

The Impact of Methylene Blue in Antimicrobial Photodynamic Therapy Against Different *Candida* Species and Its Synergy with Fluconazole

El Impacto del azul de metileno en la terapia fotodinámica antimicrobiana contra diferentes especies de *Candida* y la sinergia con fluconazol

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ABSTRACT:

Antifungal resistance in *Candida* strains has recently increased, especially in *C. non-albicans* strains, making the development of alternative treatments for fungal infections critical. Antimicrobial photodynamic therapy (aPDT) is a promising alternative treatment that has been successfully used against various infections and microorganisms, including *Candida albicans*. However, there are still limited studies on *C. non-albicans* strains, such as *Candida tropicalis* and *Candida glabrata*. In this work, the effect of aPDT was evaluated in 18 strains of 3 *Candida* species: (*C. albicans*, *C. tropicalis* and *C. glabrata*). Synergistic treatments with fluconazole and aPDT using methylene blue (MB) as photosensitizer (PS) were also performed on a *C. albicans* and a *C. tropicalis* strain. Resistance to azoles was assessed by Kirby and Bauer disk diffusion method and intracellular MB uptake by cell lysis and spectroscopic analysis. Our results showed that aPDT using MB as PS effectively inhibited the growth of all tested yeast strains *in vitro*. Even fluconazole-resistant, less-studied strains, such as *C. glabrata* or *C. tropicalis* showed significant inhibition, regardless of differences in drug resistance, strain origin, or the MB intracellular concentration. We also observed synergistic effects of aPDT with fluconazole, the combination of sublethal doses of aPDT and fluconazole can effectively eliminate fluconazole-resistant (*C. tropicalis*) or sensitive (*C. albicans*) strains. This suggests that aPDT, alone or in combination with antifungal drugs, is a viable alternative treatment for many *Candida* species.

Keywords: methylene blue, antifungal resistance, *Candida* sp., antimicrobial photodynamic therapy, fluconazole, synergistic treatment.

RESUMEN:

La resistencia antifúngica en las cepas de *Candida* ha aumentado recientemente, especialmente en las cepas de *Candida non-albicans*, lo que hace que el desarrollo de tratamientos alternativos para las infecciones fúngicas sea crítico. La terapia fotodinámica antimicrobiana (TFDa) es un tratamiento alternativo prometedor que se ha utilizado con éxito contra diversas infecciones y microorganismos, incluida *Candida albicans*. Sin embargo, todavía son limitados los estudios sobre cepas de *C. non-albicans*, como *Candida tropicalis* y *Candida glabrata*. En este trabajo se evaluó el efecto de la aPDT en 18 cepas de 3 especies de *Candida*: (*C. albicans*, *C. tropicalis* y *C. glabrata*). Además, se realizaron tratamientos

sinérgicos con fluconazol y aPDT empleando azul de metileno (MB) como fotosensibilizador (FS) en una cepa de *C. albicans* y una de *C. tropicalis*. La resistencia a los azoles se evaluó mediante el método de difusión en disco de Kirby y Bauer y la captación de MB intracelular mediante lisis celular y análisis espectroscópico. Nuestros resultados mostraron que la aPDT usando MB como FS inhibía efectivamente el crecimiento de todas las cepas de levadura probadas *in vitro*. Incluso cepas resistentes a fluconazol, menos estudiadas, como *C. glabrata* o *C. tropicalis* mostraron una inhibición significativa, independientemente de las diferencias en la resistencia a los fármacos, el origen de aislamiento de las cepas o la concentración intracelular de MB. También se observaron efectos sinérgicos de la TFDA con fluconazol, dosis subletales de aPDT más fluconazol eliminaron las cepas resistentes (*C. tropicalis*) o sensibles (*C. albicans*) a fluconazol. Lo que sugiere que la aPDT es un tratamiento alternativo viable para muchas especies de *Candida*, solo o en combinación con medicamentos antimicóticos.

Palabras clave: azul de metileno, resistencia antifúngica, *Candida* sp., terapia fotodinámica antimicrobiana, fluconazol, tratamiento sinérgico.

