Effects of Photodynamic Therapy Using Bisdemethoxycurcumin Combined with Melatonin on *Candida albicans.*

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Abstract

Photodynamic therapy (PDT) involves exposure of a photosensitizer (PS) to a harmless visible light with promising results against *C. albicans.* This study aims to determine whether bisdemethoxycurcumin (20, 40, and 60 µM) alone, melatonin (20 and 100 µM) alone or a combination of both as PS can inhibit *C. albicans* biofilm. Biofilm culture of *C. albicans* (ATCC 10231) was done on 96 glass coverslips at 37° C for 48hrs. The coverslips were divided into 11 test and 5 control groups. Test photosensitizers were added to the coverslips placed in a dark 6-well plate and incubated for 20 minutes. In a dim environment, groups containing BDMC alone were irradiated with blue LED light (λ=430nm) cm^2 while those containing melatonin alone were irradiated with red LED light (λ= 630 nm) both with a power density of 250mW/cm^2 and an energy density of 37.5J/cm^2. Groups with both photosensitizers were irradiated with blue and subsequently red LED light. The groups were incubated for 0 and 1 hour with the photosensitizers after irradiation prior to performing drop plate technique. Colony counting was performed 48hrs after. Controls include phosphate buffer saline (PBS), Nystatin and light only groups. Results showed that the median log_{10}CFU/ml/biofilm weight of groups treated with 40 µM BDMC (5.8 ± 3.58), 60 µM BDMC (5.5 ± 2.9), 60 µM BDMC+20 µM MLT (5.8±0.43), 20 µM BDMC+20 µM MLT (5.78±3.14), and 20 µM BDMC + 100 µM MLT (5.88 ± 3.54) at 1hr had higher biofilm reductions compared to PBS and were not significantly different from Nystatin (p=0.05)

***Keywords*:** *Candida Albicans, Photodynamic Therapy, Bisdemethoxycurcumin, Melatonin*

1. Introduction

Candida albicans occurs naturally in the normal flora. However, it is also an opportunistic pathogen and is the causative organism of oral candidiasis. Oral candidiasis presents as white plaques that can be rubbed off in the oral mucosa, tongue and the oro-pharynx. Studies have also shown that *C. albicans* is present in subgingival plaque and in relatively high numbers in patients with aggressive and chronic periodontitis (Slots et al., 1988). Denture wearers may also be affected by oral candidiasis (Zomorodian et al., 2011). When left untreated, it can lead to discomfort and compromised nutrition. In a study among adolescent Thai subjects in 2012, oral candidiasis was present in about 30% of the subjects, with *C. albicans* being the most prevalent specie in 88.5% of the patients (Santiwongkarn et al., 2012). Several antifungal drugs are commercially available. However, according to the Center for Disease Control, the overexposure and improper use of antifungal drugs contribute to drug resistance. This necessitates the discovery of alternatives that would not contribute to drug resistance. In a host with robust immune responses, *C. albicans* are able to survive through its ability to self-regulate in an environment with varying pH levels (Mayer et al., 2013).

Photodynamic therapy (PDT) is a novel approach in the treatment of candidiasis. The procedure has been used in cancer treatment, fungal and bacterial treatment. The mechanism of PDT involves the irradiation of a photosensitizer (PS) with a harmless visible light matching its absorption spectrum. This process produces either Type I or Type II reactive oxygen species (ROS). Type I produces superoxide anion (O_2),

hydroxyl (•OH), and hydrogen peroxide radicals (H_2O_2) while Type II produces singlet oxygen ($^1O_2$). These ROS play an important role in cellular hemostasis and key molecular functions such as metabolism, signaling and gene transcription (Dai et al., 2012). It is also involved in the host cells' protective functions against bacterial and viral growth. •OH radicals are utilized in the antimicrobial function of antibiotics while singlet oxygen, on the other hand, can inactivate enzymes, proteins and peroxidation of lipids that lead to the lysis of cell membrane, lysosomes and mitochondria (de Souza et al., 2006). There is also no known enzyme that specifically targets singlet oxygen (Dias et al., 2020).

