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White Mulberry (*Morus alba* L.) Extracts Modulate *Candida albicans* Biofilm Formation: A Biological and Genetics-Relevant Perspective

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Abstract

Morus alba L., or white mulberry, is a small deciduous shrub from the Moraceae family, with well-known medicinal properties and beneficial effects on human health. Recent studies have confirmed the bioactive potential of this plant, including its antimicrobial effects, primarily against bacteria and viruses. In contrast, data regarding its antifungal activity are scarce. The main goal of this study was to evaluate the antifungal and antibiofilm capacity of white mulberry ethanolic and methanolic extracts derived from leaves and fruits against *Candida albicans*, through the measuring of fungal growth inhibition in the agar diffusion method; determining the minimum inhibitory concentration (MIC) by the broth microdilution method; defining the minimum fungicidal concentration (MFC); evaluation of the antibiofilm capacity of the extracts through the tissue culture plate method; and calculation of the biofilm-inhibition percentage. The fruit methanolic extract achieved the largest inhibition zones (31.00±1.00 mm), followed by the fruit ethanolic extract (30.00±1.00 mm). MIC values for all tested samples were defined at 125 µg/ml, while MFC values were determined at 250 µg/ml. In this study, *C. albicans* was evaluated as a strong biofilm former, and white mulberry extracts showed the potential to change the biofilm-forming capacity in subinhibitory concentrations. Fruit methanolic extract decreased this capacity in a wide range of concentrations, with biofilm inhibition up to 92.61%. Results indicate that white mulberry represents a strong candidate for further investigation in phytotherapy.

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Keywords

Antibiofilm capacity, Bioactivity, Candida albicans, Morus alba L.

Introduction

White mulberry (*Morus alba* L.) is a plant from the Moraceae family, naturally distributed and cultivated worldwide (Chen et al., 2021). Traditional medicinal practices recognize all parts of this plant, including leaves and fruits, as valuable sources of beneficial compounds (Zhou et al., 2022). White mulberry leaves contain many polyphenolic substances, such as phenolic acids and flavonoids, and their extracts exhibit hypoglycaemic, hypolipidaemic, and anti-atherogenic properties (Sánchez-Salcedo et al., 2015a). Furthermore, white mulberry fruits are consumed in fresh and processed forms, and studies confirmed positive effects on human health, especially in cases of type 2 diabetes mellitus (Sánchez-Salcedo et al., 2015b). Antimicrobial properties of various white mulberry products have been evaluated in previous studies (Islam et al., 2008; Chen et al., 2017; Miljković et al., 2018; Aelenei et al., 2019; Thabti et al., 2020), with a main focus on bacterial and viral pathogens. A comprehensive phytochemical and pharmacological study on *M. alba* (Batiha et al., 2023) offers insight into the biological potential of this plant, and there is also a lack of data regarding its antifungal activity.

Candida albicans is a yeast, normally found on skin and mucosal surfaces as part of the regular human microbiome. However, this species has significant pathogenic potential and can cause infections under specific circumstances (Parambath et al., 2024). According to the World Health Organization's Fungal Priority Pathogens List (2022), *C. albicans* is classified in the Critical Priority Group of pathogens, along with *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Candida auris*. In addition to the fact that *C. albicans* expresses

various virulence factors and emerging patterns of resistance to antifungal drugs (Cavalheiro and Teixeira, 2018), this species is also considered one of the major biofilm producers within the genus (Chandra et al., 2001). Biofilm development involves the initial adherence of the yeast cells to the substrate, followed by the initiation step, where cells project out and continue to grow into the filamentous hyphal structure. After that, hyphae are assembled and begin depositing an extracellular matrix (ECM), which is a key step in biofilm maturation. Ultimately, non-adhering cells disperse from the biofilm (Atriwal et al., 2021), having greater pathogenicity, higher filamentation, adhesion, and biofilm formation capacity (Uppuluri et al., 2009), and also with different transcriptional profiles (Eix and Nett, 2020). Biofilm-forming capacity provides *C. albicans* with higher resistance to available antifungal agents, and that inevitably means higher mortality rates in cases of infections (Brighenti et al., 2017). According to Taff et al. (2013), acquired drug resistance in planktonic cells is associated with higher activity in efflux pumps and genetic changes in the genes that code for drug target enzymes, specifically ERG11 and FKS1. At the same time, antifungal resistance is an intrinsic characteristic of *Candida* biofilm, and it could be up to 1000-fold higher in comparison to that of planktonic cells. There are six major transcription regulators involved in biofilm formation in *C. albicans*: Efg1 and Tec1 (regulation of the cell morphology); Ndt80 (participates in biofilm formation); Bcr1 (occurrence of the cells with hyphae); Brg1 and Rob1 (both present exclusively in species closely related to *C. albicans*) (Pareira et al., 2020). Additionally, other transcription regulators were described: Gal4, Rfx2, and Flo8. Flo8 is essential for biofilm formation throughout all stages of development, while both Gal4 and