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1. Introduction

Fungi are most common pathogens in both immunocompetent and immunocompromised patient populations. Among the fungal kingdom, *Candida* spp. are the most prevalent [1-4]. These species are primarily found on the skin, mucosal surfaces, and as part of the human microbiota. *Candida albicans* remains the most common fungus, accounting for up to 50% of the reported invasive candidiasis cases [5]. However, in recent decades its significance has been decreasing due to the increase of *C. non-albicans* species. The more important *Candida non-albicans* strains are: *Candida glabrata*, *Candida parapsilosis*, *C. tropicalis*, *Candida krusei* [5-7], and more recently, *Candida auris* [8]. In addition, an increased incidence of candidemia associated with COVID-19 infection has been reported. Nucci *et al.* demonstrated the high incidence of candidemia in COVID-19 patients in a tertiary care hospital [9], and Riche *et al.* reported up to a 10-fold increase in the incidence of candidemia in hospitalized COVID-19 patients receiving corticosteroids [10]. Similarly, Alves *et al.* reported *C. tropicalis* and *C. glabrata* as the most common fungal species in oral candidiasis in critically ill patients [11]. Each *Candida* species has unique and special characteristics that determine its susceptibility to antifungal drugs [12]. *C. tropicalis* for example is a yeast of medical interest because it is associated with the highest mortality rates among *Candida* species and multidrug resistance similar to that of *C. auris* [13]. In comparison, *C. glabrata* has historically been considered a commensal species with little pathogenic risk, but with the increase and widespread use of immunosuppressive therapy in conjunction with broad-spectrum antifungals, its pathogenicity has recently increased [14, 15]. At the moment *C. glabrata* is difficult to treat as many of them are inherently resistant to azoles or develop resistance rapidly. Azoles are probably the most widely used antifungal drugs; they block the synthesis of ergosterol, a fundamental lipid in the fungal membrane. The susceptibility pattern of *Candida* species to azoles has been altered by increased drug use, geographic distribution, and specific genetic characteristics that can vary even among strains of the same species due to a high capacity for adaptation and evolution [16-18], for this reason, they could become a major public health problem in the not too distant future.



Antimicrobial photodynamic therapy (aPDT) has become one of the most promising alternative treatments for fungi infections, Unlike azoles, which can face resistance issues, aPDT shows no evidence of microbial resistance [18]. aPDT works by photoactivating a photosensitizer (PS) which, once excited, ends up in a triplet state and eventually generates reactive oxygen species (ROS) [17]. Singlet oxygen is considered to be the most important reactive species that causes irreversible damage at a variety of intracellular sites [16, 19]. aPDT has been successfully used to treat viral [20], bacterial [21], and fungal [22] infections, including antimicrobial-resistant microorganisms [23], to inactivate pathogens in blood products [24, 25], to sterilize water [26], and to disinfect and sanitize surfaces [27, 28], among others. Although there are several studies on the use of methylene blue (MB) as PS in aPDT in *C. albicans*, there are very few reports on the use of MB in *C. non-albicans* strains, like *C. tropicalis* or *C. glabrata*, which are becoming increasingly relevant in the clinical setting. There are also not enough studies on the synergistic effect of aPDT in combination with antifungals for *Candida* strains. Hence in this work, three of the most frequently pathogenic *Candida* sp (*C. albicans*, *C. tropicalis*, and *C. glabrata* laboratory reference strains and strains isolated from patient's collection) were selected to study their elimination by MB-aPDT as well as, the effect of aPDT in combined treatment with fluconazole.

2. Methodology

2.1. Biological material

Three laboratory reference strains were used: *C. tropicalis* MYA3404, *C. albicans* CAF2, and *C. glabrata* ATCC2001, and fifteen strains (five of each of the following strains: *C. tropicalis*, *C. albicans* and *C. glabrata*) isolated from the patient's collection donated by the Mycology Laboratory, Science Institute, of the University of Puebla (ICUAP-BUAP). The fungi were inoculated on dextrose Sabouraud agar medium (SDA) (MCD LAB). To perform the aPDT tests, a 12 h culture was inoculated on SDA agar at 37°C. Cell dilutions were then prepared in 1X phosphate buffer solution (PBS), pH 7.4 (OmniChem, Mexico) at a concentration of $\sim 10^6$ CFU/ml.

