

# Evaluation of the Effect of Intra-Coronal Bleaching after Using C-Phycocyanin as a Photosensitizing Agent- In vitro Study

Nawal Aidaros\*<sup>1</sup> and Heba Badra<sup>2</sup>

## ABSTRACT:

**Purpose:** This study aimed to evaluate the effect of intra-coronal bleaching after using C-Phycocyanin as a photosensitizing agent. **Materials and methods:** Fifty-one freshly extracted permanent anterior human teeth with intact crowns were used. All teeth were endodontically treated then stained with 2 mL of fresh rabbit blood and then centrifuged three times a day for 30 minutes. The teeth were randomly allocated according to the materials used for bleaching into group I (sodium perborate), group II (using C-Phycocyanin as a photosensitizer), and group III (control). The VITA easy shade V digital spectrophotometer was used for evaluation of the samples' color lightening parameter at baseline, at days 7 and 14 of the completion of the intracoronal bleaching procedure. **Results:** the sodium perborate treated group showed a significant increase in color lightening parameter at 7 and 14 days ( $P=0.048$ ), while the C-Phycocyanin treated group showed a non-significant difference at 7 and 14 days ( $P=0.505$ ). **Conclusion:** C-Phycocyanin had no satisfactory bleaching effect and is not a promising tooth-bleaching agent.

**Keywords:** Sodium perborate, Tooth bleaching, C-Phycocyanin, photosensitizer, color

---

\*<sup>1</sup> Associate Professor, Operative Dentistry Department, Dentistry Faculty, Ahran Canadian University, Giza-Egypt.

<sup>2</sup> Assistant professor, Endodontic Department, Dentistry Faculty, Ahran Canadian University, Giza-Egypt.

## INTRODUCTION:

Aesthetic dentistry has become one of the most important aspects of the dental field. Nowadays, gaining a beautiful smile has become the primary concern of most patients<sup>(1)</sup>.

Tooth discoloration is one of the challenges that encounter dentists in aesthetic dentistry<sup>(2)</sup>. Tooth discoloration could be caused by intrinsic or extrinsic factors<sup>(3)</sup>. Intrinsic discoloration can either be caused by systemic or local factors. Local factors could be due to endodontic materials and coronal filling materials<sup>(4)</sup>. Bleaching of discoloured teeth is one of the promising approaches to putting an end to this problem as it is considered a minimally invasive procedure<sup>(5-7)</sup>.

Intra-coronal bleaching (Walking Bleach) is a simple, cost-effective, and conservative method for improving the color of endodontic-treated teeth<sup>(8)</sup>. Sodium perborate and hydrogen peroxide (HP) are used as intra-coronal bleaching material when added to the pulp chamber and sealed for three to seven days<sup>(9)</sup>. Hydrogen peroxide is a potent oxidizing agent that penetrates the dentinal tubules and releases reactive oxygen species (ROS). The free oxygen molecules

split the chromophore compounds' double bonds, causing lightning of stains<sup>(10,11)</sup>.

Photodynamic therapy (PDT) is a non-invasive mode for treating many medical conditions such as tumours and skin problems<sup>(12,13)</sup>. Photodynamic bleaching of teeth was reported to be effective when photosensitizing agents (such as rhodamine-B) are added to peroxide bleaching material<sup>(14-18)</sup>. Low-intensity visible light source activates peroxide oxidation and enhances the breakdown rate of Reactive Oxygen species (ROS) speeding up the bleaching procedure<sup>(18)</sup>.

Phycocyanin is one of the natural biological molecules found in *Spirulina platensis*<sup>(19)</sup>, as a light-harvesting pigment, which possesses antioxidant effects<sup>(20)</sup>. The anionic or cationic affinity of these photosensitizers after the binding process to the calcium atoms or the phosphate atoms respectively present in hydroxyapatite crystals produces precipitates that act as a physical barrier, thus interfering with the dentin surface-resin contact<sup>(21)</sup>.