Rfx2 are necessary for proper biofilm formation during intermediate time points (Fox et al., 2015). Wang et al. (2024) discussed the gene regulation of biofilm formation in *C. albicans* through four stages: adhesion, hyphal growth and aggregation, maturation, and dispersion. Genes related to the adhesion stages comprise: *als1*, *als3* (codes for cell wall glycoproteins from the Als protein family) (Giacometti et al., 2009), *eap1* (coding for an adhesion protein essential for linkage to the medium), and *ywp1* (negative regulation in this stage, since it codes for the Ywp1, protein that inhibits the formation of biofilm). Despite their important role in this initial step of biofilm formation, they are also regulated by other genes such as *efg1* (coding for Efg1 protein included in the biofilm colonization) (Giacometti et al., 2011), or the Bcr1 factor, a zinc finger protein that positively affects the expression of *als1* and *als3* (Biswas et al., 2007) and strengthens cell adhesion via direct regulation of the *ywp1* and *eap1* genes (Davis, 2003). Gene regulation of the colonization stage is taking place by four pathways, namely Hog MAPK (included in the virulence and the oxidative stress response through the hyphal formation) (Eriksson et al., 2016), MAPK (induced by different settings of the matrix) (Vylkova and Lorenz, 2014), cAMP-PKA (participates in the hyphal elongation), and Rim101 (pH-sensing pathway that plays a role in the adaptation to alkaline environment) (Wang et al., 2024). The mature *C. albicans* biofilm is a resistant, hyphae-based structure with an ECM rich in specific proteins, primarily Glx3 (Beirão et al., 2014). Key genes in this stage include *glx3*, *adh5*, *gca2*, *gca1*, *csH1*, *ifd6*, and *erg6*, and they are mainly regulated by Zap1, a zinc-sensitive transcription factor that acts as a complex negative regulator in mature biofilms (Ramage et al., 2002). This factor can inhibit ECM formation by

suppressing some genes (*csH1* and *adh5*; reducing β -1,3 glucan), but also promote the ECM accumulation via *gca2* and *adh5* expression. Furthermore, Zap1 inhibits *erg* and *hxt* gene expression, thus inhibiting overall biofilm maturation (Wang et al., 2024). Genes that regulate dispersion of cells in biofilm are *pes1*, *nrg1*, and *ume6*, where the first two improve cell dispersion, while *ume6* enhances cell release from biofilms. As an important factor in this step, the *hsp90* gene was also identified (Wang et al., 2024). According to Costa et al. (2022), dispersed cells can upregulate multiple genes involved in adhesion, resistance, nutrition, and pathogenesis. Differences in biofilm formation in *C. albicans* are closely tied to the species' broad natural genetic variation, which leads to strain-specific patterns of gene expression (Cravener et al., 2023; Delaney et al., 2023; Gonçalves et al., 2023).

There are some reports about the antifungal activity of white mulberry products (Ahmad and Beg, 2001; Jha and Srivastava, 2013; de Oliveira et al., 2015; Lu et al., 2017), but according to our best knowledge, there are no thorough data regarding antifungal and antibiofilm effects of *M. alba* against *C. albicans*. Therefore, the present study aimed to evaluate the antifungal and antibiofilm potential of white mulberry leaf and fruit extracts against *C. albicans*.

Material and methods

Plant material and extract preparation

For the extract preparation, leaves and fruit from the healthy adult tree of white mulberry (*Morus alba* L.) located in Tuzla (Bosnia and Herzegovina) were collected. The botanical designation was conducted in the Laboratory of Plant Systematics at

the University of Sarajevo-Faculty of Science, and the herbarium specimen is stored in the scientific collection of the National Museum of Bosnia and Herzegovina (SARA). Before extraction, leaves were air-dried, while fruits were frozen. White mulberry leaf and fruit extracts were obtained using ultrasonic extraction with ethanol (Sigma-Aldrich) and methanol (Sigma-Aldrich) as solvents (Kainat et al., 2023). The experiment was implemented on the ultrasonic bath (Sonorex Digitec, DT 31 H). Finally, four extracts of white mulberry were prepared: ethanolic and methanolic extracts of leaves and fruit (Table 1). The extract yields (Mahmutović-Dizdarević et al., 2024) expressed as a percent of the weight of raw material are presented in Table 1. This study did not analyze the chemical composition, and previous data are referenced only for contextual interpretation. Obtained extracts were stored in the dark, at 4 °C. To investigate the antifungal and antibiofilm potential of the white mulberry, extracts were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) to a final concentration of 1000 µg/mL.

