ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2021, 10(1), pp. 69-78, DOI: 10.18036/estubtdc.632471

RESEARCH ARTICLE

THE EFFECT OF ANTIOXIDANTS AND PH ON PHOTOOXIDATIVE STRESS WITH METHYLENE BLUE OF *E. COLI, S. AUREUS* AND *C. ALBICANS*

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ABSTRACT

In this study, the effect of photooxidative stress on different microorganism groups (Gram positive, Gram negative and Eukaryote) and the role of pH and antioxidants on this effect were investigated. The study was performed under 3 day light fluorescent lamps (total 4950 lux) in phosphate buffer (5.0, 6.0, 7.0, and 8.0) at different pH values. As a result, the colony counts of *Escherichia coli, Sthapylococcus aureus* and *Candida albicans* were not decreased under photooxidative stress according to the starting number at pH 5.0 and 6.0. While *E. coli and S. aureus* at pH 8.0 were decreased 3 log and 3.38 log, it were decreased 1.27 and 1.56 log at pH 7.0. Similarly, *C. albicans* decreased 0.35 log at pH 7.0 and 0.75 log at pH 8.0. T_{99} value at pH 8.0 was determined as 4.0 hours for *E. coli*, 3.5 hours for *S. aureus*, and 15.7 hours for *C. albicans* ($p < 0.05$). When the effects of NaCl, Mannitol and ascorbic acid on photooxidative stressed microorganisms were examined, it was determined that NaCl protected microorganisms against photooxidative stress, and ascorbic acid and mannitol changed their effect according to microorganism. In conclusion, photooxidative stress were found to be more effective to Gram positive than Gram negative bacteria, more effective to bacteria than eukaryotes. The effectiveness of photooxidative stress has been determined to be quite high at alkaline pH. It was also determined that there is a direct relationship between the effect of photooxidative stress and pH and osmolarity. It has been found that Ascorbic acid and Mannitol are not protective against photooxidative stress in Gram-positive and eukaryotic cell. Yet the cause of this condition is unknown.

Keywords: Photooxidative stres, *E. coli, S. aureus, C. albicans*, Mannitol, Ascorbic acid

1. INTRODUCTION

There are many environmental factors that affect the life of microorganisms in the nature. The most important of these factors are photooxidative stress caused by sunlight and light-sensitive molecules. The sunlight is also effective by directly acting as a lethal effect, as well as indirectly by the effect of light, to produce radicals in many molecules [1]. Apart from sunlight, it is able to produce photooxidative stress in artificial light sources through both direct and light-sensitive molecules [2]. A variety of toxic products arise from light-sensitive molecules due to light effects. These products are singlet oxygen, H_2O_2, superoxide and hydroxyl radical, commonly known as reactive oxygen species [3]. Reactive oxygen products (ROS) that occur in photooxidation have the ability to attack all types of molecules in all cells. Amino acids, lipids, carbohydrates and nucleic acids in all proteins are damaged by these radicals [4]. In addition, it has been determined that different mutations occurred in the DNA of cells treated with methylene blue, which is a light and photosensitizer, and that similar studies revealed genetic diseases and impaired metabolic pathways [5]. In all aerobic organisms, ROS arise as an inevitable result in plant chloroplasts. In the presence of Cu and Fe-doped metals, hydroxyl radicals can also be formed directly as H_2O_2. Cells have various mechanisms that protect themselves from the effects of oxidative stress [6]. These mechanisms are divided into two as enzymatic and non-enzymatic. Enzymatic protection mechanisms include various enzymes that remove reactive oxygen species from the environment. These enzymes include catalase, superoxide dismutase, glutathione peroxidase, alkyl hydroperoxidase [6, 7]. Non-enzymatic mechanisms include various carotenoid pigments, vitamins,

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Received: 13.10.2019 Published: 25.01.2021

glutathione. In addition, life strategies developed against different environmental stresses indirectly protect against oxidative stress, and there is a global network controlled by multiregulator systems [8]. There is not enough work on these networks. Reactive oxygen derivatives are also a defense strategy that uses the immunity system in combating pathogens. In the human body, the immune system fights pathogens through ROS and allows them to be destroyed [9]. In recent years, photodynamic therapy (PDT) has also been developed as a sterilization method or treatment of various diseases [10]. For this reason, factors affect the formation of photooxidative damage in light environment were important to combat microorganisms.

