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Assessment of the *In Vitro* Antimicrobial Activity of *Ficus alba* and *Ficus carica* Latex

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Research Article

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ABSTRACT

Objective: *Ficus alba* Linn. and *Ficus carica* Linn., both belonging to the Moraceae family, have been utilized in traditional medicine for many years due to their antimicrobial, antifungal, and antioxidant properties. In the present study, the antimicrobial activities of latex samples from *Ficus alba* L. and *Ficus carica* L. were evaluated against various microorganisms, including the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853), and *Acinetobacter baumannii* (ATCC 17978). Additionally, the study assessed the activity against Gram-positive bacteria such as *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212), as well as the yeast-like fungus *Candida albicans* (ATCC 10231).
Methods: Both of the latex samples were collected from the south-western region of Türkiye. The microbial inoculums were adjusted according to the McFarland 0.5 standard and antimicrobial activity was assessed according to the standard disc diffusion method of European Committee on Antimicrobial Susceptibility Testing (EUCAST).
Results: Both types of latex samples of *Ficus species (Ficus sp.*) exhibited strong antimicrobial activity. The most potent antimicrobial activity was observed against *C. albicans* (15.3 mm). The strongest antibacterial activity was observed against *E. coli* (13.3 mm) among the six different bacterial species tested, while the weakest antibacterial effect was observed against *A. baumannii* (8.3 mm). The antimicrobial activity of *F. carica* latex was found to be more potent than that of *F. alba* latex.
Conclusion: The findings support the potential use of this latex as natural antimicrobial agents, particularly in the battle against pathogens that are developing antibiotic resistance. It is recommended that further investigation be conducted into the phytochemical components of these plants and latex, with a view to their potential future use in the pharmaceutical industry.

Keywords: Antimicrobial activity, *Ficus alba, Ficus carica*, Latex.

Ficus alba ve *Ficus carica* Latekslerinin *In Vitro* Antimikrobiyal Aktivitesinin Araştırılması

Araştırma Makalesi

Süreç

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ÖZET

Amaç: Moraceae familyasında yer alan *Ficus alba* Linn. ve *Ficus carica* Linn. bitkileri antimikrobiyal, antifungal ve antioksidan özellikleri sayesinde geleneksel tıpta uzun yıllar boyunca kullanılmaktadır. Bu çalışmada *Ficus alba* L. ve *Ficus carica* L. lateks örneklerinin antimikrobiyal aktivitesi Gram-negatif bakterilerden *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 17978) ve Gram-pozitif bakterilerden *Staphyloccoccus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212) ve maya mantarlarından *Candida albicans* (ATCC 10231)'a karşı test edilmiştir.
Yöntem: Lateks örnekleri Türkiye'nin güney-batı bölgesinden toplanmıştır. Mikrobiyal inokulumlar McFarland 0.5 standartına uyarlanarak, antimikrobiyal aktivite European Committee on Antimicrobial Susceptibility Testing (EUCAST)'in standart disk difüzyon metoduna göre araştırılmıştır.
Bulgular: Her iki *Ficus* türüne *(Ficus sp.*) ait lateks örneği de güçlü antimikrobiyal aktivite göstermiştir. En güçlü antimikrobiyal aktivite *C. albicans*'a (15.3 mm) karşı gözlemlenmiştir. Altı farklı bakteri türü arasında en güçlü antibakteriyel aktivitenin *E. coli*'ye (13.3 mm) karşı olduğu, en zayıf antibakteriyel etkinin ise *A. baumannii*'ye (8.3 mm) karşı olduğu tespit edilmiştir. *F. carica* lateksinin antimikrobiyal aktivitesinin *F. alba*'nın lateksine göre daha güçlü olduğu belirlenmiştir.
Sonuç: Sonuçlar, özellikle antibiyotik direnci gelişen patojenlerle mücadelede, bu latekslerin doğal antimikrobiyal ajanlar olarak potansiyel kullanımını desteklemektedir. Gelecekteki farmasötik uygulamalar için bu bitkilerin ve latekslerinin fitokimyasal bileşenlerinin daha detaylı araştırılması gerektiği vurgulanmıştır.