Agar diffusion method

The fungal strain used in this investigation was *Candida albicans* ATCC 10231, obtained from the American Type Culture Collection, ATCC (Manassas, Virginia, USA). The agar well diffusion method is performed according to Balouiri et al. (2016). Inoculum was prepared following the recommendations of EUCAST (2017). Overnight cultures were dissolved in sterile saline solution to achieve the final turbidity of 0.5 McFarland standard, representing the fungal cell concentration of 1.5×10^8 CFU/mL. Inoculation of the plates was performed using the sterile swabs soaked in a suspension of *C. albicans*, and the seeded plates

were left at room temperature for 15 minutes to allow better absorption of the applied inoculum. Agar plates were then drilled to make wells with 8 mm in diameter. 50 µl of white mulberry extract was transferred into the wells, and plates were incubated for 16 to 18 hours at 37 °C. The antifungal activity was evaluated based on the diameter of inhibition zones, after diffusion in the medium. This assay was performed in triplicate. The standard antifungal drug Nystatin, 100 IU (Oxoid™), was used as the positive control, while DMSO was the negative control.

Broth microdilution method and determination of the minimum fungicidal concentration

The broth microdilution method was applied to determine the minimum inhibitory concentration (MIC) of the tested extracts. Overnight cultures were adjusted in Sabouraud Glucose Broth, SGB (Sigma-Aldrich), to the density of 0.5 McFarland standard (1.5×10^6 CFU/mL). SGB was used for further dilution and the preparation of the final inoculum suspension (1×10^3 CFU/mL). Upon that, each well was inoculated with 100 µL of yeast suspension, and an equal volume of two-fold dilution of extracts (1000-1.95 µg/mL), which resulted in 5.0×10^2 cells/mL (NCCLS, 2002). Inoculated SGB medium was used as a positive control, while the SGB containing pure DMSO was taken as the negative control. Microtiter plates were incubated overnight at 35 ± 1 °C in the standard incubator with natural convection (Binder BD 53E2). Results were read on a microplate reader (Biotek Epoch Microplate Reader) at a wavelength of 595 nm. Experiments were performed in four replications. To evaluate the minimum fungicidal concentration (MFC), the content of the well, described as the MIC, and two surrounding wells

Table 1. Nomenclature of the prepared samples and extract yields

Label	Type of extract	Extract yields (% of the weight of raw material)
S1	Leaf ethanolic extract	14.56 %
S2	Leaf methanolic extract	20.03 %
S3	Fruit ethanolic extract	3.12 %
S4	Fruit methanolic extract	10.56 %

were replated on a sterile Sabouraud Glucose Agar SGA (Sigma-Aldrich). Plates were incubated overnight at 37 °C. This experiment was performed in three replications.

Evaluation of the antibiofilm potential

For the assessment of the changes in biofilm formation of *C. albicans* in the presence of white mulberry extracts, the tissue culture plate (TCP) method in 96-well plates (Merritt et al., 2005) was used, with tryptic soy broth (TSB) (Sigma-Aldrich) as the dilution medium. The initial extract concentration of 1000 µg/mL was diluted two-fold in TSB up to the end concentration of 1.95 µg/mL. The final volume of 100 µL of such dilutions was added to each well, and after that, the inoculation was performed with 10 µL of the studied fungal strain. The inoculum was prepared as previously described. The biofilm formation was determined through the adherence of fungal cells only in the presence of TSB.

After overnight incubation, the content of the plates was decanted, and the plates were first washed in Phosphate Buffered Saline, PBS (Sigma-Aldrich), and then stained with a 0.1% solution of crystal violet for 10 minutes. Before reading the plates (Biotek Epoch Microplate Reader, 595 nm), 96% ethanol was added to each well. The experiment was carried out in four replications, and the results are presented as the mean value ± STDEV. The biofilm-forming category of the investigated fungal

strain was determined according to Stepanović et al. (2007). The optical density cut-off value (ODc) was calculated as three standard deviations above the mean OD of the negative control, while the biofilm categories were determined as follows: $OD \leq ODc$: non-adherent (NA), $ODc < OD \leq 2 \times ODc$: weakly adherent (W), $2 \times ODc < OD \leq 4 \times ODc$: moderately adherent (M), and $4 \times ODc < OD$: strongly adherent (S).