Mechanisms that provide protection against individual stress factors appear to have quite different effects in the case of global regulation, ie in the presence of more than one stress factor. When there are more than one stress, it is very important to investigate the mechanisms of protection against stress conditions and the effectiveness of stress and to combat bacterial life and the conditions. For example, the effectiveness of osmotic stress at neutral pH and the mechanisms of protection, and the mechanism of protection at acidic pH and the it's effectiveness are changing. In addition, Idil et al. (2016) in their study with *E. coli* and mutants under photooxidative stress in phosphate buffer at different pH values, they found that there was no effect of photooxidative stress at pH 5 and 6 [11]. Therefore, the aim of this study is to investigate the effect of pH on the efficacy of photooxidative stress, to show the difference between the responses of different groups of microorganisms against this stress and to show their relationship with antioxidant factors. So, It was to provide preliminary evidence on effect of some factors on the efficacy of methylene blue. In addition, it is expected to be obtained in the data which will be the basis for the illumination of the mechanism of photooxidative stress.

2. MATERIAL and METHODS

2.1. Microorganisms and Culture Conditions

In this study, Gram negative *E. coli* W3110 and Gram positive *S. aureus* ATCC6535 were used as prokaryotes, while *C. albicans* ATCC10231 was used as eukaryote. Microorganisms were prepared from stock culture at -80 ºC, and incubated in nutrient agar (Merck) medium at 37 ºC for 24 hours.

2.2. Viability Experiments under Photooxidative Stress

E. coli W3110, *S. aureus* ATCC6535 and *C. albicans* ATCC10231 were incubated in Nutrient broth (Merck) medium in a shaker incubator at 37 ºC for 18 hours at 160 rpm. For life experiments, 5 ml of each microorganism was centrifuged, washed twice with pH 7.0 sodium phosphate buffer and resuspended with 5 ml phosphate buffer again. After the absorbance of bacterial and fungal suspension was adjusted to 1.0 absorbance at OD_{600}, 100 µl were transferred to 50 ml phosphate buffer with different pH values (pH 5.0, 6.0, 7.0, 8.0). Thus, approximately $5x10^6$ for bacteria and $15x10^4$ starting yeast for microorganisms were provided. After the beakers were covered with stretch film, the beakers were incubated at 25 ºC under 3 fluorescent lights (4950 ± 75 lux). In addition, dark control groups were coated with aluminum foil and used as control in the role of light effect. At different pH levels, the effect of photooxidative stress was determined by taking samples with spread plate counting method at 2-hour intervals. 100 µl of methylene blue (Merck) was taken from 0.1 g ml^{-1} stock, and was added to the 50 ml phosphate buffer for photooxidative stress. The dark control, dark + methylene blue, light controls were used to show the presence of photooxidation [12]. The studies were done as 4 independent replicates, and the data were obtained by taking the averages.

2.3. Effect of Antioxidant and NaCl on Photooxidation

NaCl (Merck), ascorbic acid (Sigma) and mannitol (Sigma) were added to the pH 8.0 phosphate buffer which revealed the presence of photooxidative stress. Ascorbic acid, mannitol and NaCl stock solutions

were prepared. It were added 0.01 M and 0.1 M NaCl, 10 mM mannitol, 5 mM ascorbic acid to pH 8.0 phosphate buffers. Samples were taken at certain intervals during the incubation period, and microorganism counts were made by spreading plate method [13].

2.4. Statistical Analysis

Vitality values were obtained by plate counting method and the obtained values were converted to logarithmic values. Also t_{99} and k values were calculated using these values. All mean data were given with standard deviation values. Differences were tested by Student's t test for statistical significance. When the significance value was less than 5%, it was considered significant.

