Antimicrobial Properties, Anti-Virulence Activities, and Physico-mechanical Characteristics of Orthodontic Adhesive Containing C-Phycocyanin: a Promising Application of Natural Products

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Abstract

Introduction: Antimicrobial orthodontic adhesives aim to reduce enamel demineralization, white spot lesions, and incipient tooth decay around bonded orthodontic brackets, but they should not imperil its mechanical properties.

Aim: To evaluate the antimicrobial and physico-mechanical properties of acrylic containing different concentrations of C-phycocyanin on Streptococcus mutans, Lactobacillus acidophilus, and Candida albicans.

Materials and methods: The mechanical properties of acrylic resins were measured by flexural strength test after preparation of acrylic resin samples with concentrations of 1%, 2%, 5%, 7.5%, and 10% of C-phycocyanin. Then we evaluated the antimicrobial effects of acrylic resin containing the maximum concentration of C-phycocyanin with clinically acceptable flexural strength and the changes in expression of virulence factors.

Results: The highest and lowest means of flexural strength were obtained in acrylic resins containing 0% and 10% concentrations of C-phycocyanin at 50.2±4.5 and 30.1±3.3 MPa, respectively. Adding 1%, 2%, and 5% of C-phycocyanin showed no significant decrease in flexural strength (p>0.05). The maximum mean diameter of the growth inhibition zone was observed around discs containing 5% of C-phycocyanin. Until day 30 of the study, no microbial biofilms were formed on any acrylic disc. Only microbial biofilms of C. albicans were able to form on discs containing 5% of C-phycocyanin at 90 days. 5% C-phycocyanin could significantly decrease the expression levels of gtfB, hsp16, and ALS9 6.1-, 7.3-, and 3.9-fold, respectively.

Conclusions: It can be concluded that the most acceptable concentration of C-phycocyanin in acrylic resin is 5% based on the results of flexural strength tests and antimicrobial activities of acrylic resin containing various concentrations of C-phycocyanin.

Keywords
acrylic resins, antimicrobial activity, C-phycocyanin, flexural strength, gene expression
INTRODUCTION

Cold-cure acrylic resin is very important in dentistry as it is used in orthodontic devices such as functional devices, retainers, and temporary prostheses. These resins are often composed of methacrylates, especially methyl methacrylate and polyethyl methacrylate, and copolymers. Polymethylene methacrylate (PMMA) has good properties including ease of use and low cost. However, the use of this substance in dentistry is not without its drawbacks. One of the major drawbacks of acrylic resins in removable orthodontic plates is the potential of this material to accumulate plaque due to the absorption of nutrients through the surface pores of this material. Plaque accumulation results in decalcification of the tooth structure and eventually decay along with gingivitis. Thus, among the important side effects of such functional devices and retainers in orthodontics, the increased risk of biofilm accumulation of carcinogenic bacteria (Streptococcus mutans and Lactobacillus acidophilus) and the formation and spread of caries, as well as fungal diseases such as denture stomatitis following the formation of fungal biofilm (Candida albicans) is expected.

The most common problem related to biofilm-forming microorganisms is their high resistance to antimicrobial compounds and even immune system factors. In addition, the bacteria present in the biofilm phase express different genes than the planktonic bacteria, which results in better adaptation of bacteria to the oral environment.

Therefore, according to the above mentioned, it is necessary to have acrylic resins with antimicrobial and anti-biofilm activities. Various methods have been proposed to solve the problem of microbial accumulation on acrylics of devices, one of which is the addition of antimicrobial agents to acrylics. Titanium dioxide (TiO₂), silicon dioxide (SiO₂), silver (Ag), zinc (Zn) and platinum (Pt) have been successfully added to acrylic resins and their antimicrobial properties have been observed. Since the above materials are of mineral and/or synthetic origin, biological concerns have been expressed about them.

Recently, marine algae have been widely regarded as a natural source of bio-products containing various biological properties. Seaweed Spirulina, which has a water-soluble blue pigment called C-phycocyanin, has a variety of biological properties including antibacterial, antifungal, anti-virus, anti-tumor and anti-inflammatory properties.

