, interference with nucleic acid synthesis, and induction of oxidative stress (Bocso & Butnariu, 2022; Sabiu, 2022). Among these, saponins, xanthones, and triterpenoids are particularly prominent. Saponins, due to their amphiphilic structure, interact with membrane lipids, increasing permeability and causing leakage of cellular contents. Xanthones have been shown to inhibit key bacterial enzymes and DNA-related processes while promoting the production of reactive oxygen species (ROS), leading to cellular damage. Triterpenoids can alter membrane structure, affect lipid bilayer dynamics, and interfere with critical metabolic functions (Haddad et al., 2003; Lacaille-Dubois et al., 2020; Zeleke et al., 2024). Collectively, these mechanisms provide a plausible explanation for the broad-spectrum antibacterial activity observed in this study.

Determination of Antifungal Activity by Agar Well Diffusion Method

The antifungal activity of the chloroform extract of *P. myrtifolia* was evaluated against *C. albicans* and *C. utilis* using the agar well diffusion method at different concentrations (2000–250 µg/mL) (Table 1 and Figure 2).

The results demonstrated that the extract inhibited both strains at all tested concentrations, with the highest activity observed at 1000 µg/mL, producing an inhibition zone of 13 mm.

Table 1. *Antifungal effects of chloroform extract of P. myrtifolia at different concentrations (mm ± SD)*

P. myrtifolia **extraction concentration (µg/mL)**	***C. albicans***	***C. utilis***
2000	12 + 0.66	11 + 0.33
1000	13 + 0.48	12 + 0.48
500	7 + 0.00	7 + 0.00
250	11 + 0.33	7 + 0.00

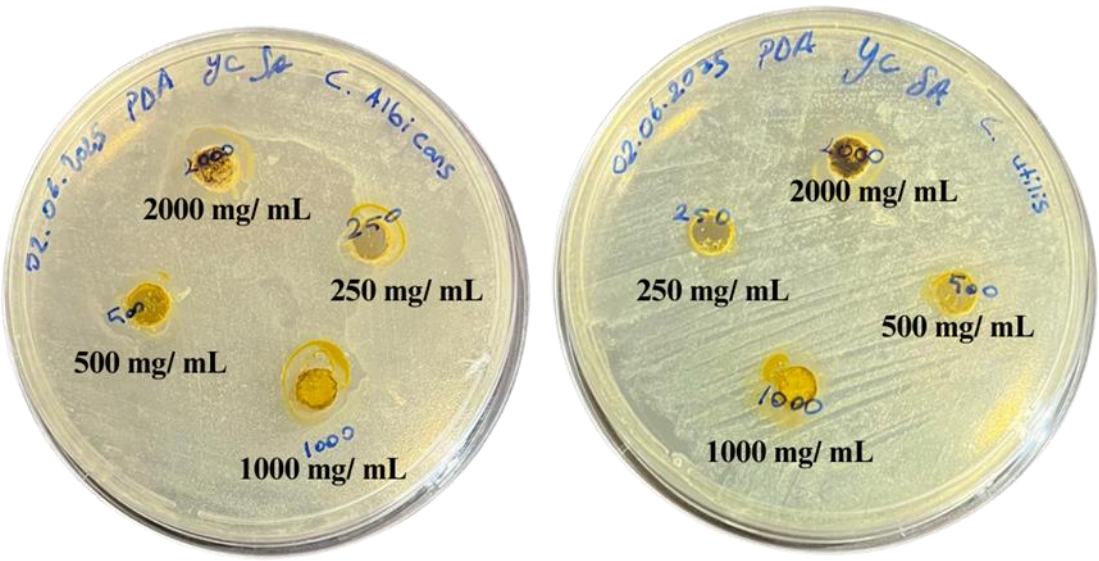

Figure 2. Representative agar well diffusion assay demonstrating the antifungal activity of *P. myrtifolia* chloroform extract against tested fungi at different concentrations (2000, 1000, 500, and 250 mg/mL). The inhibition zones observed around the wells indicate concentration-dependent antifungal effects. The image represents one experimental set from the study.