The percentage of biofilm inhibition produced by the activity of white mulberry extracts was calculated according to Jadhav et al. (2013):

$$\% = \left[\left\{ \frac{OD_{595 \text{ nm}} \text{ of experimental well with the extract}}{OD_{595 \text{ nm}} \text{ of control well without the extract}} \right\} \times 100 \right]$$

Statistical analysis

Descriptive statistical parameters (mean values and standard deviation) and the percentage of biofilm inhibition were calculated using Microsoft Office 2019 Excel (Microsoft Corporation, USA). Further statistical analysis was performed using one-way ANOVA with the post hoc Fisher's Least Significant Difference (LSD) test (STATISTICA 10; StatSoft, Inc.). Differences were considered statistically significant at the $p < 0.05$ level.

For the determination of MIC and MFC values, as well as in antibiofilm assays, data were presented descriptively, and interpretation was done according to predefined threshold values/percent of inhibition.

Results and Discussion

The first step in evaluating white mulberry extracts as antifungal agents was implementing the agar diffusion method. After measuring the generated inhibition zones (Figure 1), the results are summarized as follows (Table 2).

Diameters of inhibition zones ranged from 19.17 ± 0.76 mm caused by the S2 sample, to 31.00 ± 1.00 mm achieved by the activity of the S4. Larger inhibition zones were detected in the case of the white mulberry fruit extracts (Table 2). Nystatin induced inhibition zones of 20.80 ± 0.20 mm, while DMSO did not exhibit antifungal activity. The results of the broth microdilution method are given in Table 3. The minimum inhibitory concentration of all tested extracts was determined at $125 \mu\text{g/ml}$ (Table 3). After replating, all investigated extracts'

MFC values were determined at $250 \mu\text{g/ml}$ (Table 3). The average optical density (OD) values of quadruplets \pm STDEV for all tested concentrations of all extracts and their negative and positive controls are presented in Figure 2.

After the analysis of the biofilm-forming capacity, *C. albicans* is marked as a strong biofilm former. Mean absorbance values (MAV) \pm STDEV measured at subinhibitory concentrations of the white mulberry extracts, and used in evaluating their antibiofilm activity against *C. albicans*, are presented in Table 4. The percentage of biofilm inhibition was calculated at particular concentrations that induced a change in the biofilm-forming category. S1 sample decreased the strong *C. albicans* biofilm to moderate at the range of 31.25 - $1.95 \mu\text{g/ml}$. For this extract, the extent of the biofilm inhibition was 74.08 - 85.76% (Figure 3).

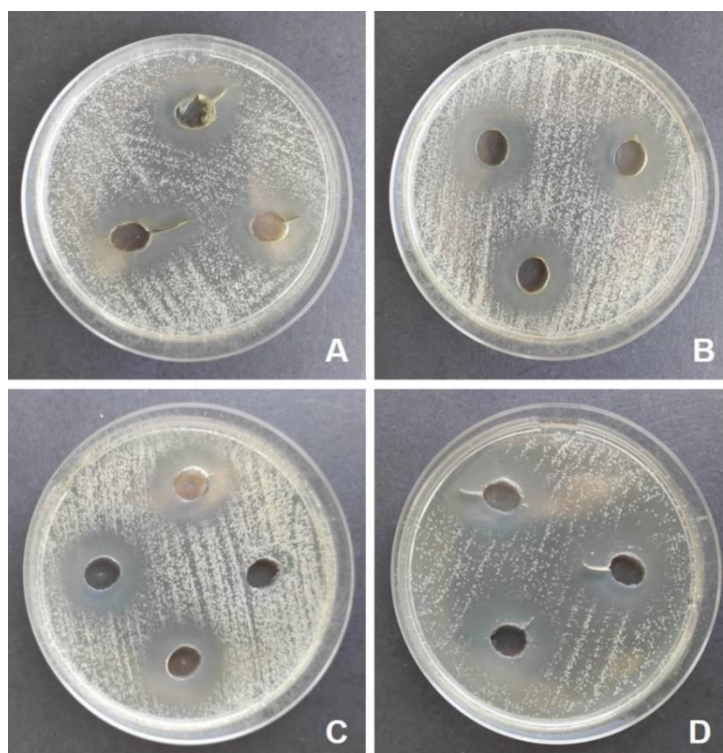


Figure 1. Growth inhibition of *C. albicans* achieved through the activity of white mulberry extracts. A-S1; B-S2; C-S3; D-S4