2.2. PS and light source

A stock solution of the PS (MB, MEYER) was prepared at a concentration of 200 μ M in 1X PBS. This solution was sterilized using syringe filters (0.22 μ m, GVS Life Sciences, USA). Subsequently, a 20 μ M solution was prepared from the stock solution to be used in all aPDT treatments. Irradiations were performed using a LED-based light device (600-650 nm) with a peak emission at 630 nm and optical power of 20 mW, producing an intensity of 60 mW/cm² at the treatment site (well microplate).

2.3. Antifungal resistance test

All strains were subjected to antifungal susceptibility testing by the Kirby and Bauer disk diffusion method. The strains were first inoculated in culture broth with yeast extract, peptone, and dextrose (YPD) (MCDLAB) culture broth, and, after 24 h of growth, the concentration was adjusted to 5 nephelometric turbidity units on the McFarland scale. The yeasts were uniformly inoculated into Petri dishes with Mueller Hinton medium (BD Bioxon), immediately afterward, discs with 50 μ g fluconazole (BIO-RAD) or discs with 50 μ g ketoconazole (BIO-RAD) were placed on the agar and finally incubated for 72 h. The results were interpreted according to the manufacturer's indications shown in **Table 1**.

Table 1. Indications for the determination of antifungal resistance.

ANTIFUNGAL	INHIBITION ZONE DIAMETER	RESULTS
Azole fungicide (ketoconazol, fluconazol)	> 20 mm	Sensitive
	20 – 10 mm	Intermediate
	< 10 mm	Resistant

2.4. Intracellular quantification of PS

Each strain was inoculated and grown for 24 h in SDA, then, a cell solution with 10^6 cells/ml was incubated (1:1) with a final concentration of 10 μ M MB solution for 30 min. After the incubation period, 2 washes with

PBS were performed and 1.5 ml of injectable water was added to lyse the cells. Finally, 200 µl of lysed cell sample was added to a 96-well microplate and the absorbance was read at 655 nm in a microplate absorbance reader (Bio-Rad imark). The experiment was repeated six times.

2.5. aPDT

For the aPDT treatment, 50 µL of yeast solution ($\sim 1 \times 10^6$ CFU/ml) was added to a 96-well microtiter plate, followed by 50 µL PS solution (final concentration of 10 µM). For yeast and light controls, 1X PBS was used instead of the MB solution. The mixture was incubated for 30 min at 37°C in the dark and then irradiated at a dose of 55 J/cm² dose. The dose of aPDT used was determined from a previously published study using a *C. albicans* reference strain [29]. The samples were collected and inoculated on SDA; after 48 h of incubation at 37°C in the dark, the efficiency of aPDT was evaluated by counting the surviving CFU. Appropriate controls were also performed: dark toxicity control, light control, and yeast control (without treatment).

2.6. aPDT combined with fluconazole

Following the procedure described above for MB-aPDT, an additional MB-aPDT was performed on reference strains of *C. albicans* and *C. tropicalis* but with sublethal doses (30 and 50 J/cm²) to observe its synergistic effect with the drug. A solution of fluconazole (10, 20, 40 and 60 µM/ml) was added to the culture medium prior to gelation. The efficacy of the treatments was evaluated by UFC counting 48 h after treatment.

2.7. Statistical analysis

All the experiments were performed 3 times in triplicate under sterile conditions. And analyzed with the one-way ANOVA technique and the Tukey test with a level of significance $p < 0.05$.

3. RESULTS

Varying degree of azole resistance was observed in all strains of *Candida* tested, except for the reference strain *C. albicans* CAF2 which showed sensitivity to ketoconazole and fluconazole. *C. glabrata* 190, *C. glabrata* 152-2, *C. glabrata* 131-2 and *C. glabrata* 219-2 showed an intermediate resistance. On the other hand, all *C. albicans* strains maintain a high concentration of intracellular MB (between 2 and 6 µM) unlike all *C. tropicalis* strains, which only maintain smaller concentrations of the PS (between 0.51 and 1.06 µM). Finally, very variable intracellular concentrations of MB were observed among *C. glabrata* species (between 0.32 y 3.06 µM) (**Table 2**).