The ROS may also damage normal cells especially when there is chronic exposure to the radicals. However, in PDT, the PS is only taken up by target cells and the ROS produced are very short-lived as the production ceases when the source of the light is removed (Dias et al., 2020). It mainly targets the cell wall structures of the pathogen and does not necessarily have to be engulfed by the cell. Therefore, developing resistance against the treatment is unlikely (Winckler, 2007). Therefore, the damage it may cause to normal cells is negligible (Sheng et al., 2015). This short life spans of ROS, existing only during illumination, makes it unlikely for the *C. albicans* to develop resistance as they are not continuously exposed to it as compared with conventional antifungal drugs. And because the treatment using PDT is localized on the affected surfaces, the ROS does not travel to other areas of the body and thereby further reduces the likelihood of resistance (Donnelly et al., 2008). As far as the author knows, there have been no reports of resistance of *C. albicans* to PDT. However, in PDT cancer treatment, mechanisms of resistance may be related to different uptake rate or efflux, altered cellular trafficking of the drug, and decreased drug activation and increased inactivation of the drug (Casas et al., 2011).

In literature, PDT when compared with nystatin has different results. Alrabiah et al., in 2019 compared the efficacy of PDT against Nystatin in patients with denture stomatitis and found that PDT was equally effective as nystatin in decreasing colony counts. However, a more recent systematic review and meta-analysis found that although PDT is effective in reducing *Candida* colony counts, it does not seem to be more effective than conventional nystatin (Firoozi et al., 2021).

Several photosensitizers have been found effective Phenothiazines is one example that includes methylene blue and toluidine blue. It has been found to be effective against *Candida albicans* in murine models at concentrations of 400-500 µg/mL when irradiated with red light achieving reductions of 2.5 $\log_{10}$ and 2.74 $\log_{10}$ respectively. Phenothiazines are also toxic to non-target cells such as red blood cells. These dyes also cause discoloration on teeth, dental restorations and prosthesis (Dias et al., 2020). Porphyrins are also another type of PS. However, porphyrins are hydrophilic and has limited ability to penetrate into deeper layers of biofilms (Santezi et al., 2018).

Curcumin, a natural photosensitizer, was also explored in more recently. It is isolated from the root of a plant called *Curcuma longa* and has been shown to have anti-oxidant, anti-inflammatory, anti-microbial and wound healing capabilities. Curcumin produces mainly hydroxyl radicals upon irradiation with blue light. A study that used 80-120 µM curcumin irradiated with blue LED light with a wavelength of 440-460 nm, power density of 22mW/cm^2 for 29 minutes producing energy density of 37.5J/cm^2 resulted in approximately 0.5 $\log_{10}$ reduction of *C. albicans* (Quishida et al., 2016). For *in vitro* studies, Pfaller et al. in 2004 suggested that a log CFU/ml reduction of at least 3 $\log_{10}$ or 99.9% is the most stringent criterion for determining fungicidal activity. The approach to inactivating biofilm forms of *C. albicans* is more complex due to the presence of extracellular matrix, among other factors, which acts as both a physical barrier to drug penetration or to immune responses and stabilizes the architecture of the biofilm. Therefore, significantly more effort and higher concentrations are needed to treat *C. albicans* in biofilm form (Dias et al., 2020). However, it has also been observed that concentrations of curcumin greater than 100 µM completely hindered light penetration and therefore inhibited ROS production (Chan & Wu, 2004). Curcumin is the stronger antioxidant compared to its derivatives. It could be possible that the ROS produced by curcumin are negated by its antioxidant capabilities (Nardo et al., 2011). Due to the limitations of curcumin, its derivatives have also been explored as photosensitizer. One of these derivatives is bisdemethoxycurcumin (BDMC) which has the least antioxidant capability among the derivatives. It has also been found to produce higher amounts of ROS with light absorption ranging from 348 nm to 423 nm (Nardo et al., 2011). Therefore, considering the advantages

of bisdemethoxycurcumin, it can be used as an alternative PS to curcumin. However, the use of bisdemethoxycurcumin on *C. albicans* has not been extensively studied.