Table 2. Diameter of inhibition zones (mm) obtained through the agar well diffusion method

S1	S2	S3	S4	Nystatin
24.33±2.52*	19.17±0.76*	30.00±1.00*	31.00±1.00*	20.80±0.20*

Results are Mean ± STDEV. DMSO did not cause growth inhibition. *Obtained values differ significantly at p<0.05 after the *post hoc* LSD test.

Table 3. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of tested extracts (µg/ml)

Determined value	S1	S2	S3	S4
MIC	125	125	125	125
MFC	250	250	250	250

Table 4. Mean absorbance values (MAV) ± STDEV in the evaluation of the antibiofilm activity of the white mulberry extract against *C. albicans*

Tested sample	Subinhibitory concentrations of the extracts (µg/ml)					
	62.50	31.25	15.62	7.81	3.90	1.95
S1	0.353±0.214	0.194±0.049*	0.265±0.110*	0.347±0.078*	0.329±0.140*	0.288±0.102*
S2	0.138±0.036**	0.122±0.020*	0.183±0.059*	0.175±0.010*	0.195±0.047*	0.342±0.117*
S3	0.143±0.017**	0.169±0.016**	0.230±0.018*	0.281±0.014*	0.648±0.213	1.508±0.075
S4	0.103±0.006**	0.104±0.011**	0.113±0.010**	0.764±0.163	1.532±0.276	1.387±0.160

*Decreasing of the biofilm-forming capacity for one category; **Decreasing of the biofilm-forming capacity for two categories; MAV ± STDEV (positive/negative control), *C. albicans*: 1.096 ± 0.505/0.075 ± 0.008.

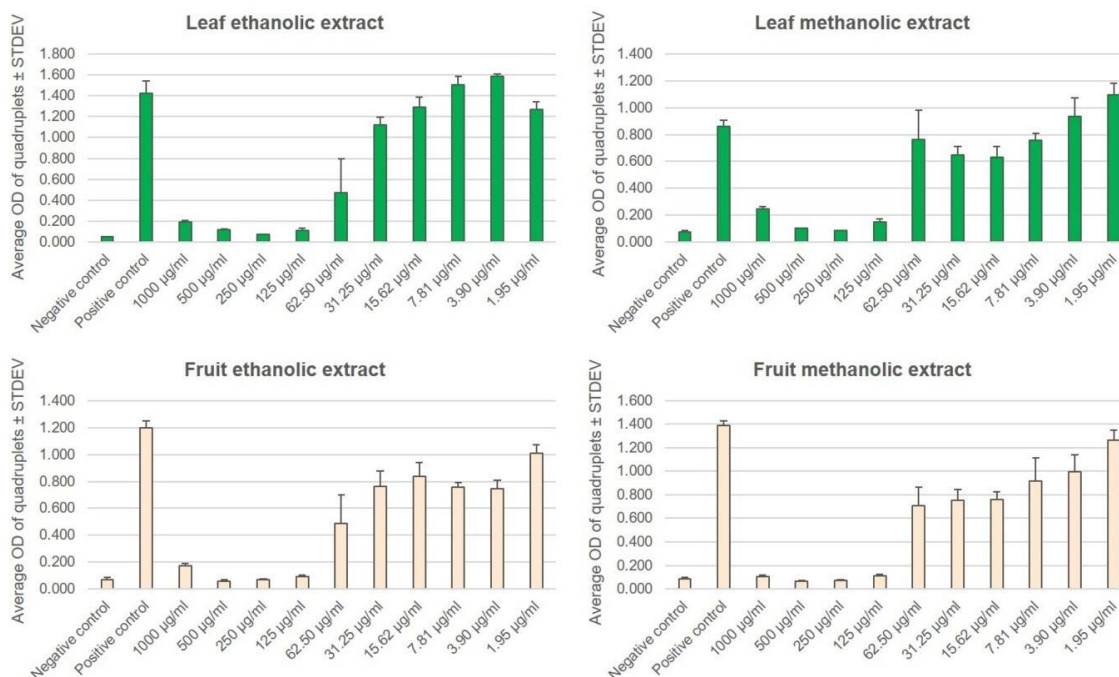


Figure 2. Average optical density values (±STDEV) for all extracts and controls used for the MIC determination