The null hypothesis is that c-phycocyanin has no intra-coronal bleaching effect. The current study compared the intra-coronal bleaching effect of PDT using C-Phycocyanin as a photosensitizer with

sodium perborate on discoloured non-vital teeth.

## **MATERIALS AND METHODS:**

### **Materials:**

(1) Sodium Perborate (SP) (Morgan Speciality Chemicals)

(2) C-Phycocyanin (C-PC) (National Research Centre)

### **Methods:**

#### **1. Ethical Approval**

The study had been approved by the Research Ethics Committee, Faculty of Dentistry, Cairo University (CREC); Research number: 35-3-22.

#### **2. Samples Size Calculation**

A minimum sample size of 51 teeth would be sufficient to detect the effect size of 0.6 and a power ( $1-\beta=0.95$ ) of 05% at a significance level of  $p<0.05$ . According to sample size calculations, each group was represented by a minimum of 17 samples. The sample size was calculated according to G\*Power software version 3.1.9.2 <sup>(22,23)</sup>

#### **3. Samples Selection and Preparation**

Anterior extracted permanent human teeth (n=51) with mature apices were used for this experimental in vitro study. Each tooth was immersed in NaOCl (5.25%) for two hours to be disinfected then stored in saline until use <sup>(6)</sup>.

Initial radiographs were taken to confirm root canal patency. All teeth were mechanically scaled using an ultrasonic scaler to remove any remaining bone, calculus, or soft tissue. Coronal access was prepared to all teeth using #12 diamond bur (MANI, GET-Egypt) with a high-speed water spray coolant.

#### **4. Root Canal System Cleaning and Shaping**

The working length (WL) was determined by subtracting one mm from the distance between the reference point and the tip of the #15 k-file just extended beyond the apical foramen. The canals were prepared using the crown-down technique utilizing rotary M-Pro nickel-titanium instruments (IMD Company, China) according to the manufacturer's instructions up to #35 instrument (MANIN, GET-Egypt). The M-Pro system was connected to a Wismy endodontic micro-motor (Bomdent, China). Each canal was irrigated with 2 ml of 5.25% sodium hypochlorite (NaOCl) at each file size using a 27-gauge needle. After root canal preparation, each canal was irrigated with 5ml of 17% ethylenediaminetetraacetic acid (EDTA) for 60 seconds, then it was obturated with gutta-percha and root canal sealer (MetaBiomed resin sealer) using lateral condensation technique. Two millimeters of

root canal filling were removed below the cementoenamel junction then 1 mm of resin-modified glass-ionomer cement (GC-FUGI, Tokyo, Japan) was packed to seal the root canal filling cervically.

### 5. Artificial Staining of The Samples

Each tooth was immersed in a test tube containing fresh rabbit blood (2 ml), then centrifuged using a blood centrifuge machine (Larksci Portable Blood Plasma Extractor PRP Kit Centrifuge 4000 rpm) at 500 X for 30 min, three times per day. The blood was changed every day to repeat the discoloration process for 7 successive days until the discoloration of all samples was similarly obtained (Fig.1). Finally, each tooth was rinsed with distilled water (10 ml), and the crown was polished with a rubber cup, and pumice<sup>(24,25)</sup>.



**Figure (1):** Test tubes containing fresh rabbit blood in the blood centrifuge machine.

### 6. Color Lightening Evaluation Before Intracoronary Bleaching

Each tooth color lightening was assessed at baseline using VITA easy shade V digital spectrophotometer (VITA Zahnfabrik, Bad Säckingen, Germany). Standardized photographs of each crown were taken using Kodak Color film under standardized lighting conditions (original color). The photographic camera (Canon T50, automatic with ocular macro-FD 100 mm, 1:4 and extension tube FD50; Canon, Japan) was placed 50 cm from the crown. These films were developed under identical conditions by a professional laboratory.