These single PS are incapable of producing both types I and II ROS. Theoretically, the production of both types of ROS may enhance the microbicidal effect of PDT. The search for the combination of PS that can produce both types I and II ROS still continues. Melatonin (MLT), an indole-based hormone produced by the pineal gland and is a strong antioxidant particularly in larger concentrations. Melatonin possesses both hydrophilic and lipophilic characteristics that enables it to penetrate all biological membranes (Reiter et al., 1997). 100 µM Melatonin was also found to produce detectable singlet oxygen upon exposure to Nd:YAG laser at a wavelength of 672 nm and a power density of 97 mW that resulted in a quantum yield of 1.14 (Maharaj et al., 2005). The use of melatonin in cancer therapy has been extensively studied but its application to antimicrobial PDT leaves more to explore. Glutaryl-melatonin, a derivative of melatonin, have been reported to inhibit *C. albicans* at concentrations of 14.2 mM without light. It was found that it was not significantly different from nystatin (Damrongrungruang et al., 2020).

The use of dual photosensitizers has been explored with the aim of achieving a significant reduction in *Candida* colony counts using the least concentration of PS. Studies on bisdemethoxycurcumin on *C. albicans* are scarce unlike curcumin. As a derivative of curcumin, it was deemed logical to use the effective concentrations of curcumin as concentrations of BDMC for this study. Based on a study by Dovigo et al., in 2013 using blue LED light with energy density of 37.5J/cm^2 and curcumin as PS on *C. albicans*-inoculated mice, the most effective dose of curcumin ranged from 20-80 µM, yielding up to 4 $\log_{10}$ reduction of *Candida albicans* colony count for groups treated with 80 µM BDMC. Another study by Ma et al. in 2019 using 60 µM curcumin alone, also using blue LED was able to reduce *C. albicans* viability by 90.87%. However, their study used a considerably lower energy density with only 13.2 J/cm^2 in 2020, a study by one of the authors using 20, 40 and 60 µM BDMC in combination with 100 µM potassium iodide (KI) irradiated with blue LED light with an energy density of 90J/cm^2 found that a combination of 40 µM BDMC with 100 µM KI could yield 3.5 $\log_{10}$ reduction in *C. albicans* colony counts. (Damrongrungruang et al., 2022) This indicates that lower concentrations of BDMC may be enhanced by a second PS. In study, we hypothesize that the addition of melatonin will also enhance the effects of BDMC and thus limited the concentrations of BDMC to 20, 40 and 60 µM using an energy density of 37.5 J/cm^2. Melatonin, on the other hand, has not been studied as a photosensitizer in PDT. As far as the author knows, data on the effective concentrations of melatonin when used as PS for PDT on *C. albicans* is non-existent. Maharaj et al., 2005 was able to detect singlet oxygen using 100 µM melatonin. Roberts et al. 2000, however, also determined that melatonin can moderately quench singlet oxygen and higher concentrations. Therefore, an extreme of 20 µM melatonin and a maximum of 100 µM melatonin will be used for this study.

One of the limitations of PDT is the cost of equipment that prohibits its wide use. The use of light emitting diodes (LED) stands as a good alternative as these are less expensive and more versatile (Sorbellini et al., 2018). In the dental field, blue LED light is commonly used as the light source to cure restorative materials. Earlier studies using blue LED light with different combinations of photosensitizers has also shown that blue LED is capable of promoting the production of ROS (Kanpittaya et al., 2021). Blue light is able to reach superficial layers of the epidermis and is thus useful in treatment of superficial conditions. Red LED light, on the other hand, has been extensively used in the field of dermatology for the treatment of conditions such as actinic keratosis (Szeimies et al., 2009).

In this study, we aimed to determine the effects of combining bisdemethoxycurcumin that mainly produces hydroxyl radicals and melatonin that produces singlet oxygen upon irradiation of light with their respective absorption wavelengths on *C. Albicans.*

2. Objectives

1) To test the anti-fungal ability of photodynamic therapy using 20, 40 and 60 µM bisdemethoxycurcumin, 20 and 100 µM melatonin or a combination of the 2 photosensitizers activated by blue, red LED and blue + red lights, respectively, on *C. albicans* biofilm in-vitro.