Additionally, S2 decreased the biofilm-forming category of the yeast to weakly adherent at 62.50 $\mu\text{g/ml}$, followed by a lowering of the category to moderately adherent up to 1.95 $\mu\text{g/ml}$. Inhibition of the biofilm was assessed at 42.87-64.45% (Figure 3). S3 led to the occurrence of weakly adherent *C. albicans* biofilm at 62.50 $\mu\text{g/ml}$ and 31.25 $\mu\text{g/ml}$, as well as to moderately adherent biofilm at 15.62 $\mu\text{g/ml}$ and 7.81 $\mu\text{g/ml}$. The inhibition percentage was 78.23-88.89% (Figure 3). The S4 sample changed the biofilm-forming category to weakly adherent in the 62.50-15.62 $\mu\text{g/ml}$ concentration range. Inhibition of the biofilm in this case was 91.89-92.61% (Figure 3).

C. albicans causes infections usually treated with antifungal drugs from a few different classes, distinguished by their key activity targets. There are four main classes of antifungal agents with anti-*Candida* activity: polyenes, azoles, echinocandins, and 5-Flucytosine (nucleoside analogs) with possible fungistatic or fungicidal activity (Bhattacharya et al., 2020). Unfortunately, there is an increasing antimicrobial resistance within the genus *Candida* (Whaley et al., 2017), and the US

Centers for Disease Control and Prevention reported in 2019 that more than 34,000 cases and 1700 deaths annually were related to drug-resistant *Candida* infections.

It has already been mentioned that *C. albicans* possesses the ability to form biofilms. Considering that approximately 80% of all microbial infections are caused by biofilm-forming pathogens (Nobile and Johnson, 2015), there is an excessive need for a definition of novel antibiofilm agents, especially for yeast, where the lack of available synthetic drugs is recognized. *C. albicans* biofilms exhibit intrinsic resistance to most antifungal drugs, which is achieved through multifactorial and complex mechanisms but mainly via the upregulation of efflux pumps, the occurrence of the extracellular matrix, and the existence of recalcitrant, metabolically inactive persister cells (Gulati and Nobile, 2016).

Antimicrobial properties of *M. alba* were examined in previous studies, but those investigations were primarily focused on antibacterial and antiviral activity. According to the available data, there are only a few investigations regarding the antifungal

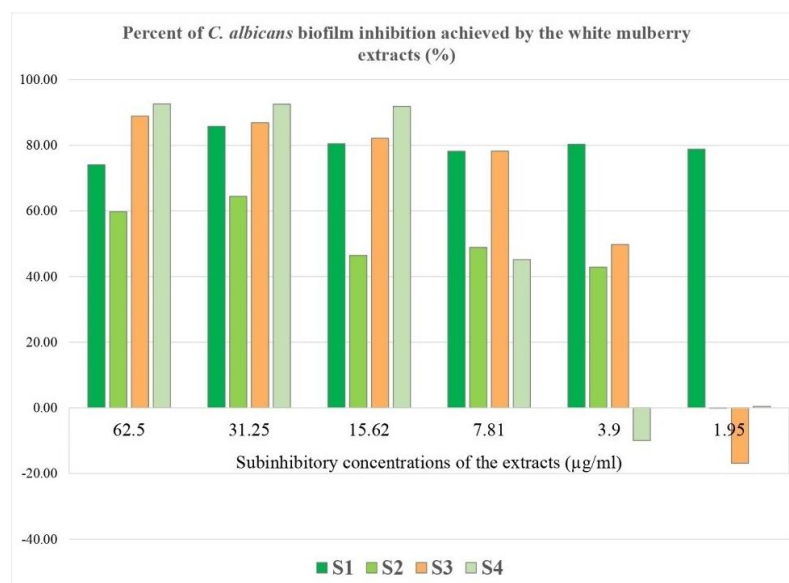


Figure 3. Percent of the biofilm inhibition due to the activity of subinhibitory concentrations of white mulberry extracts