### 7. Preparation of the Bleaching materials

#### (a) C-Phycocyanin Extract (C-PC)

The extract was prepared in the National Research Centre (NRC), Egypt. Algae was milled in 100 mL of 0.1M phosphate buffer solution (pH: 6.8), and 10 mL of Tris-HCl in the presence of acid-washed neutral sand and then filtered. The mixture was further subjected to freezing (-20 °C for 30–100 minutes) and thawing for 3–10 freeze-thaw cycles in total, stirred at 150 rpm at 4°C for 30 minutes, and sonicated ten times at 4 C- phycocyanin. Calculations were determined using the spectrophotometry-based methods on the absorbance ratio.<sup>(26)</sup>

C-Phycocyanin concentration: The C-Phycocyanin concentration (C-PC) in  $\text{mg} \cdot \text{mL}^{-1}$  was calculated from the optical densities (OD) at 652 and 620 nm, using Equation <sup>(26)</sup>:

$$\text{C-PC} = (\text{OD}_{620} - 0.474\text{OD}_{652})$$

---

5.34

The purity of C-PC preparations was evaluated supported by the ratio between absorbencies from phycocyanobilin at 620 nm and aromatic amino acids in all proteins in the preparation at 280 nm. Purity ratio of C-Phycocyanin =  $A_{620} / A_{280}$  at 0°C with ten seconds of sonication and ten-second intervals, centrifuged at 4°C for 8 minutes at 7000 rpm, and the blue-colored supernatant was taken for further investigations.

(b) Sodium Perborate (SP):

Sodium perborate powder (2 gm) was mixed with distilled water (1 mL).

### 8. Intra-coronal Bleaching

The teeth used in this in vitro study (n=51) were randomly allocated by generating numbers from 1:51 using Sequence Generator <https://www.randomizer.org/> into 3 equal groups according to the bleaching material used:

*Group I:* The pulp chamber of each root canal (n=17) was packed with sodium perborate bleaching material

*Group II:* The pulp chamber of each root canal (n=17) was packed with C-Phycocyanin <sup>(28)</sup>. C-Phycocyanin was activated using a 625-nm diode laser (Lasotranix Company) with an output of 220 mW in continuous mode for 3 min (Fig. 2). The power density of the device is  $0.34 \text{ w/cm}^2$ . Laser power was checked with a power meter (Coherent USA) before the experiment.

*Group III (Control group):* The pulp chamber of the root canal (n=17) did not receive any bleaching material .

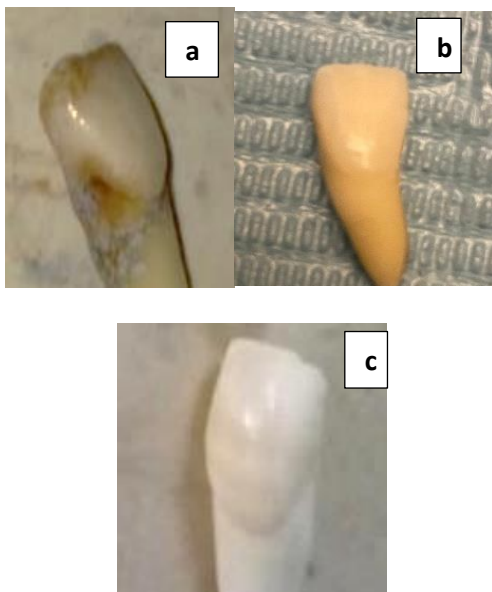
After packing the pulp chambers with the bleaching materials, Orafi-G temporary cement (Prevest, India) was used to seal the coronal access preparations. The teeth were immersed in distilled water and incubated (BTC incubator, 20L, Egypt) at 37°C, the solution was changed daily for 7 days <sup>(24, 27)</sup>.