3. RESULTS

3.1. The Effect of Photooxidative Stress on the Survival of *E. coli, S. aureus* and *C. albicans* at Different pH values

In this study, the effect of photooxidative stress on the survival of 3 different microorganisms and the effect of medium pH on the effectiveness of this stress was investigated. Light control, dark control and dark + methylene blue beakers were used as the control group. These control groups were used as a control for both the presence of photooxidative stress and the toxic effect of methylene blue. *E. coli, S. aureus* and C *albicans* at pH 5.0 and 6.0 were not affected by photooxidative stress according to the colony count results given in Table 1. There was also not the effect of methylene blue and pH in the control groups too (Table 1). We founded that cell counts decreased from 6.64 log to 5.37 log at *E. coli*, from 6.63 log to 5.07 log at *S. aureus*, from 5.20 log to 4.85 log at *C. albicans* under the effect of photooxidative stress in pH 7.0. The effect of photooxidative stress was further increased at pH 8.0 than pH 7.0. Cell counts in this pH decreased from 6.64 log to 3.66 log at *E. coli*, from 6.63 log to 3.25 log at *S. aureus*, and from 5.20 to 4.45 log at *C. albicans*. According to these results, while any photooxidative stress activity in pH 5.0 and 6.0 is not seen, its effect was started to seen at pH 7.0, an this effect was determined to go further at alkaline environment such as pH 8.0.

Table 1. The survival of *E. coli, S. aureus, C. albicans* under photooxidative stress at different pH values (pH 5.0, 6.0, 7.0 and 8.0). L + MB; Light+ Methylene Blue (MB), L; Light, D + MB; Dark + Methylene Blue (MB), D; Dark Control

Microorganism	Stress	pH 5				pH 6			
		0	2	4	6	0	2	4	6
E. coli	L+MB	6,64±0,05	6,75±0,08	6,79±0,11	**6,71**±0,06	6,64±0,05	6,56±0,10	6,59±0,17	**6,66**±0,09
	L	6,64±0,05	6,73±0,06	6,73±0,15	6,68±0,02	6,64±0,05	6,65±0,04	6,56±0,13	6,51±0,10
	D+MB	6,64±0,05	6,78±0,05	6,78±0,11	6,70±0,05	6,64±0,05	6,77±0,11	6,63±0,09	6,66±0,11
	D	6,64±0,05	6,75±0,05	6,74±0,04	6,73±0,06	6,64±0,05	6,70±0,02	6,65±0,13	6,68±0,12
S. aureus	L+MB	6,63±0,07	6,63±0,08	6,30±0,10	**6,24**±0,06	6,63±0,07	6,36±0,45	6,12±0,40	**6,04**±0,40
	L	6,63±0,07	6,64±0,10	6,55±0,07	6,50±0,12	6,63±0,07	6,54±0,23	6,59±0,29	6,50±0,05
	D+MB	6,63±0,07	6,69±0,05	6,64±0,10	6,75±0,10	6,63±0,07	6,69±0,05	6,64±0,11	6,69±0,10
	D	6,63±0,07	6,66±0,07	6,69±0,09	6,68±0,05	6,63±0,07	6,66±0,04	6,65±0,07	6,66±0,02
C. albicans	L+MB	5,20±0,03	5,20±0,10	5,27±0,06	**5,30**±0,08	5,20±0,03	5,20±0,09	5,23±0,07	**5,27**±0,08
	L	5,20±0,03	5,37±0,07	5,32±0,18	5,28±0,05	5,20±0,03	5,37±0,07	5,32±0,18	5,25±0,06
	D+MB	5,20±0,03	5,44±0,10	5,47±0,03	5,29±0,03	5,20±0,03	5,30±0,07	5,30±0,13	5,16±0,04
	D	5,20±0,03	5,34±0,04	5,35±0,06	5,24±0,03	5,20±0,03	5,26±0,06	5,22±0,04	5,25±0,13