Table 2. Intracellular MB concentration in *Candida* strains.

TRAIN		ABSORBANCE, STANDARD DEVIATION	MB CONCENTRATION (µM)
Reference strain	<i>C. albicans</i> CAF2	0.096, 0.008	6.008
	<i>C. albicans</i> 56	0.075, 0.008	4.693
Patients strains	<i>C. albicans</i> 44	0.050, 0.007	4.129
	<i>C. albicans</i> 43	0.036, 0.005	2.253
	<i>C. albicans</i> 46	0.073, 0.006	4.568
	<i>C. albicans</i> 2641	0.074, 0.004	4.631
Reference strain	<i>C. tropicalis</i> MYA3404	0.014, 0.003	0.876
Patients strains	<i>C. tropicalis</i> 2130	0.014, 0.002	0.51
	<i>C. tropicalis</i> 7809	0.014, 0.003	0.846
	<i>C. tropicalis</i> 7806	0.013, 0.003	0.81
	<i>C. tropicalis</i> 71	0.017, 0.002	1.063

	<i>C. tropicalis</i> 2131	0.009, 0.002	0.563
Reference strain	<i>C. glabrata</i> ATCC2001	0.020, 0.002	1.251
	<i>C. glabrata</i> 20	0.014, 0.003	0.876
	<i>C. glabrata</i> 190	0.049, 0.004	3.066
Patients strains	<i>C. glabrata</i> 152 – 2	-	-
	<i>C. glabrata</i> 131 – 2	0.043, 0.005	2.691
	<i>C. glabrata</i> 219 – 2	0.005, 0.001	0.312

MB-aPDT in *Candida* species showed satisfactory inhibition. In *C. albicans*, was observed that aPDT can inhibit $\geq 98.5\%$ in all strains, with a statistically significant difference ($p < 0.05$). MB was not toxic to the fungi because all dark toxicity controls did not show an inhibition effect; however, light controls show considerable inhibition on *C. albicans* 43 and *C. albicans* 44 and a minor inhibitory effect on *C. albicans* 2641 (average viability for 3 experiments by triplicates was 78%, 69%, and 89% respectively). For *C. tropicalis* strains, aPDT the inhibition of all strains $\geq 93.4\%$ with a statistically significant difference ($p < 0.05$). Dark toxicity controls showed a slight inhibition ($< 18\%$) as opposed to *C. albicans* where a slight increased cell viability was observed. The light control had a major inhibitory effect on the growth of most of the *C. tropicalis* strains tested, including the reference one (MYA3404). The light controls reduced the cell viability ranging from 17% to 37%. Finally, in *C. glabrata*, it was also observed that aPDT inhibited the growth of all strains with a statistically significant difference ($p < 0.05$). The dark toxicity controls show little toxicity in all strains (Figure 1).