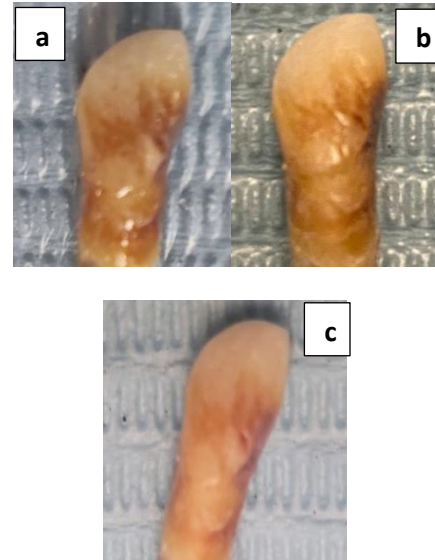
**Figure (2):** Diode Laser device (a) and its handle (b) used to irradiate and activate C-Phycocyanin in the pulp chamber of the tooth (c).

### 9. Color Lightening Evaluation after Intra-coronal Bleaching

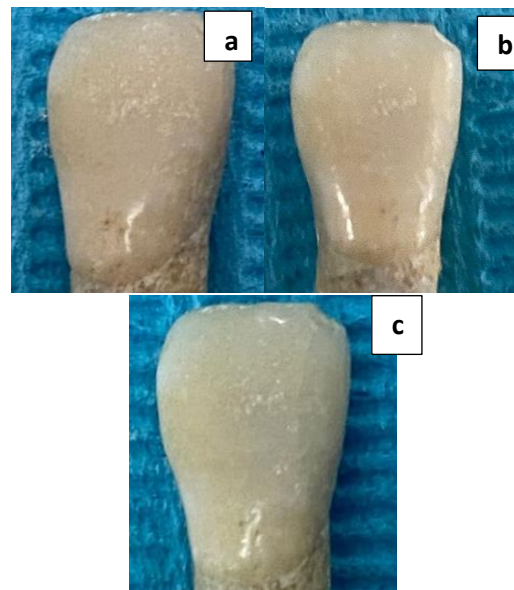
On day 7, the teeth' color lightening was assessed after the cement removal and the bleaching material washing away with water. The assessment was performed by the same investigator using the Vita shade guide in the same examination room against a black background in a dark box. Then, a fresh portion of the bleaching agent was again packed into the pulp chambers of the teeth. The teeth were immersed again in distilled water and incubated (BTC incubator, 20L, Egypt) at 37°C, the solution was changed daily for 14 days. The teeth' color lightening was again assessed for 14 days. (Fig. 3,4, 5.)



**Figure (3):** Tooth color treated with sodium perborate (a) at baseline, (b) at 7 days, (c) at 14 days.



**Figure (4):** Tooth color treated with C-Phycocyanin a) at baseline, (b) at 7 days, (c) at 14 days.



**Figure (5):** Tooth color treated with saline a) at baseline, (b) at 7 days, (c) at 14 days.

### 10. Statistical Analysis:

One-way ANOVA is proposed to evaluate the effect of intra-coronal bleaching after using phycocyanin as a photosensitizing

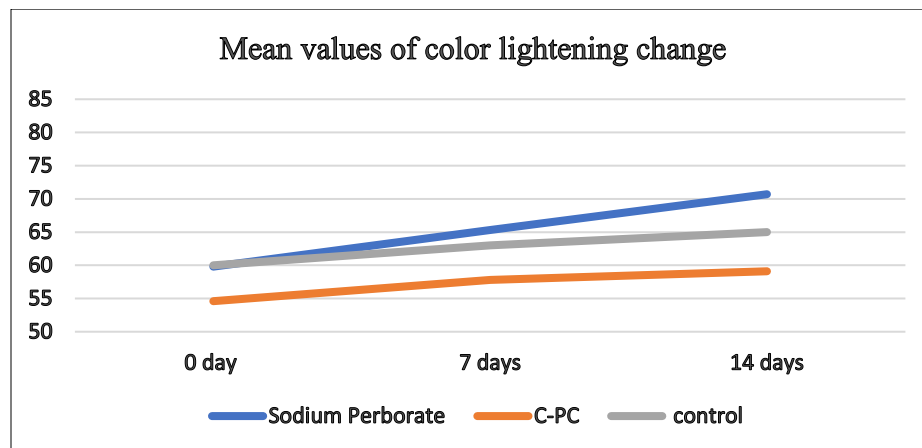
agent. The sample size was estimated based on the data extracted from Carrasco et al 2007<sup>(28)</sup>. Different groups including positive and negative control groups will lead to a large effect size ( $f=0.62$ ) on the delta E.