2) To compare the log reduction of *C. albicans* colony forming units/ml biofilm weight among groups after PDT.

3. Materials and Methods (Flow of the methods shown in Figure 1)

Candida albicans Biofilm Preparation

The present procedure has been developed based on the study of Jin et al. in 2003 and Thein et al. in 2007, with some necessary modifications. *Candida albicans* ATCC 10231 was grown in Sabouraud dextrose broth. The culture was then blended using a high-speed blender to ensure a homogenous mixture and then centrifuged at 3000 x *g* for 10 minutes at 25ºC. The culture was then washed with phosphate buffer saline (PBS). The optical density of the washed culture was then adjusted to 0.380 using an absorbance spectrophotometer at a wavelength of 530 nm to achieve 10^7 cells/ml in the final solution.

Within a 6-well plate, 1 ml of the culture was placed in each well, containing a pre-weighed glass coverslip. The plate was then incubated for 1.5 hours at 37ºC in an orbital shaker at 75 rpm to promote yeast adherence. After incubation, the wells were washed twice with PBS to remove any loosely attached cells. Subsequently, 2 ml of 1 M yeast nitrogen base (YNB) and 50 mM of glucose solution was added to promote biofilm growth and incubated further in the orbital shaker with the same settings to allow the biofilm to mature.

On the day of the experiment, the glass coverslips were washed with PBS to remove the YNB+glucose solution and any loose cells. These were then re-weighed to determine the weight of the formed biofilm.

Photodynamic Therapy

A total of 11 test groups and 5 control groups with 6 biofilm cultures each were performed. As an in-vitro experiments, all conditions are controlled and thus, a higher number of samples than 5 is generally performed to ensure the reliable results. This sample size is consistent with the study conducted by Jordão et al. in 2020 on the effect of PDT on gene expression of *C. albicans*. Individual concentrations of test photosensitizers, 20 µM, 40 µM and 60 µM of BDMC (MedChem Express, NJ, USA) as well as 20 µM and 100 µM melatonin (Faculty of Pharmaceutical Science, Khon Kaen University, Thailand) and 6 combinations were prepared using 1% ethanol and phosphate buffer saline (PBS). 1 ml of these test photosensitizer solution and 1 ml of Sabouraud dextrose broth were added into the 6 well plate containing the glass coverslips with the *Candida albicans* biofilm. The plate was then incubated at 37ºC for 20 minutes to facilitate photosensitizer uptake by the biofilm.

The test groups containing BDMC were irradiated for 150 seconds using dental blue light (3M™ Elipar™ DeepCure LED-L Curing Light) with a tip diameter of 1 cm, a wavelength of 430 nm, and a power density of 250 mW/cm^2 and an energy density of 37.5J/cm^2. The light source was placed at a distance of 5 cm from the bottom of the plates. The procedure was carried out in a dim environment to minimize the impact of ambient light. The test groups containing melatonin also went under the same procedure but irradiated with red LED light source (Faculty of Engineering, Khon Kaen University, Thailand) with a wavelength of 630 nm, a similar power density, energy density, distance, duration, mode and environment using a customized light box. For the groups containing both BDMC and melatonin were first irradiated with blue LED light and immediately followed by red LED light with the same light and environment set-up. Nystatin 1:100,000 U/ml and PBS were used as positive and negative control respectively without light irradiation. In addition, a separate group that was treated with blue, red, and a combination of both light sources without any photosensitizer was also performed. After treatment, the wells were incubated for 0 and 1 hour after treatment, as singlet oxygen production was found to last more than 30 minutes (Santos et al., 2022). After incubation, the 6-well plates were washed with PBS. Then, 2 ml of PBS was then added and placed in a sonicator at 75 rpm for 15 minutes to separate the cells from the glass coverslips. The cell suspension underwent serial dilutions of up to 1:1000 and plated in Sabouraud dextrose agar at 37ºC. After 48 hours, colonies were counted and a logarithmic transformation of the CFU/ml/biofilm weight was performed.