The chloroform leaf extracts of *P. myrtifolia* demonstrated antifungal activity against both fungal strains, indicating that their efficacy is governed not only by concentration but also by physicochemical properties of the active compounds and their interactions with fungal cell wall structures. These findings highlight the potential of *P. myrtifolia* as a valuable natural candidate for the development of antifungal agents.

Plant secondary metabolites apply antimicrobial effects by disrupting cellular structures and key metabolic processes. Among these, saponins and xanthones play major roles in antifungal activity (Johann et al., 2011; Lacaille-Dubois et al., 2020). Saponins interact with membrane sterols, increasing permeability and causing leakage of intracellular contents (Haddad et al., 2003). Xanthones inhibit essential enzymatic functions and induce oxidative stress, leading to fungal cell damage.

Triterpenoids also destabilize membrane structure and interfere with metabolic pathways (Johann et al., 2011; Motsei et al., 2003; Šovljanski et al., 2025). Xanthones are key constituents of *Polygala Radix* and are widely recognized for their pharmacological activities, particularly their antifungal potential. Due to its bioactive properties, *P. Radix* has been incorporated into the Chinese Pharmacopeia as a medicinal plant, with traditional use primarily focused on its dried roots (Garg et al., 2024; Johann et al., 2011). However, Song et al. found that the aerial parts also contain significant levels of active compounds, including xanthones, indicating a broader utilization potential (Song et al., 2016; Zhang et al., 2015). Overall, these observations highlight the role of xanthones in the antifungal efficacy of Polygala species and reinforce their importance in the development of plant-based therapeutic agents.

Accumulating evidence indicates that members of the Polygala genus possess significant antifungal activity against opportunistic pathogens, particularly *Candida spp*. In vitro studies on species such as *P. campestris, P. cyparissias, P. paniculata, P. pulchella*, and *P. sabulosa* have consistently shown that both crude extracts and their fractions are active against a broad spectrum of Candida species, *including C. krusei, C. tropicalis, C. glabrata, C. albicans*, and *C. parapsilosis* (Johann et al., 2011). However, these activities are often reported at relatively high concentrations, generally exceeding 1000 µg/mL when extracts are obtained using solvents such as hexane, ethanol, or ethyl acetate.

In contrast, the comparatively stronger antifungal activity observed in the current study suggests that the extraction strategy plays a decisive role in determining biological efficacy. Specifically, the use of chloroform may have facilitated the enrichment of lipophilic bioactive compounds, thereby enhancing antifungal potency. These findings underscore the critical role of solvent polarity in shaping the phytochemical profiles of extracts and highlight the importance of optimizing extraction conditions to maximize antifungal activity.

Determination of Antibiofilm Activity by Crystal Violet Assay

A concentration-dependent increase in antifungal activity was observed for all tested strains except *S. enteritidis*. The highest biofilm inhibition was observed at 500 µg/mL, whereas the lowest inhibition was observed at 15.625 µg/mL (Figure 3). Notably, the extract exhibited the highest inhibition of biofilm formation against *E. durans* (93.6%).

The concentration-dependent nature of biofilm inhibition suggests that the extract may act through a similar underlying mechanism across different bacterial strains. Plant-derived secondary metabolites are recognized as key bioactive agents with potent antibacterial and antibiofilm properties, primarily through their ability to target multiple cellular processes simultaneously (Flemming et al., 2016; Sun et al., 2013). Among these compounds, saponins may play an important role in antibiofilm activity due to their amphiphilic structure, which has been reported to interact with membrane lipids, increase membrane permeability, and potentially destabilize the biofilm matrix.

In addition, previous studies have suggested that saponins may interfere with initial bacterial adhesion and quorum sensing mechanisms, thereby contributing to the inhibition of biofilm formation (Chen et al., 2024; Tatli Cankaya & Somuncuoglu, 2021). Similarly, xanthones and triterpenoids have been associated with antibiofilm activity through possible effects on microbial metabolism and biofilm architecture (Lacaille-Dubois et al., 2020; Trentin et al., 2011). However, since no phytochemical profiling or mechanistic analyses were performed in the present study, these mechanisms remain hypothetical.