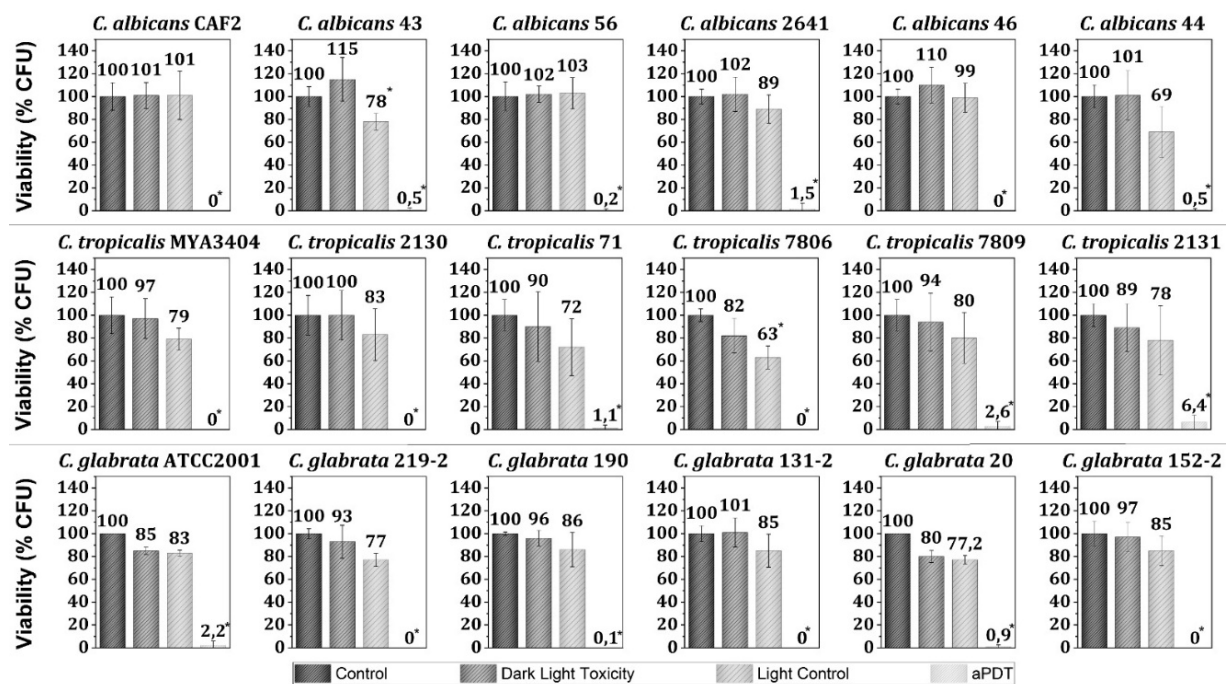


Figure 1. Viability of *C. albicans*, *C. tropicalis* and *C. glabrata* strains compared to no treatment control, dark toxicity control (10 μ M MB), light control (55 J/cm²) and MB-aPDT (10 μ M MB and 55 J/cm² red light). *Statistically significant difference from control, $p < 0.05$.

C. albicans and *C. tropicalis* responded differently to fluconazole (Figure 2). *C. albicans* had a survival rate between 8.5% and 0.5% as the fluconazole dose was increased from 10 to 60 μ g/ml, respectively. In contrast, *C. tropicalis*, has a survival rate of 95% at all fluconazole doses used. Despite the observed drug resistance level, both *Candida* species responded satisfactorily to MB-aPDT. In addition, the combination of sublethal aPDT and fluconazole resulted in potentiation of inhibition in all cases. This effect was particularly evident in *C. tropicalis*, where a statistically significant reduction was observed when comparing aPDT alone with aPDT plus fluconazole, especially when the highest doses were used (aPDT: 10 μ M MB and 50 J/cm²

with 40 or 60 µg/ml fluconazole). In *C. albicans*, complete inhibition was achieved with the highest dose of aPDT (10 µM MB and 50 J/cm² with all doses of fluconazole used) (**Figure 2**).