All experiments were conducted in duplicates. Statistical analyses were performed using Kruskal-Wallis and Dunn's test with adjusted $p = 0.05$ through IBM SPSS Statistics (Version 28)

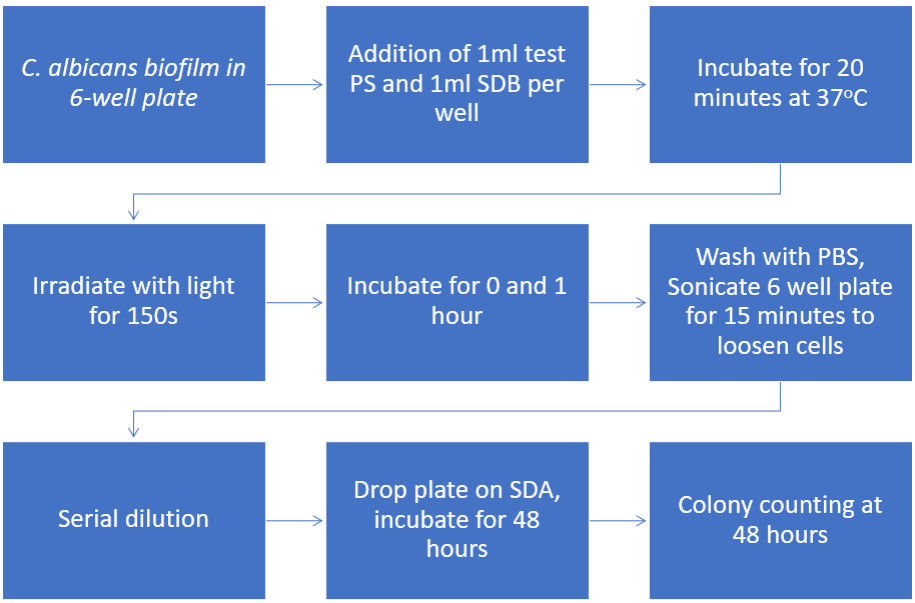

Figure 1 Flow chart of photodynamic therapy. PS= photosensitizer, SDB= Sabouraud dextrose broth, SDA= Sabouraud dextrose agar.

4. Results and Discussion

At 0 hour of incubation, most of the groups were able to yield > 3 log10 CFU/ml/biofilm weight inhibition of *C. albicans.* (Figure 2). There was a significant difference in the log CFU/ml reductions among groups based on the Kruskal Wallis test, $\chi^2(15) = 56.86$, $p = 0.05$. A pairwise comparison using Dunn's (1964) test was performed with a Bonferroni correction for multiple comparisons was made with adjusted *p*-values. None of the test photosensitizers were able to significantly inhibit *C. albicans* when compared to the controls.

Although several groups were able to obtain the desired reduction levels at 0 hours of treatment, statistically significant outcomes may not have been achieved due to the limited sample size (Fig. 2). The duration of incubation may also be insufficient to result in statistically significant inhibitions of the fungi. In the studies evaluated by Dias et al. in 2020 where an extensive review on the use of Curcumin in PDT was done, most of the studies evaluated the inhibitory effects at 0 hour of treatment. ROS production continues for more than 30 minutes; therefore, the incubation time with the PS after irradiation may not be sufficient (Santos et al., 2022). In 2021, Kanpittaya et al. combined bisdemethoxycurcumin with erythrosine and nano-titanium dioxide and blue LED light and was successfully able to produce both types of ROS and resulted in approximately 1.1$\log_{10}$ reduction of *C. albicans in biofilm form* compared to controls treated with only PBS. However, the test groups in this study were not statistically significant from nystatin (median = 8.9 $\log_{10}$ CFU/ml/biofilm weight). This may be attributed to the small sample size.

