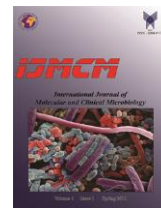




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Antibacterial effect of brilliant blue food dye

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ABSTRACT

Brilliant blue is largely found in ice cream, canned processed peas, packet soups, blue raspberry flavored products, dairy products, sweets and drinks. It is also used in soaps, shampoos, mouthwash and other hygiene and cosmetics applications (Archived from Florida). This study was aimed to investigate the antimicrobial effects of Brilliant blue on *Bacillus subtilis* as a gram positive bacterial strain and *Pseudomonas aeruginosa* as a gram negative bacterial strain with MIC, MBC, and disk diffusion assay. MIC values of Brilliant blue against *Bacillus subtilis* was 5 µg/ml, and MBC values was 60 µg/ml and MIC values of Brilliant blue against *Pseudomonas aeruginosa* are 10 µg/ml, and MBC values was 80 µg/ mL. In this study Brilliant blue had only a bacteriostatic effect on two bacterial strains. The result of disk diffusion assay showed that Brilliant blue (0.625, 1.25, and 1.875 µg) has not antimicrobial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*. It can be concluded that Brilliant blue might not pose a potential risk for our bacterial flora. However, it must be evaluated with different new studies.

1. Introduction

There are many components used to make foods and some drinks. The Food and Drug Administration (FDA) Provide a list of about 3000 components in its database with name "Everything Added to Food in the United States", a large number of which we utilize at our foods (sugar, vanilla, baking soda, salt and colors) (FDA, 2010). Food dyes or color additive are any dyes, pigments or components that convey color when it is added to food or any drink. They come in many forms consisting of liquids, powders and gels. Food dyes is using in commercial food production and in domestic

cook processing. Food dyes are also using in a variety of non-food applications including cosmetics, pharmaceuticals and medical devices (FDA, 2012). Food coloring with natural substances has been used since approximately 1500 years before Christ in Egypt and has been regulated from the time of England's King Edward I, in the 13th century (Burrows, 2009). In the United States, the following seven artificial colorings are generally used in some drinks and foods: 1: Brilliant blue, 2: Indigotin, 3: Fast Green, 4: Erythrosine, 5:

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Allura Red, 6: Tartrazine, 7: Sunset Yellow (FDA, 2015; FDA, 2007).

Brilliant blue is an organic compound classified as a triarylmethane color, reflecting its chemical structure. Known under various commercial names, it is a dye for foods and drinks. It is denoted by E number E133 and has a color index of 42090. It has the appearance of a reddish-blue powder. It is soluble in water, and the solution has a maximum absorption at about 628 nanometers (Bassam et al., 2005). Like other blue color, Brilliant blue is largely found in ice cream, canned processed peas, packet soups, bottled food colorings, icings, ice pops, blue raspberry flavored products, dairy products, sweets and drinks, especially the liqueur blue Curaçao. It is also used in soaps, shampoos, mouthwash and other hygiene and cosmetics applications (Archived from Florida). In 2006, Neveen reported that brilliant blue were mostly attributable to hepatocellular damage, renal failure and decrease in spermatogenesis process (Nevveen, 2006). Hansen et al. (1966) showed that Brilliant blue had non-significant effect on fetuses. On the other hand Durne et al. (1995) concluded that the synthetic dye brilliant blue had no significant effect on chromosomal aberrations.

Brilliant blue is widely consumed by people and no published antibacterial study of Brilliant blue against *Bacillus subtilis* and *Pseudomonas aeruginosa* were found in the literature. Consequently, this study was aimed to investigate the antimicrobial effects of Brilliant blue on *Bacillus subtilis* as a gram positive bacterium and *Pseudomonas aeruginosa* as a gram negative bacterium with detection of MIC, MBC, and disk diffusion assay.