MATERIALS AND METHODS

Preparation of acrylic samples for flexural strength measurement

Acrylic resin samples were manufactured in accordance with ISO:20795-1 (2008). To prepare acrylic samples with different concentrations of C-phycocyanin, C-phycocyanin (Sigma, Germany) was mixed in acrylic monomer (SR Triplex cold, Ivoclar Vivadent, Liechtenstein, Germany) at 5 concentrations 1%, 2%, 5%, 7.5%, and 10%. To prepare the 1% concentration, 0.03 g of C-phycocyanin was added to 1 mL of monomer. To prepare the 2% concentration, 0.06 g of C-phycocyanin was added to 1 mL of monomer. To prepare 5% concentration, 0.15 g of C-phycocyanin was mixed with 1 mL of monomer. To prepare the 7.5% concentration, 0.25 g of C-phycocyanin was added to 1 mL of monomer, and to prepare the 10% concentration, 0.3 g of C-phycocyanin was mixed with 1 mL of monomer. Sonication with the power of 100 W and frequency of 30 kHz for 3 minutes was used to mix C-phycocyanin in the monomer. According to ISO:20795-1 (2008), the metal model was first prepared in a larger dimension of 54×14×7.3 mm for the manufacture of acrylic resin samples containing C-phycocyanin in dimensions of 5×10 (±0.2)×3.3 (±0.2) mm with smooth surface without porosity and precise angles and with acrylic shrinkage when setting. After the acrylic resins were set, the samples were removed from the mold and cut to the desired dimensions (Fig. 1).

For flexural strength testing based on the ISO:20795-1 (2008), prepared specimens were immersed in water at 37°C for 50±2 hours before testing to provide similar conditions to the oral environment. Each specimen was immediately placed in a ZWICK Z250 (Fig. 2). Then, the flexural strength of each specimen was calculated in megapascals (MPa).

Preparation of acrylic samples for measuring the antimicrobial activity

Following the method described above, acrylic samples containing selected C-phycocyanin (acrylic resin containing the highest percentage of clinically acceptable flexural strength) were made for antimicrobial tests. The mixture of each concentration was poured into a pre-made mold with 5 mm in diameter. After polymerization, the samples were incubated at room temperature for 24 hours to fully set. The samples were finally extracted and polished using Exsys disc and Superfingrit disc (3 ME SPE, USA). After polishing, the specimens were 10 mm in diameter and 4 mm in thickness (Fig. 3). All samples were sterilized by gamma rays at a dose of at least 25 kGy at the Iranian Atomic Center.

AIM

The aim of this study was to evaluate the antimicrobial, anti-biofilm and physico-mechanical properties of acrylic resins containing 1%, 2%, 5%, 7.5%, and 10% concentrations of C-phycocyanin on S. mutans, L. acidophilus, and C. albicans microorganisms to obtain the most appropriate concentration used in orthodontics.
Microorganisms and growth conditions

Standard strains of *S. mutans* ATCC 35668, *L. acidophilus* ATCC 314 and *C. albicans* ATCC 14053 were prepared from Microbial Institute of Pasteur, Iran. *S. mutans* and *L. acidophilus* were incubated in Tryptic Soy Broth (TSB; Merck, Germany) in the presence of 5% CO₂ that was provided by gas-pack and *C. albicans* in aerobic conditions for 48 hours at 37°C.

Artificial aging on acrylic discs containing selected C-phycocyanin

One hundred fifty acrylic discs containing selective C-phycocyanin sterile in 24-well microplate wells containing 2 ml of sterile artificial saliva (0.2 g NaCl, 0.2 g KCl, 0.453 g CaCl₂ • 2H₂O, 0.345 g NaH₂PO₄ • 2H₂O, 0.0025 g Na₂S • g of urea in 1000 mL of DI water; pH 7) were stored at 35°C (Fig. 4). At 0, 15, 30, 60 and 90 days, the discs were removed from each well and used for antimicrobial and anti-biofilm experiments.

Evaluation of antimicrobial effects of acrylic discs containing selected C-phycocyanin

Antimicrobial effects of selected C-phycocyanin (acrylic resin containing maximum C-phycocyanin content with clinically acceptable flexural strength) were evaluated by two different tests:
Assessment of the virulence-associated genes expression by quantitative real-time PCR (qRT-PCR)

In the present study, the changes in gene expression were investigated following the isolation of microorganisms from acrylic discs containing 5% concentration of C-phycocyanin. Briefly, after isolation of each microorganism, the total RNA extraction was performed using the GeneAll Hybrid-R™ RNA purification kit (GeneAll Biotechnology Co. Ltd, Seoul, Korea) according to the manufacturer’s instructions. The complementary DNA (cDNA) was synthesized through a Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, US) with random primers after the elimination of the residual genomic DNAs by RNase-free DNase I treatment (Thermo Scientific GmbH). The primers that were designed using the Primer3 software version 4.0 (http://bioinfo.ut.ee/primer3/) are presented in Table 1.