Data were checked for normality using the Shapiro-Wilk test. One-way ANOVA was used to compare tested groups followed by Tukey's HSD test for pairwise comparison. For comparison between time intervals, repeated measure ANOVA was used followed by multiple comparisons with Dunn Bonferroni. A significant level was set at 0.05.

## **RESULTS:**

Regarding color lightening change ( $\Delta L$ ) results, the sodium perborate treated group showed a significant increase in color lightening parameter at 7 and 14 days ( $P=0.048$ ), while C-Phycocyanin treated group showed a non-significant difference at 7 and 14 days ( $P=0.505$ ).

Comparing the three groups, a non-significant difference in color lightening change was observed on days 7 and 14 days. Non-statistically significant changes were noted in the control teeth, as seen in table (1) and Fig. 6.



**Figure (6):** Mean values of color lightening change ( $\Delta L$ )

**Table (1):** Mean values and standard deviation of color lightening change ( $\Delta L$ ).

	0 day		7 days		14 days		p-value
	Mean	SD	Mean	SD	Mean	SD	
<b>Sodium perborate</b>	59.8	10.5	65.3	12.5	70.7	5.9	0.048*
<b>C-Phycocyanin</b>	54.6	14.1	57.8	13.7	59.1	14.1	0.505 NS
<b>Control</b>	60.0	6.0	63.0	8.7	65.0	10.3	0.256 NS
	0.264 NS		0.807 NS		0.760 NS		

*p-value* \* = Significant difference. NS indicate non-significant.

## DISCUSSION:

Teeth bleaching is a minimally invasive aesthetic dental treatment in recent years<sup>(29, 30)</sup>. The teeth bleaching depends on the reactive oxygen species released through the oxidation process of hydrogen peroxide. These ROS split the dark complex molecules of chromophore into light simple molecules leading to teeth bleaching<sup>(31)</sup>. The bleaching materials are able not only to decompose the pigment molecules but also destroy the organic and inorganic components in the

tooth structure, resulting in harmful side effects<sup>(32,33)</sup>.

C-Phycocyanin is one of the natural biological molecules present in the algae *Spirulina platens*<sup>(34)</sup>. C-Phycocyanin is water soluble and found to be non-toxic, biocompatible, and does not affect the teeth' flexural strength<sup>(35,36)</sup>. Therefore, the present study aimed to evaluate the ability of C-Phycocyanin (C-PC) as a natural extract to accomplish effective teeth bleaching.



The cervical sealing of the root canal filling material is a crucial step in the intracoronal bleaching technique as it prevents leaching the bleaching material through the dentinal tubules to the periodontal tissue. Therefore, a resin-modified glass ionomer was used in this study as a cervical seal to prevent external cervical root resorption as well as the possible risk associated with hydrogen peroxide<sup>(37-39)</sup>.

In the present study, the teeth were artificially stained by rabbit blood as it simulates one of the main causes of intrinsic tooth discoloration, which is the oxidation of haemoglobin inside dentinal tubules after pulp haemorrhage in traumatized teeth. It is an effective method for comparing intracoronal bleaching materials<sup>(24)</sup>.

The specimens were kept in distilled water for the duration of the application because storing them in artificial saliva could have resulted in the formation of a protective salivary film<sup>(27)</sup>

The spectrophotometer gives an absolute color shade providing an accurate and reproducible exact shade of teeth before and after bleaching. Therefore, VITA easy shade V digital spectrophotometer was used in this study to evaluate the color changes of each tooth<sup>(40)</sup>.