2. Materials and Methods

2.1. Materials

In this study, Mueller Hinton broth and Mueller Hinton agar were purchased from Merck and the test substance, Brilliant blue was purchased from Sigma-Aldrich and its properties and molecular structure is shown in Figure 1: (Material Safety Data Sheet, 2016)

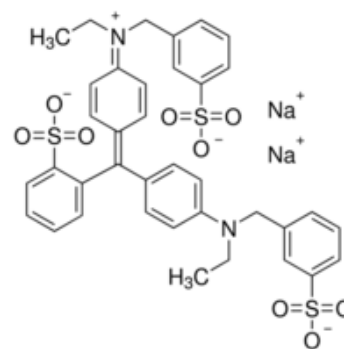


Fig.1. The structure of Brilliant blue CAS Number: 3844-45-9 Yes PubChem CID: 19700 Chemical formula: $C_{37}H_{34}N_2Na_2O_9S_3$ Molar mass: 792.85 g/mol Solubility in water: soluble IUPAC name: disodium;2-[[4-[ethyl-[(3-sulfonatophenyl)methyl]amino]phenyl]-[4-[ethyl-[(3-sulfonatophenyl)methyl]azaniumylidene]cyclohexa-2,5-dien-1-ylidene]benzenes-ulfonate.

2.2. Bacterial Strains

The test bacteria strains used in this study were obtained from Pamukkale University (Bacteriology Lab) and Bulent Ecevit University (Medical Microbiology Lab); *Bacillus subtilis* as a gram positive bacterium and *Pseudomonas aeruginosa* as a gram negative bacterium. Both bacterial strains were maintained in viable state via inoculation on Mueller-Hinton Agar and overnight incubation at 37°C.

2.3. MIC and MBC assay

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Brilliant blue were determined by dilution method. Different concentrations of Brilliant blue (final concentrations of 0.625 to 240 µg/ml) were prepared and added to different tubes with 4 ml Mueller Hinton broth. So, a microbial suspension of 0.5 McFarland turbidity standard of bacterial strains were inoculated in Mueller Hinton broth medium containing different concentration of Brilliant blue tubes (Mariselvam et al., 2014; Yehia and Al-Sheikh, 2014). Visual observation of bacterial growth was performed after overnight incubation at 37°C. MIC values were identified as the minimum concentration at which no visible bacterial growth was recorded. For determining concentration–response relationships of Brilliant blue we used spectrophotometric analysis to measure the optical density at 600

nm of bacterial cultures to monitor bacterial growth at different concentrations.

MBC were observed as the lowest concentration of test substance (Brilliant blue) that completely inhibited the bacteria growth. At this time an aliquot of 100 μL of all tubes showing no visible bacterial growth were cultured on Mueller Hinton Agar plates and incubated at 37°C for 12 hours.

2.4. Disk Diffusion Method

Disk diffusion method was used to test antimicrobial activity of Brilliant blue. A stock solution of Brilliant blue was prepared by dissolving 625 μg of Brilliant blue with 10 mL of 10, 20 and 30 μL of distilled water (0.625, 1.25, and 1.875 μg) were spotted alternately on both sides of the discs and allowed to dry. Distilled water were used as negative controls. Antimicrobial effect analysis was evaluated performed by measuring the diameter (millimeter) of the inhibition zone around the discs. Antimicrobial effect of Brilliant blue was expressed as the mean zone of inhibition diameters of around discs (Souto et al., 2006).

3. Results

The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of Brilliant

blue against *Bacillus subtilis* and *Pseudomonas aeruginosa* is shown in figure 2-3.

MIC value of Brilliant blue against *Bacillus subtilis* was 5 $\mu\text{g}/\text{ml}$, and MBC value was 80 $\mu\text{g}/\text{ml}$ and antibacterial activity of Brilliant blue for MIC values of Brilliant blue against *Pseudomonas aeruginosa* was 10 $\mu\text{g}/\text{ml}$, and MBC value was 60 $\mu\text{g}/\text{ml}$. In this study we observed that *Pseudomonas aeruginosa* was more sensitive to Brilliant blue than *Bacillus subtilis*.

In this study, Brilliant blue concentrations (0.625, 1.25, and 1.875 μg) were selected for disk diffusion assay. The disk diffusion values of Brilliant blue against *Bacillus subtilis* and *Pseudomonas aeruginosa* is shown in Figures 4-5. The result of this study showed that Brilliant blue has not antimicrobial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*, because diameter of inhibition zone at all concentration of Brilliant blue against *Bacillus subtilis* and *Pseudomonas aeruginosa* was zero mm.