Table 1. continued..,		pH 7				pH 8			
		0	2	4	6	0	2	4	6
E. coli	L+MB	6,64±0,05	6,53±0,10	6,04±0,10	**5,37**±0,15	6,64±0,05	5,46±0,06	4,42±0,18	**3,66**±0,18
	L	6,64±0,05	6,55±0,05	6,34±0,11	6,36±0,07	6,64±0,05	6,58±0,10	6,22±0,10	6,38±0,07
	D+MB	6,64±0,05	6,60±0,13	6,44±0,16	6,31±0,08	6,64±0,05	6,59±0,02	6,34±0,19	6,39±0,08
	D	6,64±0,05	6,60±0,13	6,58±0,06	6,46±0,05	6,64±0,05	6,61±0,26	6,38±0,03	6,40±0,10
S. aureus	L+MB	6,63±0,07	6,38±0,16	5,71±0,14	**5,07**±0,10	6,63±0,07	4,67±0,32	3,44±0,23	**3,25**±0,13
	L	6,63±0,07	6,52±0,09	6,19±0,11	6,12±0,09	6,63±0,07	6,28±0,15	6,08±0,17	6,32±0,04
	D+MB	6,63±0,07	6,58±0,22	6,50±0,05	6,38±0,01	6,63±0,07	6,38±0,16	6,21±0,03	6,34±0,06
	D	6,63±0,07	6,67±0,11	6,59±0,08	6,40±0,10	6,63±0,07	6,30±0,11	6,26±0,10	6,45±0,08
C. albicans	L+MB	5,20±0,03	5,37±0,14	5,26±0,39	**4,85**±0,19	5,20±0,03	4,93±0,03	4,70±0,24	**4,45**±0,06
	L	5,20±0,03	5,51±0,18	5,27±0,06	5,27±0,07	5,20±0,03	5,28±0,21	5,09±0,16	4,81±0,17
	D+MB	5,20±0,03	5,31±0,12	5,38±0,13	5,28±0,08	5,20±0,03	5,14±0,15	5,30±0,10	5,19±0,16
	D	5,20±0,03	5,32±0,07	5,31±0,07	5,31±0,11	5,20±0,03	5,10±0,17	5,12±0,29	5,19±0,04

T_{99} and k values are given in Table 2. The t_{99} value refers to the time that a bacterial culture decrease 2 logarithmic from the starting number at a given incubation time. According to these results, while *S. aureus* decreased 2 log at 3.5 h. *E. coli* showed this decrease at 4.2 hours. Thus, *E. coli*, a gram negative bacterium against photooxidative stress, is more resistant than *S. aureus*, a gram-positive bacterium. Similarly, *C. albicans* showed a decrease of 2 log in 15.7 h. *C. albicans*, a eukaryotic organism, was found to be more resistant than *E. coli* and *S. aureus*, which are prokaryotic microorganisms. In the control group dark and methylene blue t_{99} value was 47.3 h. for *E. coli* and 40.8 h. for *S. aureus*. Also, there is no significant difference between all dark controls and light control. For *C. albicans*, the t_{99} value was found to be 1084.5 h in the dark and methylene blue environment, while the light control decreased in a relatively short time of 30.6 h. These results indicate that *C. albicans* has a lower chance of life in the light and alkaline pH than in the dark. As a result, it has been determined that photooxidative stress is highly effective at alkaline pH and has no effect on acidic pH. In addition, *S. aureus* was more affected by photooxidative stress than *E. coli*. *C. albicans*, an Eucaryotic organism, were also less affected than bacteria.

Table 2. T_{99} and k values of photooxidative stress in pH 8.0 phosphate buffer.