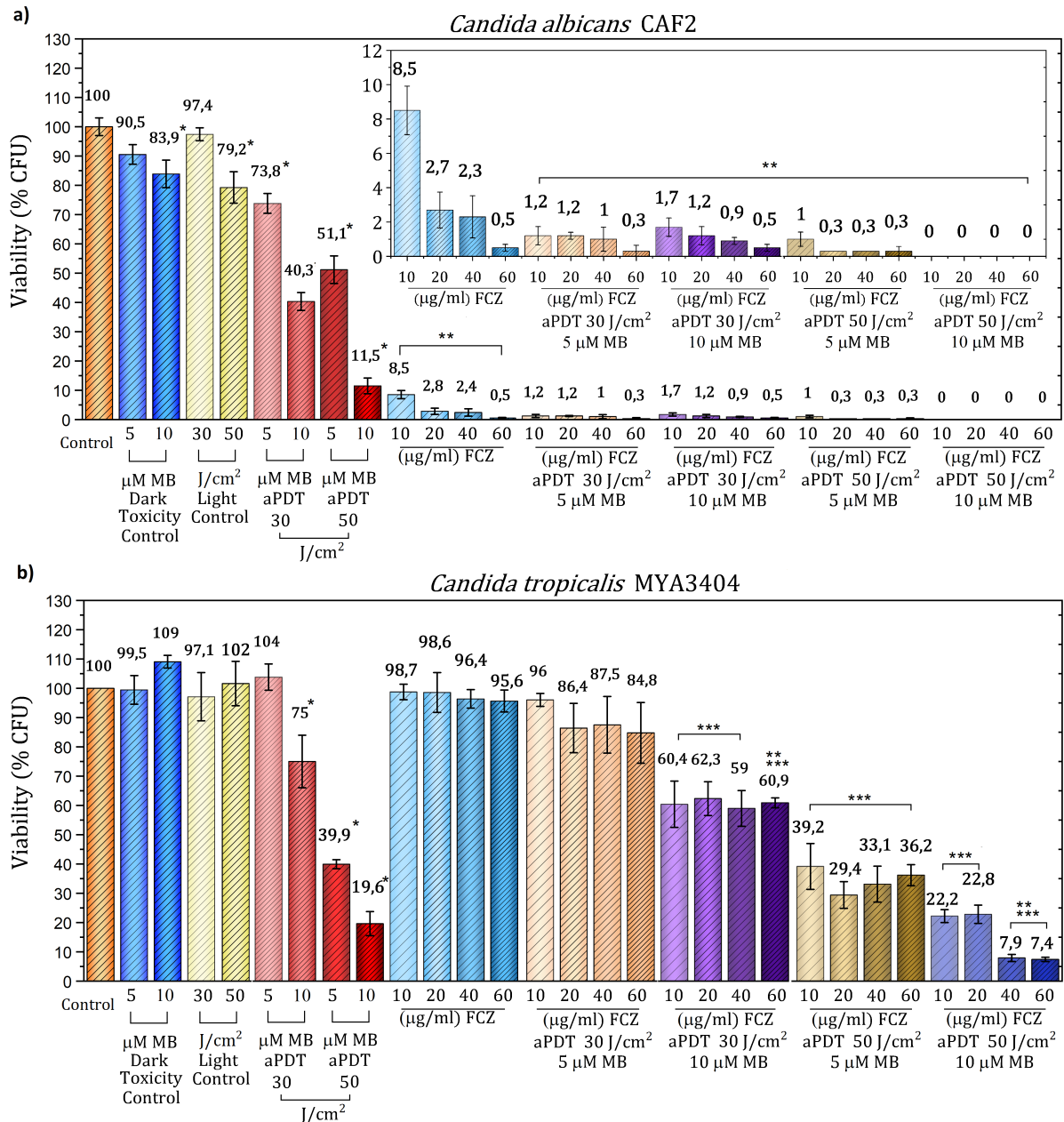


Figure 2. Synergistic effect of aPDT and fluconazole on a) *C. albicans* and b) *C. tropicalis* strains. There is a significant difference, $p < 0.05$, between all treatments and control (*), between combination treatments and aPDT alone (**), and between combination treatments and fluconazole alone (***).

4. DISCUSSION

The uncontrolled use of antifungals has been extensively documented to contribute to the rise in drug resistance among filamentous and yeast fungi [30], along with an escalation in virulence and tissue invasion [31]. Our findings reveal distinct levels of azole resistance among different species and even among strains within the same species. It has been noted that not only structural differences among species but also environmental conditions can influence their response to drugs [32, 33].