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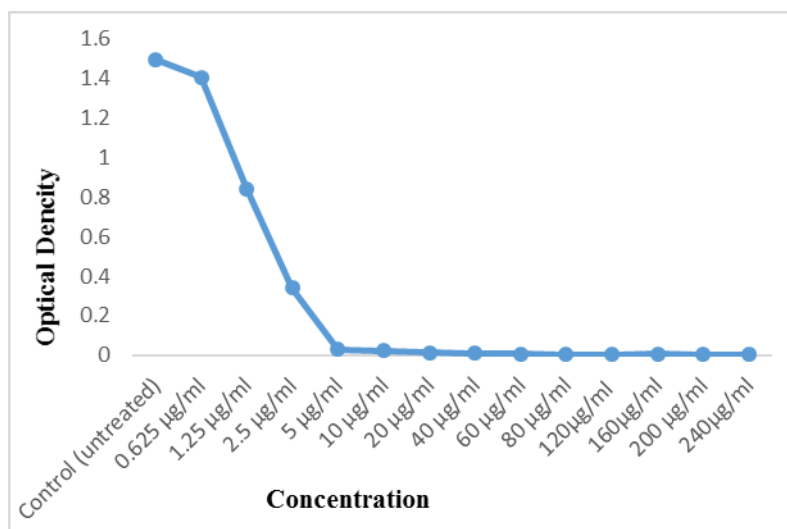


Figure 2. Antibacterial effect of Brilliant blue on *Bacillus subtilis* in Mueller Hinton broth at different concentrations (MIC value was 5 $\mu\text{g}/\text{ml}$).

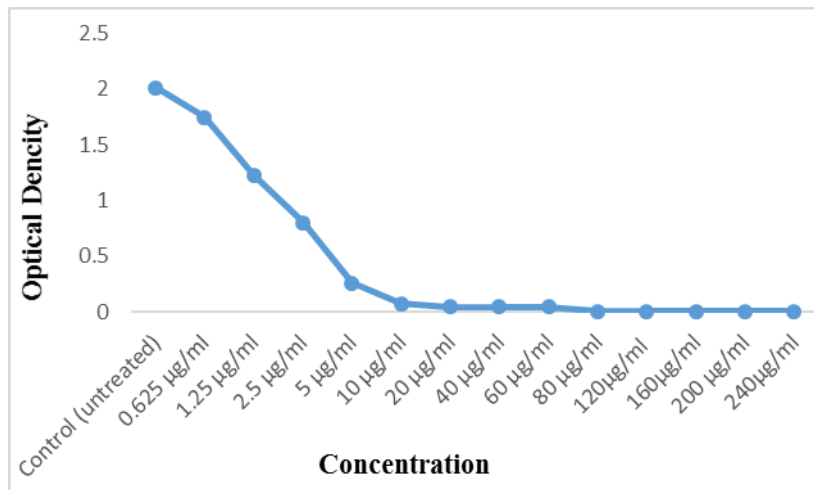


Figure 3. Antibacterial effect of Brilliant blue on *Pseudomonas aeruginosa* in Mueller Hinton broth at different concentrations (MIC value was 10µg/ml).



Figure 4. Disk Diffusion result of Brilliant blue on *Bacillus subtilis* (C: Control, 1: 0.625, 2: 1.25 and 3: 1.875 µg/ m.).



Figure 5. Disk Diffusion result of Brilliant blue on *Pseudomonas aeruginosa* (C: Control, 1: 0.625, 2: 1.25 and 3: 1.875 µg/ ml).

4. Discussion

Antibiotic is important medication for the treatment of many infectious diseases. Excessive consumption of these medications results in bacterial resistance. In this way, researchers have prioritized studies on different compounds with medicinal uses in order to discover new medication for treatment of microbial infections.