Microorganism	Stress	0. h	6. h	k	T_{99} (h)
E. coli	L+MB	6,64±0,05	**3,66**±0,18	-0,4971	**4,02**
	L	6,64±0,05	6,38±0,07	-0,0422	47,40
	D+MB	6,64±0,05	6,39±0,08	-0,0423	47,30
	D	6,64±0,05	6,40±0,10	-0,0405	49,40
S. aureus	L+MB	6,63±0,07	**3,25**±0,13	-0,1270	**3,50**
	L	6,63±0,07	6,32±0,04	-0,0653	38,60
	D+MB	6,63±0,07	6,34±0,06	-0,0018	40,80
	D	6,63±0,07	6,45±0,08	-0,0014	41,80
C. albicans	L+MB	5,20±0,03	**4,45**±0,06	-0,5642	**15,70**
	L	5,20±0,03	4,81±0,17	-0,0519	30,60
	D+MB	5,20±0,03	5,19±0,16	-0,0490	1084,50
	D	5,20±0,03	5,19±0,04	-0,0479	1450,60

3.2. The Effect of Antioxidants on the Effect of Photooxidative Stress at Alkaline pH.

In our study, it has been determined that photooxidative stress is highly effective at alkaline pH. NaCl, ascorbic acid and mannitol were added to the environment in order to determine the effect of photooxidative stress in the presence of some antioxidant substances. In pH 8.0 buffer, these substances were incubated with addition of methylene blue under the fluorescence lamp by addition to the determined final concentrations According to the results of colony counts, the added NaCl protected all of microorganisms from photooxidative stress. In *E. coli*, the value of t_{99} was 7.95 h in the presence of 0.01 M NaCl, and 8.08 h in the presence of 0.1 M NaCl. In the same NaCl values, *S. aureus* was determined as 4.37 and 8.52 h., respectively. As can be seen from Table 3, *C. albicans* was determined as 39.43 and 26.66 h in same NaCl concentrations respectively. According to these results, it has been determined that the presence of NaCl reduces the lethal effect of the photooxidative stress and provides protection of the cell. While the protection formed by the addition of 0.01 M NaCl in *E. coli* was the same as 0.1 M, *S. aureus* was not affected much when 0.01 M NaCl was added, while It was more protected at 0.1 M NaCl. When 10 mM Ascorbic acid was added to the phosphate buffer, the effect of the photooxidative stress decreased for *E. coli* (from 4.02 h to 7.10 h), while *S. aureus* was not protected from the effect of photooxidative stress. Interestingly, the addition of ascorbic acid increased the efficacy of photooxidative stress for *C. albicans*. When ascorbic acid was added, the cell count of *C. albicans* decreased at 7.78 h, but this count was 15.7 h without ascorbic acid. In the same way, it was determined that *E. coli* was protected from the effect of photooxidative stress in mannitol added samples (from 4.02 to 17.96 h.). However, *S. aureus* (3.55 h to 3.75 h.) and *C. albicans* (from 15.7 h to 14.45 h.) were interestingly found to be not protected from photooxidative stress with mannitol. It was determined that mannitol had no effect on these two microorganisms.

Table 3. The role of antioxidants added to the light medium containing methylene blue in pH 8.0 phosphate buffer

	Without antioxidant			0.01 M NaCl			0.1 M NaCl	
Microorganism/Time	0. h	6. h	T_{99}	0. h	6. h	T_{99}	6. h	T_{99}
E. coli	6,64±0,05	**3,66**±0,18	**4,02**	6,64±0,05	5,13±0,05	**7,95**	5,16±0,05	**8,08**
S. aureus	6,63±0,07	**3,25**±0,13	**3,55**	6,63±0,07	3,89±0,05	**4,37**	5,23±0,05	**8,52**
C. albicans	5,20±0,03	**4,45**±0,06	**15,7**	5,20±0,03	4,90±0,05	**39,43**	4,75±0,05	**26,66**
	Without antioxidant			Ascorbic acid			Mannitol	
Microorganism/Time	0. h	6. h	T_{99}	0. h	6. h	T_{99}	6. h	T_{99}
E. coli	6,64±0,05	**3,66**±0,18	**4,02**	6,64±0,05	4,95±0,05	**7,10**	5,98±0,05	**17,96**
S. aureus	6,63±0,07	**3,25**±0,13	**3,55**	6,63±0,07	3,53±0,05	**3,87**	3,43±0,05	**3,75**
C. albicans	5,20±0,03	**4,45**±0,06	**15,7**	5,20±0,03	3,66±0,05	**7,78**	4,37±0,05	**14,45**