Anahtar Kelimeler: Antimikrobiyal aktivite, *Ficus alba, Ficus carica*, Lateks

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Introduction

The *Ficus* species, belonging to the Moraceae family, includes species *Ficus alba* L. (*F. alba* L., white fig) and *Ficus carica* L. (*F. carica* L., black fig). These plants hold significant importance among medicinal species due to their diverse biological activities.[1] *Ficus* species produce '*latex*', a rubber-like vascular fluid.[2] The latex is a viscous white liquid flowing from the immature fruit stalks of *F. alba* and *F. carica*. The antimicrobial, antifungal, and antioxidant properties discovered through studies on the latex of these plants have led to their extensive utilization in traditional medicine for many years. The biological activities of *Ficus sp.* latex are attributed to the flavonoids, polyphenols, proteolytic enzymes, and other secondary metabolites in their biochemical structure.[1,3] The latex of *F. alba* reported to exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria.[4] Research indicates that the latex of *F. carica* exhibits strong antimicrobial activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli*.[1] Additionally, it is effective against fungal infections and show antifungal activity.[5] Furthermore, it has been demonstrated that the latex of these plants exhibits synergistic effects with traditional antibiotics against multidrug-resistant (MDR) pathogens, thereby enhancing the efficacy of antibiotics.[6] Understanding the potential antimicrobial effects of latex derived from *F. alba* and *F. carica* is considered a crucial step for future pharmaceutical applications.

Microorganisms develop antimicrobial resistance in response to the uncontrolled use of conventional antimicrobial agents for treating infectious diseases. Additionally, the side effects and rare complications associated with certain antimicrobial agents have led researchers to scrutinize the antimicrobial efficacy of medicinal plants. Given the rising prevalence of antimicrobial resistance and the diminishing effectiveness of traditional antibiotics, investigating the antimicrobial effects of these plant latex is crucial for the discovery and development of natural antimicrobial agents. This study aimed to evaluate the *in vitro* antibacterial and antifungal activities of the latex derived from *Ficus alba* and *Ficus carica*. There are numerous studies conducted both globally and within our country that investigate the antimicrobial activity of leaf extracts from *Ficus species*.[7,8] Most research has been conducted on the *F. carica* species, whereas studies involving *F. alba* species are limited. A study conducted in our country evaluated the antimicrobial activity of *F. alba* and *F. carica* against foodborne pathogens.[9] It can be asserted that our research is one of the pioneering studies investigating the antimicrobial activity of *F. alba* and *F. carica* against medical pathogenic bacteria.

Materials and Methods

Materials

The latex samples of *F. alba* and *F. carica* used in this study were collected in August-2024 from the south-western region of Türkiye (Ortaca, Muğla, Türkiye: 36° 50' 20" N, 28° 45' 52" E). The latex fluids were obtained by squeezing the sap from the stems of unripe green fruits. The samples were stored in two separate brown glass bottles with droppers at +4°C in a refrigerator until the experimental analysis was completed.

Test Microorganisms

In the present study, the bacteria recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and known for their sensitivity were used.[10] Gram-negative bacteria; *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Acinetobacter baumannii* (ATCC 17978), and *Pseudomonas aeruginosa* (ATCC 27853); Gram-positive bacteria; *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) ant the yeast-like fungus *Candida albicans* (ATCC 10231) were used.

Microorganism Inoculums

The bacterial cultures were prepared overnight on 5% sheep blood agar at 37°C, while yeast cultures were cultivated on yeast peptone agar at the same temperature for 48 hours. Bacterial colonies from these young cultures were diluted in sterile saline solutions (0.9% NaCl) to adjust the McFarland standard of No. 0.5 (~10^8 colony-forming units (CFU)/mL) for bacterial suspensions and (~10^7 CFU/mL) for yeast suspensions.

Antimicrobial Test Discs

The latex samples, stored in a refrigerator (Arçelik, Türkiye) at +4°C, were allowed to reach room temperature for 30 minutes, alongside blank antibiotic discs (Bioanalyse, Türkiye). The latex samples were then filtered through sterile filters with a pore size of 0.22 µm (IsoLab, Germany). Subsequently, 200 µL of each latex sample was applied to sterile antibiotic discs with a diameter of 6 mm, which were placed in sterile petri dishes (FıratMed, Türkiye). The discs were permitted to absorb the latex fluids at room temperature for 15 minutes before being stored at +4°C for an additional 45 minutes.[11]