The results of this study showed that Brilliant blue has not antibacterial effect against *Bacillus subtilis* and *Pseudomonas aeruginosa*. According to our knowledge, this is the first study that addresses the antibacterial effects of Brilliant blue against *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Brilliant blue uses in health sciences and industrial issues. Brilliant blue FCF or E133 in the European numbering system) is one of the most common dyes used in food and cosmetic preparations and medicines. Brilliant blue was approved in various countries to be used as a food additive in dairy products, candies, cereals, cheese, toppings, jellies, liquors, and soft drinks. This dye is also used in cosmetics such as shampoos, nail polishes, lip gloss, lipsticks and in the textile sector (Watharkar et al., 2013). Brilliant blue was shown to be efficient in blocking P2X7R-induced cytotoxicity in retinal cell, microglial cell, and astrocyte cell (Suzuki et al., 2004; Jacques et al., 2004; Zhang et al., 2005). However, there are divergences concerning the applicability of Brilliant blue in human cells. Eschke et al. demonstrated a reduction in activity in human macrophage cells following Brilliant blue exposure (Eschke et al., 2002). We are using food dye in our life but some of food coloring have toxic effects, for this reason we need to study about food coloring. Chromosome aberration (CA) assay is one of methods to measure cytotoxicity and genotoxicity (Norizadeh Tazehkand and Topaktas, 2015). For example, 4-methylimidazole is one of food dyes that using in foods and drinking. 4-MEI tested by CA and another methods, the result of these study showed that 4-MEI can be has risk for humans and different bacterial strains (Norizadeh Tazehkand et al., 2016 a; Norizadeh Tazehkand et al., 2016 b; Norizadeh Tazehkand et al., 2017; Norizadeh Tazehkand, 2017). Also, some of the food colors don't have any risk or antibacterial effect against different bacterial strains. For

example, Dyes obtained from catechu (*Acacia catechu*) and myrobalan (*Terminalia chebula*) don't have antimicrobial effect against tested bacterial strains. Similarly, antimicrobial effect of phloxine B was studied on *Salmonella choleraesuis*, *E. coli* and *Shigella*. The results obtained from this study showed that phloxine B does not have antimicrobial activity on gram negative bacterial strains.

In contrast to Brilliant blue, some food dyes have antibacterial effect on gram positive and gram negative bacterial strains. Yolmeh et al. (2014) observed that annatto dye have antimicrobial activity on *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, and *Streptococcus pyogenes*. Gul and Bakht (2015) reported that turmeric dye inhibited *Escherichia coli* and *Staphylococcus aureus* growth by disk diffusion analysis. In another study antibacterial effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* was analyzed by Pintado et al. (2011). The result of this study showed that saffron has antibacterial effect against *Salmonella enterica*.

Conclusion

There was no more reports regarding to the antimicrobial effect of Brilliant blue in the available literatures. As a result, Brilliant blue don't have antibacterial effect on *Bacillus subtilis* and *Pseudomonas aeruginosa*. Therefore, it can be concluded that Brilliant blue might not pose a potential risk for different bacterium. However, it must be evaluated with different new studies.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

- Bassam, M., El Ali, B., Mohammad Farahat A. (2005). Handbook of industrial chemistry: organic chemicals. New York: McGraw-Hil. ISBN 0-07-141037-6.
- Burrows, A. (2009). Palette of our palates: a brief history of food coloring and its regulation. *Compr Rev Food Sci Food Saf.* 8:394–408.