Table 1. Mean flexural strength of acrylic resin components containing different concentrations of C-phycocyanin studied; 0.0%: No C-phycocyanin

| Groups | MPa (mean ± SD) | P value |
|--------|----------------|---------|
| 0%     | 50.2±4.5       | -       |
| 1%     | 47.4±2.5       | 0.128   |
| 2%     | 43.5±2.3       | 0.091   |
| 5%     | 40.3±2.9       | 0.078   |
| 7.5%   | 34.7±3.0       | 0.042   |
| 10%    | 30.1±3.3       | 0.012   |

The qRT-PCR analysis was performed on a Line-GeneK Real-Time PCR system. The expression levels of luxI gene were calculated relative to the calibration sample (an endogenous control 16S rRNA) to normalize the sample input. Eventually, the relative expression ratio was analyzed using the method proposed by Livak and Schmittgen.20

Statistical analysis

SPSS v. 23 was used for data analysis. Descriptive statistics were used to describe the data and one way ANOVA, repeated measures analysis of variance, and post hoc Bonferroni test were used to analyze the data. The level of significance was set at $p<0.05$.

RESULTS

Flexural strength measurement

The flexural strength of the study groups is shown in Table 2. As can be seen, the highest and the lowest mean
The flexural strengths belong to the control group at 50.2±4.5 MPa and 10% concentration of C-phycocyanin at 30.1±3.3 MPa, respectively. The results also showed that the flexural strength of acrylic resin did not decrease significantly (p<0.05) by adding 1%, 2%, and 5% of C-phycocyanin, while adding 7.5% and 10% of C-phycocyanin significantly decreased flexural strength (p>0.05). Therefore, according to the results, 1%, 2%, and 5% concentrations of C-phycocyanin were used for microbial tests with acceptable flexural strength.

### Antimicrobial effect of C-phycocyanin by DAD test

The antimicrobial effects created by the release of selected C-phycocyanin particles from acrylic discs on MHA were measured by DAD test. According to Table 3, the maximum mean diameter of growth inhibition zone against all 3 microorganisms was observed around discs containing 5% of C-phycocyanin. As can be seen in Table 3, the changes in the diameter of the growth zone are time-dependent. As the incubation time of the discs containing C-phycocyanin increased, the diameter of the growth zone decreased, so that the growth rate of all microorganisms reduced at 90 days. According to the results, acrylic resins containing 5% of C-phycocyanin were able to inhibit the growth of *S. mutans*, *L. acidophilus* and *C. albicans* more than other groups up to 30 days of study on MHA.

### Table 2. The diameter of growth zone with acrylic discs containing different concentrations of C-phycocyanin studied using DAD test

| Strains   | C-phycocyanin concentrations (%) | Days | 1    | 15   | 30   | 60   | 90   | 1    | 15   | 30   | 60   | 90   | 1    | 15   | 30   | 60   | 90   |
|-----------|----------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|           |                                  |      | 1    |      |      |      |      | 2    |      |      |      |      | 5    |      |      |      |      |
| S. mutans |                                  |      | 7    | 7    | 6    | 6    | 5    | 10   | 10   | 10   | 9    | 6    | 12   | 12   | 11   | 10   | 6    |
| L. acidophilus |                    |      | 7    | 7    | 7    | 6    | 6    | 10   | 10   | 10   | 9    | 7    | 12   | 12   | 11   | 10   | 7    |
| C. albicans |                              |      | 5    | 5    | 5    | 5    | 4    | 9    | 9    | 8    | 6    | 5    | 11   | 11   | 11   | 11   | 5    |

### Antimicrobial effect of acrylic discs containing selected C-phycocyanin on inhibition of microbial biofilm formation

The anti-biofilm effects created by C-phycocyanin in acrylic discs were measured at 1, 15, 30, 60 and 90 days. According to the results (Table 4), no microbial biofilm was formed on any acrylic disc at concentrations of 1%, 2%, and 5% of C-phycocyanin by the 30th day of the study. Microbial biofilm from *S. mutans* and *L. acidophilus* strains were formed on the acrylic discs at 1% concentration on days 60

### Table 3. Microbial biofilm formed by the studied microorganisms on acrylic discs containing different concentrations of C-phycocyanin

| Strains   | C-phycocyanin concentrations (%) | Days | 1    | 15   | 30   | 60   | 90   | 1    | 15   | 30   | 60   | 90   | 1    | 15   | 30   | 60   | 90   |
|-----------|----------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|           |                                  |      | 1    |      |      |      |      | 2    |      |      |      |      | 5    |      |      |      |      |
|           |                                  |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| S. mutans |                                  |      | NG   | NG   | NG   | 1.18±1.4 | 2.35±1.4 | NG   | NG   | NG   | 1.89±1.1 | NG   | NG   | NG   | 6.88±3.5 |
| L. acidophilus |                              |      | NG   | NG   | NG   | 1.04±1.3 | 2.08±0.8 | NG   | NG   | NG   | 1.45±1.4 | NG   | NG   | NG   | 6.54±4.2 |
| C. albicans |                                |      | NG   | NG   | NG   | 2.41±0.6 | 3.12±1.2 | NG   | NG   | NG   | 1.16±1.6 | 2.13±1.8 | NG   | NG   | NG   | 6.23±4.1 |