properties of white mulberry, particularly against *C. albicans*. Ahmad and Beg (2001) found no antifungal activity against *C. albicans*, while de Oliveira et al. (2015) noted inhibition of *C. albicans*, with the comparatively high MIC value of 256 µg/ml. In our study, the tested *M. alba* extracts exhibited lower MIC and MFC values of 125 µg/ml and 250 µg/ml, respectively. Jha and Srivastava (2013) and Lu et al. (2017) reported the antifungal activity of *M. alba* extracts against non-*Candida* fungi, including *Saccharomyces cerevisiae* and *Trichophyton rubrum*, with relatively high MIC values obtained. It is possible that the enhanced efficacy of white mulberry extracts used in our study could be related to optimized extraction. This investigation employed the ultrasonic extraction with ethanol and methanol, which represents a broader approach when compared to earlier studies. It should be noted that the extract yield and mass fraction of bioactive compounds describe two different parameters. Extract yield refers to the amount of extract obtained relative to the weight of the raw plant material, whereas mass fraction denotes the concentration of specific bioactive compounds per gram of dry extract. Accordingly, a lower extract yield does not necessarily reflect a lower content of bioactive constituents. In our previously published study (Mahmutović-Dizdarević et al., 2024), fruit extracts, particularly methanolic ones, showed higher mass fractions of phenolic compounds despite the lower extraction yields reported in the present study. This suggests that fruit extracts are chemically more concentrated, which may partly explain their pronounced antifungal and antibiofilm activity. Results obtained earlier (Mahmutović-Dizdarević et al., 2024) detected the mass fractions of extracts as follows: 31.20±2.00 mg/g sample for the leaf ethanolic extract; 105.56±1.90 mg/g sample for the leaf

methanolic extract; 145.58±1.40 mg/g sample in the fruit ethanolic extract; and 200.29±1.70 mg/g sample in the case of fruit methanolic extract.

Besides the antifungal screening, our study explored the antibiofilm activity of *M. alba* extracts, which is important in terms of the emerging drug resistance and the biofilm-forming capacity in *C. albicans*. According to our best knowledge, this is the first study to evaluate the impact of *M. alba* extracts on *C. albicans* biofilms. This study differs from previous reports in the fact that it offers a quantitative assessment of *M. alba* antibiofilm effects, which is achieved through the quantification of the changes in biofilm-forming categories as well as through the calculation of inhibition percentages. In this experiment, the tested *C. albicans* strain showed strong biofilm-forming capacity in the positive control. Subinhibitory concentrations of the white mulberry extracts exhibited the potential to change this ability, mainly by decreasing the strength of the biofilm. An important finding was in the case of the methanolic fruit extract (S4), which significantly reduced strong biofilm formation to weakly adherent states. Inhibition rates were above 91%, and such an effect was not previously documented for *M. alba*. This result achieved *in vitro* could be encouraging since *C. albicans* biofilm formation *in vitro* is well correlated with *in vivo* and *ex vivo*, having a similar time course in development and looking architecturally similar to those isolated from infected patients (Gulati and Nobile, 2016). Previously reported HPLC analysis of the *M. alba* ethanolic and methanolic leaf and fruit extracts (Mahmutović-Dizdarević et al., 2024) revealed the presence of different polyphenolic compounds, with quercetin and pterostilbene concentrated in leaf methanolic extracts, while resveratrol, fisetin, pterostilbene, luteolin, and hesperidin were

abundant in fruit extracts. Quercetin is recognized as a potential antifungal and antibiofilm agent against *C. albicans*, especially when combined with synthetic drugs such as amphotericin B and fluconazole (Oliveira et al., 2016; Ghao et al., 2016). Tan et al. (2023) noted that quercetin inhibits biofilm formation, adhesion, and invasion of *C. albicans in vitro*, with emphasis on *in vivo* anti-inflammatory activity in the candidiasis treatment. Regarding the intracellular activity of quercetin, Kwun and Lee (2020) described that this compound increases the intracellular level of Mg²⁺, which is associated with mitochondrial dysfunction in *C. albicans*, and ultimately leads to apoptosis due to overall disruption in antioxidant systems and DNA fragmentation. Earlier analysis (Mahmutović-Dizdarević et al., 2024) revealed the presence of quercetin in the white mulberry leaf methanolic extract in the amount of 16.708±0.082 mg/g dry extract. In addition, pterostilbene was also detected (Mahmutović-Dizdarević et al., 2024) in the white mulberry fruit ethanolic extract (0.082±0.005 mg/g dry extract), as well as in the leaf methanolic extract (10.483±0.056 mg/g dry extract). Pterostilbene is a phytoconstituent with recognized antibiofilm potential on *C. albicans* (Hu et al., 2017). This stilbene-derived phytoalexin inhibits the biofilm formation of *C. albicans* and destroys the maintenance of mature biofilms by decreasing the cellular surface hydrophobicity, suppressing hyphal formation, altering the expression of genes involved in the morphological transition, ergosterol biosynthesis, oxidoreductase activity, and heat shock proteins unfolding processes. Many filamentation-related genes are downregulated upon pterostilbene treatment, which suggests that the antibiofilm effects of this substance are related to the Ras/cAMP pathway (Li et al., 2014), a major determinant of intrinsic stress resistance in yeasts