4. DISCUSSION

The effectiveness of each stress varies depending on the presence of other stress factors or other chemicals in the environment. Therefore, the effectiveness of stress factors and the resistance mechanisms of microorganisms against these stresses must be explained in the global network. Photodynamic therapy is seen as one of the methods used in the fight against microorganisms. Therefore, the advantages and disadvantages of this method, the mechanism of action and the mechanisms of protection should be established. The survival of microorganisms under different stress conditions requires specific genes and their expression at different levels. This synthesis is controlled by multiple regulator mechanisms. The explored of global network gene arrangements involving these multiple regulatory systems will shed light on the lives of bacteria in natural environments. In addition, since *E. coli*, *S aureus* and *C albicans* are quite different microorganisms, the differences between different

organisms and their response to stress have been investigated. Also, the efficacy of methylene blue in different pH and in antioxidants have been investigated.

At the end of the study, it was determined that the microorganisms studied in the presence of light + methylene blue were killed, and the control groups were not affected. Also pH was found to be very important in the efficacy of photooxidative stress. As a result, in our study, photooxidation with methylene blue did not have any effect at pH 5 and pH 6, whereas it had effects at pH 7 and especially at pH 8. It is thus determined that photooxidation of methylene blue is more effective at alkaline pH. The effectiveness of photooxidative stress increases as it moves towards the alkaline environment. Eisenberg et al. 1987 [14] reported a very strong correlation with pH values and to be more efficient of MB inactivation at a basic pH. This study was done at pH 4, 7 and 10 values. They found a strong connection between pH and sensitization. But Cooper and Goswami (2002) demonstrated that there was no a connection for photooxidation in the presence of MB and sunlight at different pH (7 and 10) [15]. Also Cooper and Goswani (2002) demonstrated that MB (10 mg/L) effected in dark on *E. coli*, but our study demonstrated that 0.2 mg/ml MB did not effect on bacteria in dark. Our before study demonstrated that same results on dark [11]. Acher et al. 1990 [16] and 1994 [17] demonstrated that increasing the effluent pH from 7.1-7.6 to 8.6-8.9 brought about improved and reproducible results. The increase in the pH resulted in an increase in the effect of photooxidation on coliform, enterococcus, fecal koliform. Our study determined that alkaline pH was more effect than acidic pH on effectiveness of photooxidative stress via MB, and also gram positives are more affected by photooxidation than Gram negatives.

Brovko et al. (2009) demonstrated that malachite green was active against Gram positive bacteria under illumination, and it did not affect Gram negative bacteria or yeasts [18]. Souza et al. (2010) investigated that the *Candida albicans* photoactivation using methylene blue, toluidine blue and malachite green under low-power GaAlAs laser irradiation. This study demonstrated that this photooxidation at a concentration of 0.1 mg mL^{-1} effected on *C. albicans* [19]. The highest reduction in log CFU mL^{-1} after PDI was observed in the presence of toluidine blue, followed by methylene blue and malachite green. However, there was no significant statistical difference between the dyes studied. Vilela et al. (2012) compared the action of malachite green with the phenothiazinic photosensitizers (methylene blue and toluidine blue) on *Staphylococcus aureus* and *Escherichia coli* biofilms [20]. The best results for both microorganisms were obtained with photosensitizer concentrations of approximately 300 mM MB, with microbial reductions of 0.8–1.0 log_{10}; 150 mM TB, with microbial reductions of 0.9–1.0 log10; and 3000 mM MG, with microbial reductions of 1.6-4.0 log10. Rolim et al. (2012) compared the PDI with methylene blue, toluidine blue and malachite green at the same concentration (163.5 mM) against the Gram positive bacteria *Streptococcus mutans* [21]. They used two different light sources, a red LED lamp (636 nm, 80 mW) and a blue curing light (570 nm, 800 mW). In addition, the singlet oxygen production of each photosensitizer was determined by tryptophan photooxidation. PDI with irradiation in the presence of the photosensitizers TBO and MG was effective in reducing *S. mutans* counts by 3 and 1.4 logs, respectively, compared to their respective untreated controls. Although MG reduced the number of *S. mutans*, this photosensitizer did not produce singlet oxygen, indicating that the antimicrobial activity of PDI may also be promoted by other ROS. Prates et al. (2007) studied the photoinactivation of a Gram negative oral bacteria *Actinobacillus actinomycetemcomitans* using MG associated with a low-power red laser [22]. They observed 99.9% cell viability inhibition using MG 0.01% (w/v) and after 5 minutes of radiation. Junqueira et al. (2010) investigated the photodynamic antimicrobial effects of the MG on thirty-six microbial strains, including Gram positive and Gram negative bacteria, and yeasts [23]. The results indicate that Gram positive bacteria were more sensitive to MG–mediated photosensitization, followed by Gram negative species, and the yeast species. Here, the highest reduction of *E. coli* viability (94.6%) was achieved by photosensitization with MB at an energy density of 335.8 J cm-2. Our study demonstatd that gram positive *S. aureus* was more sensitive than gram negative *E. coli* and eukaryote *C. albicans*. The fungicidal effect of MB has been demonstrated on various species of the Candida genus (*C. albicans, C. dubliniensis, C. krusei* and *C.*