Antimicrobial Susceptibility Testing

The standard disc diffusion method recommended by EUCAST was employed to assess the antibacterial and antifungal activities.[11,12] Bacterial suspensions were inoculated onto Mueller Hinton Agar (MHA) (Merck,

Germany) medium. After a 15-minute absorption period, latex-impregnated discs were placed on the inoculated media, with four discs placed in each culture plate. Antibiotic discs, including Ampicillin (20 µg/disc) (Bioanalyse, Türkiye), and Trimethoprim (25 µg/disc) (Bioanalyse, Türkiye), served as positive controls for bacterial growth, while Fluconazole (25 µg/disc) (Bioanalyse, Türkiye) was utilized as a positive control for yeast. Blank antibiotic discs acted as negative controls. All plates were incubated at 37°C for 18 to 24 hours. Each test was conducted in triplicate. Figure 1 illustrates the experimental protocol for testing the antimicrobial activity of *F. alba* and *F. carica* latex.

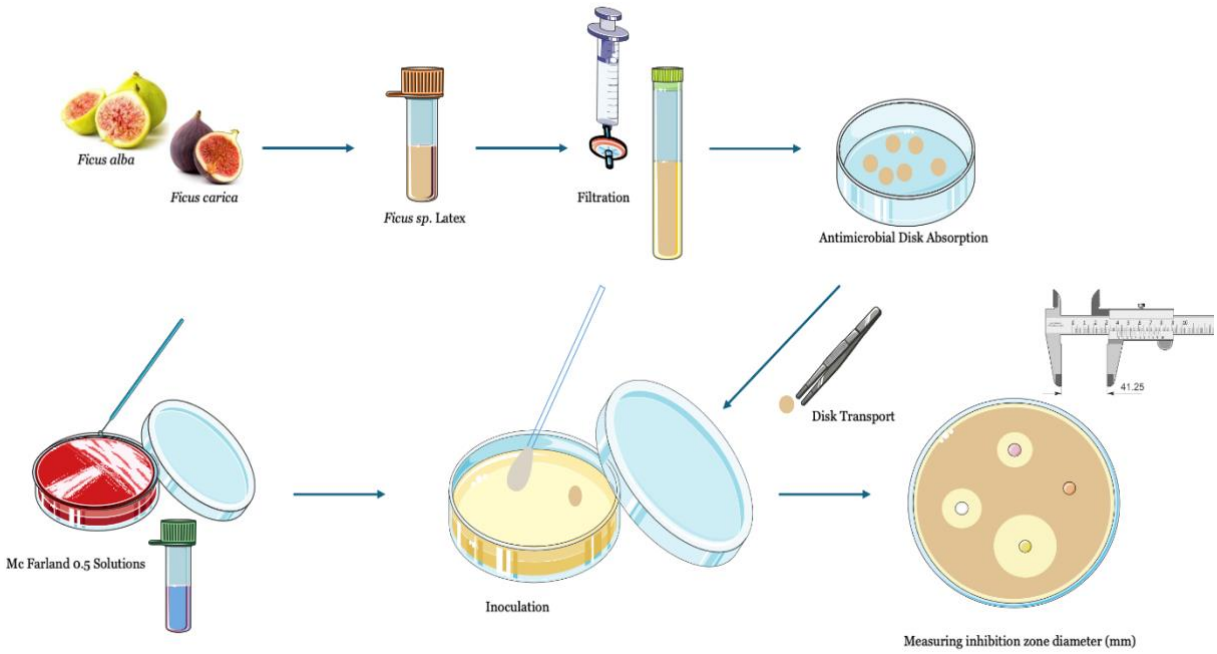

Figure 1. Schematic diagram of the antimicrobial activity test protocol for F. alba and F. carica latex. (The figure was created by Servier and is licensed under a Creative Commons Attribution 4.0 Unported License).