The photosensitizer used in this study is C-Phycocyanin which showed no significant bleaching effect on days 7 and 14 of bleaching. The color lightness changes values were shown to be less evident during the 2 weeks of bleaching. This could be due to the high viscosity of C-Phycocyanin which reduced the ROS generation. This study was in agreement with previous study results that reported that the viscosity of the bleaching material had an inverse relation to the bleaching effect<sup>(41)</sup>.

However, sodium perborate reported a significant tooth bleaching ability after 7 and 14 days. The results were consistent with previous studies<sup>(42, 43)</sup>. Significant

differences were observed in the color lightness values after 7 days when compared to color lightness values after 14 days (second session). The significant increase in color lightness after 7 days could be due to the complete penetration and diffusion of hydrogen peroxide into the deep layers of enamel and dentin owing to its low viscosity and high flowability. The oxidation of the bleaching agent leads to an expansive reaction that could dislodge the temporary restoration and decrease the effectiveness of the bleaching action after 14 days<sup>(44)</sup>.

The control group reported a non-significant color change after 7 and 14 days. The result was in agreement with a previous study that used distilled water as a bleaching material and had a non-significant increase in color lightening effect<sup>(27)</sup>.

#### **CONCLUSION:**

Under the limitations of this in vitro study, it could be concluded that C-Phycocyanin had no satisfactory bleaching effect and could not

be used as a competitor tooth-bleaching material. Thus, the study failed to reject the null hypothesis.

#### **ACKNOWLEDGMENT:**

*The authors would like to thank Dr.*

*Shaimaa Rohayem; assistant professor in Operative Dentistry at Ahram Canadian University; for her continuous help and guidance.*

#### **CONFLICT OF INTEREST:**

None declared.

#### **FUNDING:**

No funding was received for this study.

#### **REFERENCES:**

1. Barakah R, Alwakeel R. Non-vital endo-treated tooth bleaching with sodium perborate. *Curr Health Sci J.* 2019; 45:329.
2. Meraji N, Bolhari B, Sefideh MR, Niavarzi S. Prevention of tooth discoloration due to calcium-silicate cements: a review. *Dent.Hypotheses J.* 2019 ;10 :4-8

3. Bosenbecker J, Barbon FJ, De Souza Ferreira N, Morgental RD, Boscato N. Tooth discoloration caused by endodontic treatment: A cross-sectional study. *J Esthet Restor Dent.* 2020; 32:569-74
4. Oliveira LV, Da Silva GR, Souza GL, Magalhães TE, Barbosa GL, Turrioni AP, A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials. *Int Endod J.* 2020; 53:1140-52.
5. Alali JE, Alanazi HA, Alyousef HK, Alali OF, Alzwayyid SA. Teeth discoloration removal and management: a review. *Int J Med Dev Ctries.* 2020; 4:1070-4.
6. Behl M, Patnana AK, Khanna V, Chaudhry K. Evaluation of three different bleaching agents in permanent and primary teeth: an in vitro study. *Int J Clin Pediatr Dent* 2020; 13:130-35.
7. Coelho AS, Garrido L, Mota M, Marto CM, Amaro I, Carrilho E, Paula A. Non-vital tooth bleaching techniques: a systematic review. *Coatings.* 2020; 10:61.
8. Banomyong D. Clinical considerations for non-vital tooth walking bleaching. *Thai Endod J* 2022; 1:33-42
9. Hirata BS, Berger SB, Guiraldo RD, Felizardo KR, Ramos NB, Lopes MB. Assessing the bleaching effect of an experimental stabilized chlorine dioxide agent used for internal bleaching purposes. *Braz J of Oral Sci* 2022; 21.
10. Frank AC, Kanzow P, Rödiger T, Wiegand A. Comparison of the bleaching efficacy of different agents used for internal bleaching: A Systematic Review and Meta-Analysis. *J Endod.* 2021; 48: 171-8.
11. Daltro TW, De Almeida SA, Dias MF, Lins-Filho PC, Da Silva CH, Guimarães RP. The influence of violet LED light on tooth bleaching protocols: In vitro study of bleaching effectiveness. *Photodiagnosis Photodyn. Ther J.* 2020; 32:102052.