tropicalis) [24, 25]. Gram negative bacteria have an outer membrane and this property has a protective role from photooxidative stress [26].

Rolim et al. (2012) demonstrated that MB generated 1.3 times more $(1)O^{(2)}$ than TBO, and both produced significantly higher concentrations of singlet oxygen than the other photosensitizers [21]. When sensitizer dyes, such as methylene blue or rose bengal, are treated with light, it was seen that a singlet oxygen generally occured [10]. However, different factors may affect the effectiveness of oxidative stress. Alkaım et al (2014) demonstrated that the photocatalyst of ZnO in different pH of solution was found to be more efficient for the degradation of the methylene dye as compared to other catalysts used (TiO_2, CdS and Co_3O_4 powder catalysts) [27].

When the efficacy of photooxidative stress is examined in the presence of ascorbic acid, osmotic stress-forming NaCl or mannitol, which can be used as a nutrient, NaCl is very important in protecting from photooxidative stress. Further protection of *S. aureus* in the presence of 0.1 M NaCl may be attributed to the ability of this bacterium to survive in higher salt concentrations than *E. coli*. *S. aureus*, it is known that protective mechanisms against osmotic stress are induced at higher salt concentrations [28]. Therefore, *S. aureus* can live in high salt concentrations and is used as a distinguishing feature in the media used for this isolation. Therefore, if there is salt in alkaline pH environment, photooxidative stress loses its effectiveness. Ascorbic acid is a well-known molecule for many years as an antioxidant [29]. However, ascorbic acid has been found to be pro-oxidant under certain conditions such as the presence of metal ions, low concentrations, unsuitable pH. Buettner et al. (1984) reported the formation of hydrogen peroxide and hydroxyl free radicals by methylene blue in the presence of ascorbate [30]. Adding ascorbic acid during the photolytic treatment of catechin hydrate decreased the dimer formation, suggesting that ascorbic acid can suppress the photosensitive oxidation of catechin [31]. Mannitol can be used as a food source by microorganisms. *C. albicans* begins to use mannitol when glucose is depleted. *C. albicans* shows dioxic growth in the presence of glucose and mannitol [32]. Brancini et al. (2016) demonstrated that the addition of 50 mM mannitol prior to PDT on *C. albicans* could not prevent cell death nor proteomic alteration to any extent, but azid could prevent cell death [33]. Mannitol is a hydroxyl radical scavenger [34].

5. CONCLUSION

As a result, It was determined that pH was highly effective on the efficacy of photooxidative stress. Alkaline pH enhances the effect of photooxidative stress. Photooxidative stress is very effective especially in pH 8. Gram positives between different microorganism groups were more sensitive. Antioxidant substances had different effects on microorganism groups. NaCl protected from photooxidative stres to microorganisms.

ACKNOWLEDGEMENTS

This study was supported by the BAP project of Amasya University with FMB-BAP code 14-084.

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