- Durne, V.A., Oreshenko, A.V., Kulavko, A.V., Bresten, N.F. (1995). Analysis of Cytogenetic Activity Material Safety Data Sheet. Erioglucine disodiumsalt.
- Eschke, D., Wüst, M., Hauschildt, S., Nieber, K. (2002). Pharmacological characterization of the P2X7 receptor on human macrophages using the patch-clamp technique. *Naunyn Schmiedebergs Arch Pharmacol.* 365(2):168–171.
- FDA. Color Additive Regulations. CFR Title 21 Part 70. 2012.
- FDA. Color Additive Status List.US FDA Color Additive Status List 2015.
- FDA. Red No. 3 and Other Colorful Controversies. Archived from the original on 2007-08-09.
- FDA. International Food Information Council (IFIC) and U.S. Overview of Food Ingredients, Additives & Colors 2010.
- Gul, P. and Bakht, J. (2015). Antimicrobial activity of turmeric extract and its potential use in food industry. *J Food Sci Technol.* 52(4):2272-9.
- Hansen, W.H., Fitzhugh, O.G., Nelson, A.A., Davis, K.J. (1966). Chronic Toxicity of Two Food Colors Brilliant Blue FCF and Indigotine. *Toxicological. Applied Pharmacy.* 8: 29.
- Jacques-Silva, M.C., Rodnight, R., Lenz, G., et al. (2004). P2X7 receptors stimulate AKT phosphorylation in astrocytes. *Br J Pharmacol.* 141(7):1106–1117.
- Mariselvam, R.A., Ranjitsingh Nanthini, J.A., Kalirajan, K., Padmalatha, C., and Selvakumar, P.M. (2014). Green synthesis of silver nanoparticles from the extract of the inflorescence of *Cocos nucifera* (Family:Arecaceae) for enhance antibacterial activity. *Spectrochimica Acta Part A: Spectrochim Acta A Mol Biomol Spectrosc.* 129, 537–541.
- Neveen, H.M. (2006). Toxic Effects of the Synthetic Food Dye Brilliant Blue on Liver, Kidney and Testes Functions in Rats. *J. Egypt. Soc. Toxicol.* 34: 77-84.
- Norizadeh Tazehkand, M., Topaktas, M., Yilmaz, M.B., Hajipour, O., Valipour, E. (2016). Delineating the antigenotoxic and anticytotoxic potentials of 4-methylimidazole against ethyl methanesulfonate toxicity in bone marrow cell of swiss albino mice. *Bratisl Lek Listy.* 117(5):290-4.
- Norizadeh Tazehkand, M., Topaktas, M. (2015). The in vitro genotoxic and cytotoxic effects of remeron on human peripheral blood lymphocytes. *Drug Chem Toxicol.* 38(3): 266-71.
- Norizadeh Tazehkand, M. (2017). Antiproliferative effect of a food coloring on colon cancer cell line. *Bratisl Lek Listy.* 118(5):265-268.
- Pintado, C., Miguel, A.D., Acevedo, O., Nozal, L., Novella, J.L. and Rotger, R. (2011). Bactericidal effect of saffron (*Crocus sativus* L.) on *Salmonella enterica* during storage. *Food Control.* 22: 638-642
- Souto, R., Andrade, A.F.B., Uzeda, M., Colombo, A. P.V. (2006). Prevalence of “non-oral” pathogenic bacteria in subgingival biofilm of subjects with chronic periodontitis. *Braz J Microbiol.* 37:208-215.
- Suzuki T, Hide I, Ido K, Kohsaka S, Inoue K, Nakata Y. (2004). Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. *The Journal of Neuroscience.* 24(1):1–7.
- Tazehkand, M.N., Hajipour, O., Moridikia, F., Moridikia, A., Valipour, E., Yilmaz, M.B., Topaktas, M. (2017). Investigation of anticarcinogenic and antioxidant effects of -methylimidazole. *Bratisl Lek Listy.* 118(3):149-152.
- Tazehkand, M.N., Moridikia, A., Hajipour, O., Valipour, E., Timocin, T., Topaktas, M., Yilmaz, M.B. (2016). The effects of 4-MEI on cell proliferation, DNA breaking and DNA fragmentation. *Bratisl Lek Listy.* 117(7):371-5.
- Watharkar, A.D., Khandare, R.V., Kamble, A.A., Mulla, A.Y., Govindwar, S.P., Jadhav, J.P. (2013). Phytoremediation potential of *Petunia grandiflora* Juss., an ornamental plant to degrade a disperse, disulfonated triphenylmethane textile dye Brilliant Blue G. *Environ Sci Pollut Res Int.* 20(2):939–949.
- Yehia, R.S. and Al-Sheikh, H. (2014). Biosynthesis and characterization of silver nanoparticles produced by *Pleurotus ostreatus* and the iran ticandidal and anticanceractivities. *World J Microbiol Biotechnol.* 30: 2797–2803.
- Yolmeh, M., Habibi Najafi, M.B. and Farhoosh, R. (2014). Optimization of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food Chemistry.* 155:319–324.
- Zhang, X., Zhang, M., Laties, A.M., Mitchell, C.H., (2005) Stimulation of P2X7 receptors elevates Ca²⁺ and kills retinal ganglion cells. *Invest Ophthalmol Vis Sci.* 46(6):2183–2191.