12. Kwiatkowski S, Knap B, Przystupski D, Saczko J, Kędzierska E, Knap-Czop K, et al. Photodynamic therapy—mechanisms, photosensitizers and combinations. *Biomed pharmacother J.* 2018; 106:1098-107.
13. Karges J. Clinical development of metal complexes as photosensitizers for photodynamic therapy of cancer. *Angew Chem Inter Ed J.* 2022; 61: e202112236.
14. Kuzekanani M, Walsh LJ. Quantitative analysis of KTP laser photodynamic bleaching of tetracycline-discolored teeth. *Photomed Laser Surg J.* 2009; 27:521-5.
15. Kwon SR, Cortez E, Wang M, Jagwani M, Oyoyo U, Li Y. Systematic review of in vitro studies evaluating tooth bleaching efficacy. *Am J Dent.* 2020; 33:17-24.
16. Maran BM, Ziegelmann PK, Burey A, De Paris Matos T, Loguercio AD, Reis A. Different light-activation systems associated with dental bleaching: a systematic review and a network meta-analysis. *Clin Oral Investing J.* 2019; 23:1499-512.
17. De Almeida Guanaes BK, Duarte TN, Correr GM, Da Rosa Kaizer M, Gonzaga CC. In-office dental bleaching with violet light emitting diode: bleaching efficacy and pulpal temperature rise. *Restor Dent Endod J.* 2022; 47.
18. De Souza Rastelli AN, Dias HB, Carrera ET, De Barros AC, Dos Santos DD, Panhóca VH, et al. Violet LED with low concentration carbamide peroxide for dental bleaching: A case report. *Photodiagnosis Photodyn Ther J.* 2018; 23:270-2.
19. Pleonsil P, Soogarun S, Suwanwong Y . Anti-oxidant activity of holo-and apo-c-phycocyanin and their protective effects on human erythrocytes. *Int. J. Biol. Macromol.* 2013 ;60:393–8
20. Abd El-Baky HH . Over Production of Phycocyanin Pigment in Blue Green Alga *Spirulr'na* sp. and It's Inhibitory Efect on Growth of Ehrlich Ascites Carcinoma Cells. *J. Med. Sci.* 2003 ; 3:314–24.

21. H Strazzi-Sahyon, P da Silva, J Nakao, P da Silva, L Nunes, M Seron, L Cintra, P dos Santos, G Sivieri-Araujo, "Influence of two photodynamic therapy sessions and different photosensitizers on the bond strength of glass-fiber posts in different regions of intraradicular dentin," *Photodiagnosis and Photodynamic Therapy* 2021.
22. Faul F, Erdfelder E, Lang AG, Buchner A. G\* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods*. 2007 ;39 :175-91.
23. Rietveld CA, Medland SE, Derringer J, Yang J, Esko T, Martin NW, Westra HJ, Shakhbazov K, Abdellaoui A, Agrawal A, Albrecht E. GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. *science*. 2013 ;340:1467-71.
24. Özer SY, Kapısız E. Comparison of Walking-Bleaching and Photon-Initiated Photoacoustic Streaming Techniques in Tooth Color Change of Artificially Colored Teeth. *Photo biomodulation, Photomed Laser Surg J*. 2021;39:355-61.
25. Santos MR, De Carvalho FB, Cangussu MC, Barros LR, Trindade RF. Spectrophotometric analysis of the effectiveness of bleaching agents used for non-vital teeth bleaching. *IJHBS*. 2020; 8:1-6.
26. Bennett A, Bogorad L. Complementary chromatic adaptation in a filamentous blue-green alga. *J. cell biol* .1973 ;58(2):419-35.
27. Mousa EA, El-Toukhy RI, Ali AI. Effect of Over-the-Counter Whitening Products on Enamel Color Change and Surface Roughness-A laboratory Study. *Group*.;1(13.74):5-75.
28. Carrasco LD, Guerisoli DM, Rocha MJ, Pécora JD, Fröner IC. Efficacy of intracoronary bleaching techniques with different light activation sources. *Int Endod* 2007; :204-8.