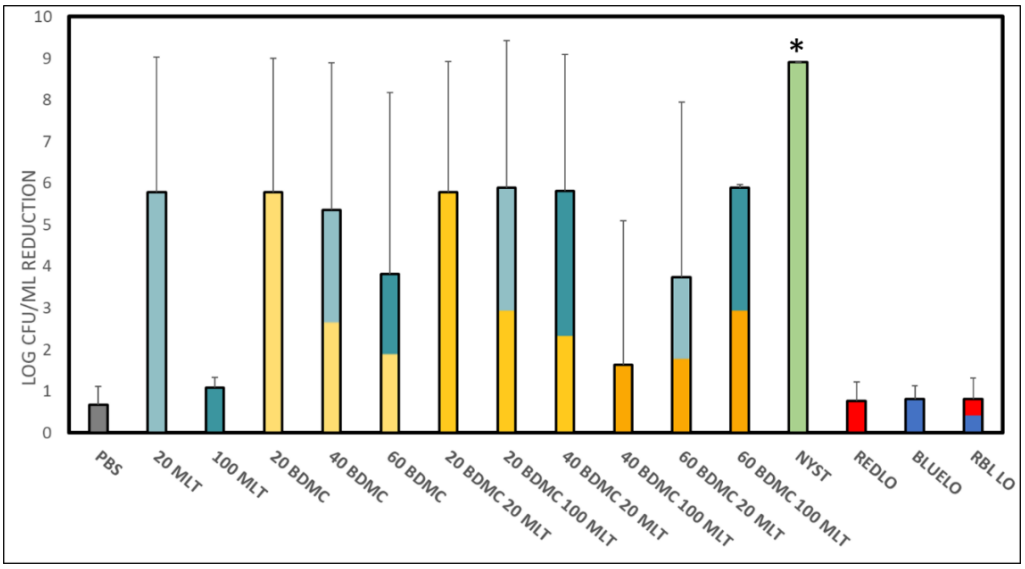

Figure 2 Comparison of median $\log_{10}$ CFU/ml/biofilm weight reductions from baseline values after 0 hours of incubation among 20 and 100 µM melatonin irradiated with red light (λ = 630 nm, 250 mW/cm^2, 37.5 J/cm^2), 20, 40 and 60 µM bisdemethoxycurcumin irradiated with blue light (λ = 430nm, 250 mW/cm^2, 37.5 J/cm^2), and different combinations of photosensitizers irradiated with blue and subsequently red light with the same wavelength, power density and energy densities. Error bars represent interquartile range computed from 6 measurements. *= significant difference at ($p < 0.05$) from PBS. PBS = phosphate buffer saline (negative control), MLT = melatonin, BDMC = bisdemethoxycurcumin, NYST=nystatin, REDLO=red light only, BLUELO=blue light only, RBL LO=red and blue light only

More test groups had significantly high $\log_{10}$ CFU reductions after 1 hour of incubation (Figure 3). There was a significant difference in the log CFU/ml reductions among groups based on the Kruskal Wallis test, $\chi^2(15) = 58.33$, $p < 0.05$ A pairwise comparison using Dunn's (1964) test was performed with a Bonferroni correction for multiple comparisons was made with adjusted *p*-values presented. The post hoc test revealed a significantly differently higher reductions in $\log_{10}$ CFU/ml/biofilm weight was seen in the 40 µM BDMC (median = 5.36 ± 3.52 $\log_{10}$CFU/ml/biofilm weight, $p = 0.035$), 60 µM BDMC (median = 3.82 ± 4.36 $\log_{10}$CFU/ml/biofilm weight, $p = 0.026$), 60 µM BDMC + 20 µM MLT (median = 3.73 ± 4.02 $\log_{10}$ CFU/ml/biofilm weight, $p = 0.002$), 20 µM BDMC + 20 µM MLT (median = 5.78 ± 3.14 $\log_{10}$ CFU/ml/biofilm weight, $p = 0.024$), 20 µM BDMC + 100 µM MLT (median = 5.88 ± 3.54 $\log_{10}$ CFU/ml/biofilm weight, $p = 0.036$) when compared to PBS (median = 0.67 ± 3.14 $\log_{10}$ CFU/ml/biofilm weight) group. The 60 µM BDMC + 20 µM MLT group was also significantly differently higher than the red light only group ($p = 0.021$). While 40 µM BDMC + 20 µM MLT (median = 5.81 ± 3.28 $\log_{10}$ CFU/ml/biofilm weight, $p = 0.031$), and 40 µM BDMC + 100 µM MLT (median = 1.63 ± 3.46 $\log_{10}$ CFU/ml/biofilm weight, $p = 0.031$) were statistically significantly lower than groups treated with nystatin (median = 8.9 $\log_{10}$ CFU/ml/biofilm weight). Other test groups were not statistically significant when compared to nystatin. (median = 8.9 $\log_{10}$ CFU/ml/biofilm weight).