However, despite the variations in antifungal resistance, aPDT proved to be a very efficient alternative for the elimination of the different strains of *Candida*. In this work, an average viability after aPDT under the same conditions was found to be 0.45% in *C. albicans*, 0.53% in *C. glabrata*, and 1.68% in *C. tropicalis*. *C. tropicalis* presented some strains with a slightly lower sensitivity to aPDT compared to *C. albicans* or *C. glabrata*. These results are in agreement with those reported by Dovigo *et al.* showing that *C. tropicalis* was less susceptible to aPDT than *C. albicans* [34]. The lower inhibition could be attributed to the fact that *C. tropicalis* strains exhibited low intracellular concentrations of MB after 30 min of incubation with the PS (**Table 3**). Unlike *C. albicans*, all strains had higher intracellular MB concentrations and good inhibition after aPDT. The differences in the ability to capture and/or retain MB between *Candida* species could be due to differences in cell membranes composition. Navarro-Arias *et al.* performed a comparative study analyzing the cell wall composition and organization of different *Candida* species and suggested that the cell wall and innate immune recognition are different between *C. tropicalis* and *C. albicans* [35]. On the other hand, it's known that *C. glabrata* has abundant genetic variation that has allowed them to adapt and evolve in clinical settings [36, 37]. This may be one of the reasons why the intracellular accumulation of MB is so variable among *C. glabrata* strains (**Table 3**).

Although several works have already evaluated the efficacy of the MB-aPDT against *C. albicans*, most of them were in biofilms [38-44], and some in planktonic growth (**Table 3**). Furthermore, little is known about aPDT with MB in *C. non-albicans* strains, such as *C. tropicalis* or *C. glabrata*. In addition, there is no consensus about the optimal light dose or PS concentration, ranging from 7-100 000 µg/mL and 2-180 J/cm², respectively, as shown in **Table 3**. This work confirms that aPDT is rather effective in *C. albicans* and *C. non-albicans* species using one of the lowest PS concentrations reported so far and modest light dose, although there may be variations between strains and species. Thus, it can be safely assumed that aPDT can become one of the best alternatives for the treatment of candidiasis caused by different *Candida* species regardless their antifungal resistance level.

Table 3. Studies of aPDT using MB as a PS in planktonic cultures of *Candida* sp. strains.

REFERENCE	BIOLOGIC AGENT	MB DOSES	LIGHT DOSES
Ahmed <i>et al.</i> [41]	<i>C. parapsilopsis</i> and <i>C. tropicalis</i>	0.1 mg/mL	90 and 180 J/cm ²
Lyon <i>et al.</i> [45]	<i>C. albicans</i>	8, 16, and 32 µg/mL	180 J/cm ²
Prates <i>et al.</i> [46]	<i>C. albicans</i>	10, 25 and 50 mg/mL	60 J/cm ²
Queiroga <i>et al.</i> [47]	<i>C. albicans</i> : ATCC76645, ATCC13803, LM018F, LMMD37, LM968. <i>C. tropicalis</i> : LM28, LM708, LM01, LM759, LM14. <i>C. krusei</i> : ATCC6258, ATCC6358, LM08, LM12, M120. <i>Candida guilliermondii</i> : LM70, LM405, LM01, LM2101, LM708	150 µg/mL	60 J/cm ² , 120 J/cm ² and 180 J/cm ²
Lyon <i>et al.</i> [48]	<i>C. albicans</i> , <i>C. glabrata</i> and <i>C. krusei</i>	64, 32, 16, 8 and 4 µg/mL	90 and 180 J/cm ²
Carvalho <i>et al.</i> [49]	<i>C. albicans</i>	0.01 to 0.1 mg/ml	28 J/cm ²
da Collina <i>et al.</i> [50]	<i>C. albicans</i>	20 mg/L	4.68 J/cm ²
Daliri <i>et al.</i> [51]	<i>C. albicans</i>	0.01% and 0.02%	6 and 10 J/cm ²
Giroldo <i>et al.</i> [52]	<i>C. albicans</i>	0.05 mg/ml MB energy	28 J/cm ²
Sousa <i>et al.</i> [53]	<i>C. albicans</i>	0.1 mg/ml	15.8 J/cm ² , 26.3 J/cm ² and 39.5 J/cm ²
Pupo <i>et al.</i> [54]	<i>C. albicans</i>	100 mg/mL	53 J/cm ²

Peloi *et al.* [55] *C. albicans* 7 to 140.8 μM 2, 4, 6 and 12 J/cm²

Additionally, there is substantial evidence supporting the synergy between various drugs and antimicrobial aPDT for microorganism eradication, particularly in bacteria [Formatting Citation]. However, literature on the synergistic effects in yeasts is scarce, with almost all reports focusing on *C. albicans* (Table 4). In this study, we investigated the synergistic effect of MB-aPDT combined with fluconazole and compared the outcomes between a drug-sensitive reference strain of *C. albicans* and a drug-resistant reference strain of *C. tropicalis*. The enhanced inhibition observed in both *C. albicans* and *C. tropicalis* with combination treatments, as opposed to aPDT or fluconazole alone (Figure 2), underscores the existence of a synergistic interaction between aPDT and the antifungal agent fluconazole. These findings offer promising prospects for more effective treatments against *Candida* infections.