Table 1. Antimicrobial Activities of Ficus alba and Ficus carica Latex (Mean ± SEM)

Microorganisms	Diameter of Inhibition Zone* (mm)							
	Ficus alba				*Ficus carica*			
	Z_1	Z_2	Z_3	$Z_{\bar{x}}$	Z_1	Z_2	Z_3	$Z_{\bar{x}}$
Escherichia coli	12	14	14	13.3 ±1.15	14	13	10	12.3±2.08
Staphylococcus aureus	13	11	11	11.6±1.15	11	12	13	12±1.0
Klebsiella pneumoniae	9	10	8	9±1.0	11	11	9	10.3±1.15
Acinetobacter baumannii	8	9	8	8.3±0.57	9	9	10	9.3±0.57
Pseudomonas aeruginosa	10	10	9	9.6±0.57	11	9	10	10±1.0
Enterococcus faecalis	9	10	8	9±1.0	10	10	8	9.3±1.15
Candida albicans	14	16	14	14.6±1.15	15	15	16	15.3±0.57

Z: Zone, $Z_{\bar{x}}$: Arithmetic mean of zones, (Mean ±SEM, mm), *Diameter of inhibition zones including the diameter of the disc (6 mm)

Evaluation of Test Results

After the incubation period, the inhibition zone diameters surrounding the latex discs were measured in millimeters (mm) using a ruler. The diameter of the discs (6 mm) was factored into the measurements. The average inhibition zone for each type of latex was then calculated.

Statistical Analysis

The data obtained from this study were analyzed using the GraphPad-Prism (v10.0) software (Boston, USA). Arithmetic means and standard deviations were used to report the results.

Results

In the present study, it was observed that the latex of *F. alba* and *F. carica* exhibited significant antimicrobial activity against the tested microorganisms. The inhibition zone diameters for *F. alba* were as follows: *E. coli* (13.3 mm), *S. aureus* (11.6 mm), *K. pneumoniae* (9 mm), *A. baumannii* (8.3 mm), *P. aeruginosa* (9.6 mm), *E. faecalis* (9 mm), and *C. albicans* (14.6 mm). The inhibition zone diameters for *F. carica* were as follows: *E. coli* (12.3 mm), *S. aureus* (12 mm), *K. pneumoniae* (10.3 mm), *A. baumannii* (9.3 mm), *P. aeruginosa* (10 mm), *E. faecalis* (9.3 mm), and *C. albicans* (15.3 mm). The results of the antimicrobial activity are presented in Table 1.

The strongest antimicrobial activity for both types of latex was observed against *C. albicans.* Among the bacterial species tested, the most potent antibacterial activity was noted against *E. coli*, while the weakest activity was observed against *A. baumannii*. The antimicrobial activity of *F. carica* latex was stronger than that of *F. alba*. The antimicrobial activities of *F. alba* and *F. carica* latex are shown in Figures 2 and 3.

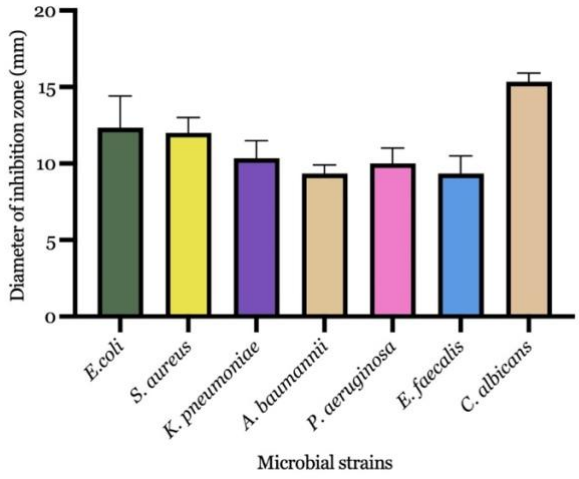

Figure 2. Antimicrobial Activities of Ficus alba Latex Samples. The values represent the means ± standard deviation of data independent experiments.

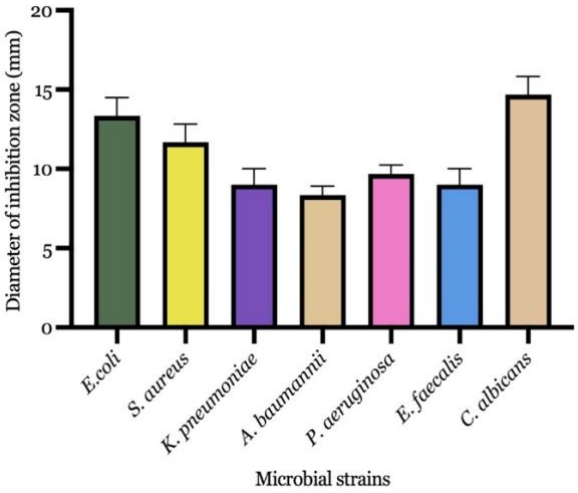

Figure 3. Antimicrobial Activities of Ficus carica Latex Samples. The values represent the means ± standard deviation of data independent experiments.