(Park et al., 2005). The study of Mahmutović-Dizdarević et al. (2024) noted that the white mulberry fruit ethanolic extract showed positive results for resveratrol in the amount of 0.026±0.005 mg/g dry extract. Earlier studies (Weber et al., 2011; Collado-González et al., 2012) indicated that resveratrol is not effective against *C. albicans in vitro*, but its antifungal activity is frequently researched (Salehi et al., 2018). Recent investigations suggest that resveratrol has the potential to inhibit existing and under-forming *C. albicans* biofilms (Okamoto-Shibayama et al., 2021), as well as to act synergistically with azoles and enhance their efficacy against fluconazole-resistant isolates (Wang et al., 2021). Furthermore, fisetin was detected in fruit ethanolic and methanolic extracts of white mulberry, with a content of 0.969±0.025 mg/g dry extract and 0.910±0.010 mg/g dry extract, respectively (Mahmutović-Dizdarević et al., 2024). Fisetin is a flavonoid with antioxidant and anti-inflammatory activities, and its antifungal activity is related to the biosynthesis of ergosterol (Reis et al., 2016). In *C. albicans*, fisetin is involved with membrane permeabilization and disruption of pH homeostasis (Kim, 2024). Luteolin was found in the fruit methanolic extract of white mulberry (Mahmutović-Dizdarević et al., 2024) with 0.192±0.010 mg/g dry extract. Fu et al. (2021) described that luteolin can inhibit the adhesion of *C. albicans* culture, and it represents a potent agent in the control of initial and intermediate stages of *C. albicans* biofilm development. This study also found that luteolin decreases the levels of exopolysaccharides, proteins, and eDNA in the matrix, which ultimately leads to the blocking of biofilm formation and increases the pathogen's susceptibility to antimicrobial drugs. Our prior results showed a large presence of hesperidin in

both ethanolic and methanolic white mulberry fruit extracts (Mahmutović-Dizdarević et al., 2024). Man et al. (2019) reported the antifungal activity of hesperidin against *C. albicans*, but the literature is generally scarce regarding this matter.

Future investigations should be oriented towards the precise definition of antifungal and antibiofilm activity of particular compounds detected in white mulberry, as well as towards the understanding of molecular mechanisms involved in these processes. Although this research represents the original work, there are some limitations of the study. It should be noted that this investigation was conducted under *in vitro* settings, which may not entirely mimic the processes occurring *in vivo*, especially in terms of microbial biofilms. Even though we are far away from a complete understanding of the numerous elements controlling the dynamic interactions between the host and microbial biofilms, the extension of the study through the *in vivo* models could be useful in further elucidation of the mentioned process. Considering the clinical relevance and emergence of antimicrobial resistance in different fungal species, further investigations could include more strains to gain a broader insight into the issue.

Conclusions

In conclusion, this study addressed the white mulberry, *Morus alba* L., as a potential antifungal and antibiofilm agent against *Candida albicans*. Although this edible plant has a long history of use in traditional medicine, its antifungal potential against *C. albicans*, particularly with respect to biofilm formation, has been insufficiently explored. Our results indicate that extracts obtained from white mulberry leaves and fruits can effectively inhibit fungal growth and, in most cases, reduce the

biofilm-forming capacity of *C. albicans*. Fruit methanolic extract showed the most pronounced activity. Considering the increasing resistance of *C. albicans* to conventional antifungal agents, these findings highlight *M. alba* as a promising natural source of antifungal and antibiofilm compounds. To the best of our knowledge, this is the first report describing both antifungal and antibiofilm effects of white mulberry extracts against *C. albicans*. Further studies are warranted to elucidate the underlying mechanisms of action and to identify the specific phytoconstituents responsible for these effects.

Authors' contributions

I. Mahmutović-Dizdarević:

Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

A. Jerković-Mujkić:

Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing – original draft; Writing – review & editing.

B. Žujo:

Formal analysis; Investigation; Writing – original draft; Writing – review & editing.

M. Avdić Obučić:

Conceptualization; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Writing – original draft; Writing – review & editing.

M. Salihović:

Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Supervision; Validation; Writing – original draft; Writing – review & editing.

Conflict of interest

Authors declare no conflict of interest.

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