29. Afrasiabi S, Pourhajibagher M, Chiniforush N, Aminian M, Bahador A. Anti-biofilm and anti-metabolic effects of antimicrobial photodynamic therapy using chlorophyllin-phycoyanin mixture against *Streptococcus mutans* in experimental biofilm caries model on enamel slabs. *Photodiagnosis photodyn Ther J*. 2020; 29:101620.
30. Bersezio C, Estay J, Jorquera G, Peña M, Araya C, Angel P, et al. Effectiveness of dental bleaching with 37.5% and 6% hydrogen peroxide and its effect on quality of life. *Oper Dent*. 2019;44: 146-55.
31. Farawati FA, Hsu SM, O'Neill E, Neal D, Clark A, Esquivel-Upshaw J. Effect of carbamide peroxide bleaching on enamel characteristics and susceptibility to further discoloration. *J prosthet Dent*. 2019; 121:340-6.
32. Bersezio C, Martín J, Angel P, Bottner J, Godoy I, Avalos F, et al. Teeth whitening with 6% hydrogen peroxide and its impact on quality of life: 2 years of follow-up. *Odontology*. 2019;107: 118-25.
33. Crastechini E, Borges AB, Torres CR. Effect of remineralizing gels on microhardness, color and wear susceptibility of bleached enamel. *Oper Dent* 2019; 44:76-87.
34. Lena C, Esteve I, Forner L. Effects of in-office bleaching on human enamel and dentin. Morphological and mineral changes. *Ann Anat J*. 2018 ;217: 97-102.
35. Wu XJ, Yang H, Chen YT, Li PP. Biosynthesis of fluorescent  $\beta$  subunits of *c*-phycoyanin from spirulina subsalsa in *escherichia coli*, and their antioxidant properties. *Molecules*. 2018;23: 1369.
36. Li Y. The Bioactivities of Phycocyanobilin from Spirulina. *J. Immunol. Res*. 2022.
37. Pourhajibagher M, Bahador A. Antimicrobial properties, anti-virulence activities, and physico-mechanical

- characteristics of orthodontic adhesive containing C-phycoerythrin: a promising application of natural products. *Folia Med J.* 2021; 63:113-21.
38. Li W, Lu L, Liu B, Qin S. Effects of phycoerythrin on pulmonary and gut microbiota in a radiation-induced pulmonary fibrosis model. *Biomed Pharm Ther J.* 2020;132: 110826.
39. Sakalli B, Basmaci F, Dalmizrak O. Evaluation of the penetration of intracoronal bleaching agents into the cervical region using different intraorifice barriers. *BMC Oral Health J.* 2022; 22:1-8.
40. Malik R. Dental bleaching: an update. *Dent. Update J.* 2018; 45:44-50.
41. Sürmelioglu D, Özçetin HK, Özdemir ZM, Yavuz SA, Aydın U. Effectiveness and SEM–EDX analysis following bleaching with an experimental bleaching gel containing titanium dioxide and/or chitosan. *Odontology* 2022; 109:114-23.
42. Li Z, Wu Z, Wang J, Huang M, Lin M. Expanding the applications of photodynamic therapy—tooth bleaching. *Clin Oral Investig J.* 2022;26: 2175-86.
43. Carrasco LD, Fröner IC, Corona SA, Pécora JD. Effect of internal bleaching agents on dentinal permeability of non-vital teeth: quantitative assessment. *Dent Traumatol.* 2003; 19: 85-9.
44. Pallarés-Serrano A, Pallarés-Serrano S, Pallarés-Serrano A, Pallarés-Sabater A. Assessment of Oxygen Expansion during Internal Bleaching with Enamel and Dentin: A Comparative In Vitro Study. *Dent. J.* 2021 24; 9:98.