Discussion

The development of antimicrobial resistance to conventional antimicrobial agents used in the treatment of infectious diseases is an escalating concern. The rise in antimicrobial resistance among pathogenic bacteria complicates infection management, prolongs hospital stays, and increases morbidity and mortality rates. Consequently, this situation has resulted in significant economic losses for healthcare systems. The resistance to conventional antibiotics, coupled with the lengthy and costly process of developing new antimicrobial drugs, has prompted scientists to explore alternative agents with antimicrobial potential. In particular, the emergence of MDR pathogens has spurred the investigation of a variety of alternative antimicrobial agents for therapeutic applications. In this context, agents with minimal side effects and toxicity are being prioritized in research as potential alternative antimicrobial solutions.[13,14]

In this study, the antibacterial and antifungal activities of *F. alba* and *F. carica* latex samples were investigated using the disc diffusion method. The findings indicated that the antimicrobial activity of *F. carica* latex was stronger than that of *F. alba*. For both types of latex, antifungal activity was observed to be higher than antibacterial activity. The results of this study are largely consistent with both national and international research.[15-18] In a study investigating the antibacterial activity of *F. carica* latex using the disc diffusion method, the standard test microorganisms were employed, similar to our study, and an inhibition zone diameter ranging from 10.16 to 14.5 mm was observed. It has been stated that *F. carica* latex can be regarded as an antibacterial agent against infections caused by test organisms.[19] Raskovic et

al. tested the antifungal activities of *F. carica* latex at different times of the year, reporting that latex collected in the spring exhibited higher antifungal activity than that collected in the summer.[20]

Aref et al. reported significant antimicrobial inhibition against human pathogens for four distinct species of *F. carica* in their study. Similar to our study, they demonstrated the most potent antimicrobial activity against *C. albicans*.[6] In a study investigating the antibacterial and antibiofilm activities of *F. carica* extracts formulated as nanoparticles, the most significant antibacterial effect was observed against *P. aeruginosa*, which contrasts with the findings of our study.[21] Rashid et al. investigated the latex and leaf extracts of *F. carica* and observed strong antibacterial and antifungal activity, which is consistent with our study. They reported that the antifungal activity was higher than the antibacterial activity.[8]

In the literature, it has been observed that there are more studies on the biological properties of *F. carica* compared to *F. alba.*[22] Akarca and Tomar reported that the antimicrobial activity of *F. carica* was higher than that of *F. alba* in their research involving the latex of both species, which is consistent with the findings of our study.[9] Studies have reported that *F. carica* has a higher glucose content than *F. alba*.[23] Nafis et al. investigated the combinational antimicrobial activity of *F. carica* leaf essential oil and conventional antibiotics in their study. They found that, in the presence of this combination, the Minimum Inhibition Concentration (MIC) value decreased significantly by 1 to 16-fold, particularly against Gram-positive bacteria, indicating a synergistic interaction.[4]

Limitations

The phytochemical composition of plants comprises various bioactive components. Although this study demonstrated the antimicrobial potential of *F. alba* and *F. carica* latex, a phytochemical analysis of these latexes should be conducted to identify the specific biological components responsible for their antimicrobial activity. Further research is necessary to ascertain the phytochemical composition of *Ficus sp.* latex.

Conclusion

The results of this study demonstrate the significant antimicrobial activity of *F. alba* and *F. carica* latex. Given the rising issue of antimicrobial resistance, these latexes may serve as alternative agents in the treatment of microbial infections. In the present era, where the search for alternative agents to conventional commercial antimicrobials continues unabated, it is critical to determine the phytochemical content and active biochemical components of these latexes through chemometric analyses. Further studies should investigate the phytochemical composition, potential toxic effects and possible combinational-synergistic interactions with conventional antibiotics.

Ethics Committee Approval

This study does not require approval from an Ethics Committee.

Acknowledgment

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Declaration of Competing and Financial Interest/Funding

The author declare that they have no known competing financial interests or personal relationships that could be perceived as influencing the work reported in this paper. No funding.

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