P. boliviensis and *P. violacea* have been reported to exhibit significant antibacterial and antibiofilm activities, particularly against biofilm-forming pathogens such as *Staphylococcus epidermidis*. Extracts derived from different plant parts (leaves, branches, and roots) tested at 0.4 mg/mL and 4.0 mg/mL (Trentin et al., 2011) generally show a concentration-dependent increase in antibiofilm activity, with higher concentrations leading to more pronounced inhibition.

In contrast, the present study's findings indicate a stronger antibiofilm effect at lower concentrations. This difference may be associated with variations in plant material and extraction solvent, which can influence the profile and availability of bioactive compounds, thereby affecting the overall biological activity.

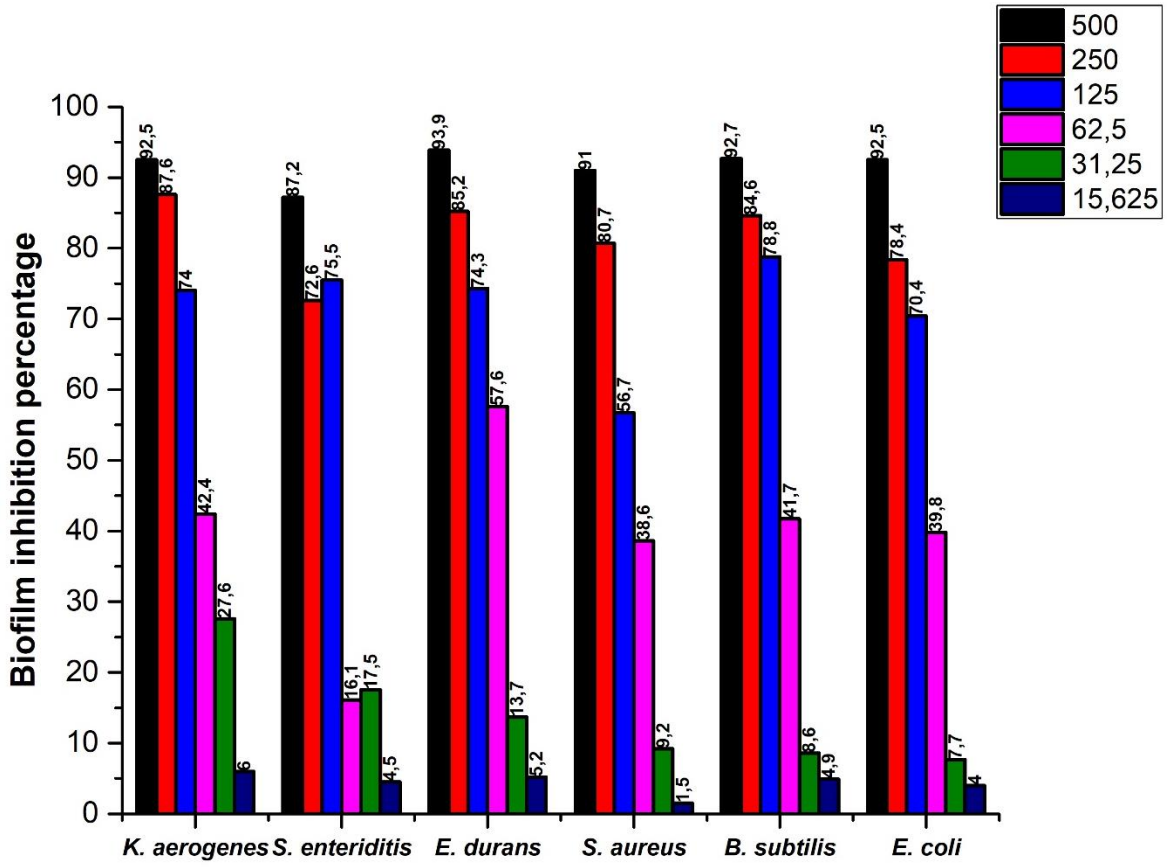

Figure 3 Concentration-dependent antibiofilm activity of *P. myrtifolia* chloroform extract against Gram (+) and Gram (-) bacterial strains at different concentrations (500–15.625 µg/mL).