Several studies have reported the use of curcumin at concentrations of 20-80 µM against planktonic fungi, which resulted in reductions ranging from < 1 $\log_{10}$ – 4 $\log_{10}$ CFU/ml. However, in the present study, using 40 µM BDMC, 60 µM BDMC, 60 µM BDMC + 20 µM MLT, 20 µM BDMC + 20 µM MLT, 20 µM BDMC + 100 µM MLT against *C. albicans* biofilm, higher reductions were achieved than with curcumin. Interestingly, the combination of 60 µM BDMC + 20 µM MLT produced slightly higher reductions than 60 µM BDMC alone, although this difference was not statistically significant ($p = 1.00$). The same trend was observed when comparing the efficiency of 20 µM BDMC alone and when combined with MLT.

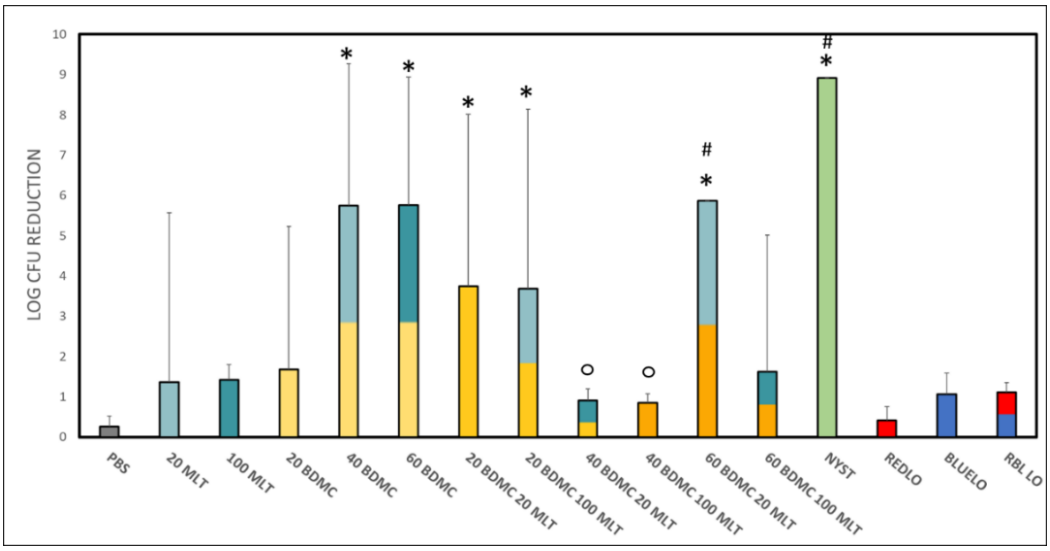

Figure 3 Comparison of median $\log_{10}$ CFU/ml/biofilm weight reductions from baseline values after 1 hour of incubation among 20 and 100 µM melatonin irradiated with red light (λ = 630 nm, 250 mW/cm^2, 37.5 J/cm^2), 20, 40 and 60 µM bisdemethoxycurcumin irradiated with blue light (λ = 430nm, 250 mW/cm^2, 37.5 J/cm^2), and different combinations of photosensitizers irradiated with blue and subsequently red light with the same wavelength, power density and energy density. Error bars represent interquartile range computed from 6 measurements. *= significant difference at (p < 0.05) from PBS. # = significant difference at (p < 0.05) from red light only. O = significant difference at (p < 0.05) from nystatin. PBS= phosphate buffer saline (negative control), MLT = melatonin, BDMC = bisdemethoxycurcumin. NYST=nystatin, REDLO= red light only, BLUELO=blue light only, RBL LO=red and blue light only