Table 4. Studies of synergistic aPDT with drug treatments for *Candida* spp.

REFERENCE	MICROORGANISM	PS	DRUG
Biazzi de Lapena <i>et al.</i> [58]	<i>C. albicans</i>	MB	chitosan
Cai <i>et al.</i> [59]	<i>C. albicans</i>	ALA	Itraconazole
Ferreira <i>et al.</i> [60]	<i>C. albicans</i>	MB	fluconazole
Hsieh <i>et al.</i> [61]	<i>C. albicans</i>	Cur	fluconazole
Lyon <i>et al.</i> [48]	<i>C. albicans</i> , <i>C. glabrata</i> and <i>C. krusei</i>	MB	fluconazole
Mendonca Dias <i>et al.</i> [62]	<i>C. albicans</i>	Photodithazine (PDZ)	fluconazole
Quiroga <i>et al.</i> [63]	<i>C. albicans</i>	tetracationictentacl e porphyrin	fluconazole
Rimachi Hidalgo <i>et al.</i> [64]	<i>C. albicans</i>	PDZ	nystatin
Snell <i>et al.</i> [65]	<i>C. albicans</i>	porphyrin TMP-1363 and MB	ketoconazol, clotrimazole and miconazole
Tavares <i>et al.</i> [66]	<i>Fusobacterium nucleatum</i> and <i>Porphyromonas gingivalis</i>	PDZ	metronidazole

The high genetic and metabolic diversity among *Candida* strains may lead to the production of specific or characteristic secondary metabolites unique to each species or strain. Some fungal metabolites, such as cytochromes like cytochrome C or antioxidants like catalases, could potentially serve as chromophores [33, 67]. Since fungi produce catalases to shield themselves from the effects of ROS [68], and red light activates these catalases [69], it is reasonable to speculate that these metabolites, along with the abundance of catalases present, may influence the extent of cellular inhibition upon light interaction. This aspect is particularly important to consider for *Candida* strains that exhibit some degree of inhibition in light controls, such as *C. tropicalis*, whose inherent susceptibility to red light has been previously documented [70]. Dovigo *et al.* also observed statistically significant differences between the red light control and the no light control, suggesting a potential toxic effect of light [34]. Furthermore, Souza *et al.* reported a decrease in the CFU/mL values of *C. tropicalis* upon red laser irradiation [70]. They proposed the presence of endogenous PS in *Candida* species, which could account for these effects [53].

5. Conclusions

Our findings highlight the remarkable efficacy of aPDT utilizing MB as a PS in inhibiting the *in vitro* growth of all tested yeast strains. Even less explored strains like *C. glabrata* or *C. tropicalis* exhibited notable inhibition comparable to that of *C. albicans*, regardless of their resistance to drugs, origin, or intracellular MB concentrations. Furthermore, we observed the potential for synergistic treatment with aPDT and fluconazole, extending to species not previously considered for combined therapies. For instance, a fluconazole-resistant strain of *C. tropicalis* displayed a significant reduction in viability following sublethal aPDT combined with fluconazole, compared to aPDT alone. Consequently, aPDT emerges as a promising alternative for combating both *C. albicans* and *C. non-albicans* species independently or in combination. The

synergistic approach involving sublethal doses of aPDT and fluconazole presents an effective strategy for eliminating strains resistant to fluconazole (e.g., *C. tropicalis*) or sensitive strains (e.g., *C. albicans*). We believe our approach could be extended to treat *C. auris* which shows multidrug resistance, responsible for severe infections, and potential for outbreaks in healthcare settings.

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