Conclusion and Recommendations

The findings of this study demonstrate that the chloroform extract of *P. myrtifolia* exhibits notable antimicrobial activity, including antibacterial, antifungal, and antibiofilm effects against clinically relevant pathogens. Notably, the extract showed strong biofilm inhibition at relatively low concentrations, suggesting its potential to target microbial virulence mechanisms rather than solely exert bactericidal effects. The observed activities are likely due to the combined action of secondary metabolites, such as saponins, xanthones, and triterpenoids. Compared to previously reported *Polygala* species, the enhanced efficacy observed in this study highlights the importance of the extraction strategy in determining biological activity. These findings indicate that *P. myrtifolia* may serve as a promising natural source for the development of antimicrobial and antibiofilm agents. Further studies focusing on compound isolation, mechanism elucidation, and *in vivo* validation are needed to define its therapeutic potential.

Author Contributions

Yusuf Ceylan: Conceptualisation, Theoretical Background, Methodology, Data Analysis, Data Visualisation, Writing, Review and Editing

Sercan Arman: Theoretical Background, Methodology, Data Collection, Funding Acquisition

İrem Nur Öztürk: Methodology, Writing, Review and Editing

Conflict of Interest

The authors declare no conflict of interest.

Plagiarism Checks

The manuscript was checked for similarity using **iThenticate** before submission.

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Artificial Intelligence Usage Statement

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or interpretation of the research data. AI-assisted language tools were used only to improve the linguistic quality of the manuscript, and the authors take full responsibility for the content of the manuscript.

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Declarations

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GENİŞLETİLMİŞ ÖZET

Araştırma Problemi

Antimikrobiyal direnç ve mikroorganizmaların biyofilm oluşturma yeteneği, enfeksiyonların kontrolünü güçleştirmekte ve yeni doğal antimikrobiyal kaynakların araştırılmasını önemli hale getirmektedir. Saponinler, ksantonlar ve triterpenoidler gibi biyolojik olarak aktif sekonder metabolitler bakımından zengin olduğu bildirilen *Polygala myrtifolia*, bu açıdan potansiyel bir bitkisel kaynak olarak değerlendirilmektedir. Bu çalışmanın amacı, *P. myrtifolia* yapraklarından elde edilen kloroform ekstraktının Gram pozitif ve Gram negatif bakteriler ile fungal mikroorganizmalar üzerindeki antibakteriyel, antifungal ve antibiyofilm aktivitelerini *in vitro* koşullarda değerlendirmektir.

Araştırma Soruları

Bu çalışma kapsamında temel olarak şu sorulara yanıt aranmıştır: *P. myrtifolia* yapraklarının kloroform ekstraktı Gram pozitif ve Gram negatif bakterilere karşı antibakteriyel aktivite göstermekte midir ve bu aktivitenin minimum inhibitör konsantrasyon (MIC) ve minimum bakterisidal konsantrasyon (MBC) değerleri nelerdir? Ekstrakt *Candida albicans* ve *Candida utilis* üzerinde antifungal aktivite göstermekte midir? Ayrıca farklı ekstrakt konsantrasyonları, test edilen bakterilerin biyofilm oluşumunu hangi düzeyde inhibe etmektedir?

Literatür Taraması

Literatür taraması, Polygala türlerinin fitokimyasal özelliklerini ve özellikle antibakteriyel, antifungal ve antibiyofilm potansiyellerini ortaya koyan önceki araştırmaların değerlendirilmesi amacıyla gerçekleştirilmiştir. Makalede literatür taraması için kullanılan belirli veri tabanları ve sistematik bir tarama protokolü tanımlanmamış olmakla birlikte, araştırma makaleleri, derlemeler ve ilgili bilimsel kaynaklardan yararlanılmıştır. Önceki çalışmalar, Polygala türlerinin saponinler, ksantonlar, fenolik bileşikler, flavonoidler ve triterpenoidler gibi çeşitli sekonder metabolitler içerdiğini ve farklı mikroorganizmalara karşı biyolojik aktivite gösterebildiğini ortaya koymaktadır. Bununla birlikte, bildirilen aktivite düzeyleri bitki

türüne, kullanılan bitki kısmına ve özellikle ekstraksiyon yöntemine ve çözücüye bağlı olarak farklılık göstermektedir. Bu durum, *P. myrtifolia* kloroform ekstraktının farklı antimikrobiyal aktivitelerinin birlikte değerlendirilmesini gerekli kılmıştır.