A study also found that PDT using curcumin was able to downregulate the expression of genes associated with biofilm formation (Jordão et al., 2020). It may be possible that melatonin was able to produce singlet oxygen after irradiation with red LED light which complementing the hydroxyl radicals produced by BDMC. It may also be possible that BDMC, was also able to inhibit gene expression associated with biofilm formation and therefore making the *Candida albicans* more susceptible to the singlet oxygen produced by MLT. This is despite the fact that melatonin is also a strong hydroxyl radical scavenger particularly in much higher concentrations (10x-100x) (Poeggeler et al., 1993). In contrast, BDMC, when combined with 100 µM of melatonin not only resulted in insignificant amounts of inhibition of *C. albicans* but also reduced the efficacy of 40 µM BDMC and 60 µM BDMC. In a study by Roberts et al. in 2000 that used perinapthenone, a derivative of melatonin, the singlet oxygen lifetime decreased with increasing concentrations of the melatonin derivatives. However, the same could not be said when 100 µM MLT was added to 20 µM BDMC. The chemical interaction between melatonin and BDMC has not been extensively studied. Perhaps the effectiveness of the combination may be influenced by the ratio of these combinations. This may also be attributed to the limited sample size and a high variation in the values of some test groups Higher reductions found after 1 hour of treatment may also be attributed to the prolonged production of ROS (Santos et al., 2022).

In literature, the effectiveness of photodynamic therapy in comparison conventional therapy varies. A systematic review of 5 randomized controlled trials (RCTs) involving a total of 168 participants with denture stomatitis showed that PDT was comparable to conventional treatment (Roomaney et al., 2021). Another systematic review by Firoozi et al., 2021 on 3 RCTs with a total of 141 participants showed that PDT is an effective treatment against fungal infections, however, it was not more effective compared to conventional nystatin treatment. The authors suggest several factors including the lack of standardization of the methods used and small sample sizes. The optimal treatment parameters for PDT have also not been

established and therefore treatment parameters vary significantly across studies. The strains of fungi also have an effect. Fluconazole-resistant strains are found to be less susceptible to PDT compared to naïve strains (Dovigo et al., 2013). The effect of local factors for each patient such as smoking, nocturnal denture wearing, oral hygiene, presence of systemic diseases such as HIV and diabetes mellitus affect the fungal load and thereby might reduce the effectiveness of PDT (Roomaney et al., 2021).

Based on this study, it may also be possible that the red LED light at 630 nm and with a power density of 250 mW/cm^2 may be sufficient to induce production of ROS by melatonin, similar to the study of Maharaj et al. in 2005 where singlet oxygen was produced after the exposure of 100 µM melatonin to 675 nm Nd: YAG laser.

5. Conclusion

In this study, we conclude that 40 µM BDMC, 60 µM BDMC irradiated with blue LED light and 60 µM BDMC+20 MLT, 20 µM BDMC + 20 µM MLT and 20 µM BDMC + 100 µM MLT irradiated with blue LED light and subsequently red LED light after 1 hour of incubation were the effective combinations to inhibit *Candida albicans* in biofilm form achieving reductions ranging from approximately 5.5 ± 2.9 $\log_{10}$ - 5.8 ± 3.58 $\log_{10}$ CFU/ml/biofilm weight and were significantly higher compared to PBS.

Although the photosensitizers used in this study may seem promising, it is also important to assess its toxicity on normal cells before considering their use in a clinical setting. It may be ideal to test C. *albicans* cultured from patients with ongoing candidiasis. It is recommended that more samples be included to the groups to ascertain the effectivity of the treatment. As the ideal protocol for photodynamic therapy has not been established, different combinations and concentrations of the photosensitizers may also be explored to determine the best protocol. Further studies to determine the types of ROS produced by this protocol should also be done to ascertain the contribution of ROS to the treatment. Although there are no reports on the resistance of *C. albicans* to PDT, further research may be done to determine such occurrence.

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