Metodoloji

Çalışma, nicel ve deneysel bir araştırma olarak yürütülmüştür. *P. myrtifolia* bitkileri Aykut Peyzaj Botanik (Antalya)'den temin edilmiş; yapraklar kurutulup öğütüldükten sonra 60 g bitki materyali 600 mL kloroform kullanılarak Soxhlet yöntemiyle 8 saat ekstrakte edilmiştir. Antibakteriyel aktivite; Gram pozitif *Staphylococcus aureus, Bacillus subtilis* ve *Enterococcus durans* ile Gram negatif *Escherichia coli, Klebsiella aerogenes* ve *Salmonella enteritidis* olmak üzere altı bakteri üzerinde araştırılmıştır. MIC ve MBC değerleri broth mikrodilüsyon yöntemiyle belirlenmiştir. Antifungal aktivite, *C. albicans* ve *C. utilis* üzerinde agar kuyucuk difüzyon yöntemi kullanılarak 2000, 1000, 500 ve 250 µg/mL ekstrakt konsantrasyonlarında değerlendirilmiş ve inhibisyon zonları ölçülmüştür. Antibiyofilm aktivitesi ise kristal viyole boyama yöntemiyle belirlenmiştir. Ekstrakt uygulanan grupların optik yoğunluk değerleri kontrol gruplarıyla karşılaştırılarak biyofilm oluşumundaki inhibisyon yüzdesi hesaplanmıştır.

Sonuçlar ve Bulgular

Antibakteriyel analizler, *P. myrtifolia* kloroform ekstraktının test edilen altı bakteri suşunun tamamı üzerinde aktivite gösterdiğini ortaya koymuştur. *K. aerogenes* ve *E. durans*, 125 ppm MIC değeriyle ekstrakta karşı en duyarlı bakteriler olarak belirlenirken, diğer bakteri suşlarında inhibisyon 250 ppm konsantrasyonda gözlenmiştir. MBC değerleri genel olarak MIC değerlerinin yaklaşık iki katı düzeyinde belirlenmiş ve özellikle *K. aerogenes* için 250 ppm MBC değeri elde edilmesi ekstraktın bu mikroorganizma üzerindeki bakterisidal etkinliğini göstermiştir.

Antifungal analizlerde ekstrakt, *C. albicans* ve *C. utilis* üzerinde test edilen tüm konsantrasyonlarda inhibisyon oluşturmuştur. En yüksek aktivite 1000 µg/mL konsantrasyonda belirlenmiş; bu konsantrasyonda *C. albicans* için 13 mm ve *C. utilis* için 12 mm inhibisyon zonu ölçülmüştür. Konsantrasyon ile antifungal aktivite arasında tamamen doğrusal bir ilişki gözlenmemesi, aktif bileşiklerin difüzyon özellikleri ve diğer fizikokimyasal faktörlerin aktiviteyi etkileyebileceğini düşündürmektedir.

Antibiyofilm analizlerinde ise genel olarak konsantrasyon arttıkça biyofilm inhibisyonunun yükseldiği ve en yüksek etkinliğin 500 µg/mL konsantrasyonda ortaya çıktığı belirlenmiştir. Test edilen bakteriler arasında en yüksek biyofilm inhibisyonu *E. durans* üzerinde %93,6 olarak saptanmıştır. Sonuçlar birlikte değerlendirildiğinde, *P. myrtifolia* kloroform ekstraktının antibakteriyel, antifungal ve özellikle antibiyofilm aktivite açısından dikkate değer bir potansiyele sahip olduğu görülmektedir. Bununla birlikte, mevcut çalışmada fitokimyasal profil çıkarılmadığından gözlenen aktivitelerin belirli saponin, ksanton veya triterpenoidlerle doğrudan ilişkilendirilmesi mümkün

değildir. Gelecekte aktif bileşiklerin izolasyonu ve karakterizasyonu, etki mekanizmalarının araştırılması ve in vivo doğrulama çalışmalarının gerçekleştirilmesi, *P. myrtifolia*'nın antimikrobiyal ajan geliştirilmesindeki potansiyelinin daha kapsamlı biçimde değerlendirilmesini sağlayacaktır.

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