### Table 4. The primer sequences in this study

| Genes      | 5’ to 3’                       | Primer 1 | Primer 2 |
|------------|--------------------------------|----------|----------|
| ALS9       | F                              | CCATATTCCAGAAAACCAAGCTTC | R       | AACTGAAGCTGTGGAGTTG |
| 28S rRNA   | F                              | TCGACGATCGTAGTTTGGAGTT | R       | AGCCCTTCCCTTCAAATT |
| hsp16      | F                              | CGTGAGCCTAAGAAAGAAGGAGAG | R       | TTGTTGTAAGTTGAGTTG |
| ldhD       | F                              | GTGGTGTGTATGTATTTTGGAGAA | R       | GGCTGGAAGTGTTGAC |
| gtfB       | F                              | GTTGGTACCTGCTAACATTTTGAGAGA | R       | GCCTAGAAAGTGTTGAC |
| I6S rRNA   | F                              | GCAGAGGGGAGGAGAAGAAGAAGA | R       | GGCTAACACCTAGAAGC |

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and 90 and on acrylic discs at 2% concentration of C-phycocyanin on day 60. However, C. albicans was able to form microbial biofilm on day 60 of study on 1, 2 and 5% acrylic discs and on day 90 of study on acrylic discs containing 5% concentration of C-phycocyanin.

**Anti-biofilm effects of acrylic discs containing selected C-phycocyanin on the biofilm-associated gene expression levels**

5% concentration of C-phycocyanin reduced significantly the expression of gtfB, hsp16, and ALS9 genes when compared to the control group. As mentioned before in the results of this study, Fig. 5 shows that the expressions of gtfB in S. mutans, hsp16 in L. acidophilus, and ALS9 in C. albicans were decreased to approximately 6.1-, 7.3-, and 3.9-fold, respectively. As the results reveal, the gene expression profiling was reduced in L. acidophilus cells with the greatest reduction, which was approximately 1.2- and 3.4-fold higher than gtfB and ALS9 in S. mutans and C. albicans, respectively. According to the results, the level of hsp16 gene expression compared with ALS9 was significantly different (p<0.05), while there was no remarkable difference in gene expression of hsp16 compared with gtfB (p>0.05).

![Figure 5](image)

**DISCUSSION**

Acrylic resins are widely used in orthodontic plates. However, the incidence of stress fractures, which are usually the middle part of the orthodontic plaque, mainly due to the low mechanical strength of acrylic resins, has long been a clinical problem. In recent years, much attention has been paid to the use of antimicrobial compounds in acrylic. Results of studies on adding nanoparticles such as Ag to acrylic suggested that these nanoparticles increase the flexural strength of Selecta Plus acrylic, but excessive addition decreases the flexural strength. In contrast, by adding Ag nanoparticles to Rapid acrylic, the flexural strength initially decreases but again increases to the initial flexural strength. Therefore, the effect of adding Ag nanoparticles on the flexural strength of acrylic depends on the type of acrylic and the percentage of the addition of nanoparticles. Another study has shown that adding TiO2 and/or SiO2 nanoparticles to the acrylic resins reduce the flexural strength and is directly related to the dose of the nanoparticles. The difference in flexural strength between acrylic containing TiO2 and SiO2 nanoparticles was not statistically significant. However, acrylic resin with TiO2 exhibits less strength than SiO2.

To the best of our knowledge, the effects of adding natural products as antimicrobial compounds to acrylic resins have not been studied so far. Therefore, in this study, antimicrobial, anti-biofilm, and physico-mechanical characteristics of orthodontic adhesive containing C-phycocyanin were investigated.

Flexural strength test was performed to investigate the effects of adding the C-phycocyanin at different concentrations of 1%, 2%, 5%, 7.5%, and 10% to the orthodontic adhesive on the mechanical properties of acrylic resins used in orthodontics. The results of our study showed that adding 1%, 2%, and 5% of C-phycocyanin showed no significant decrease (p<0.05) in flexural strength compared to C-phycocyanin-free acrylic, while adding 7.5% and 10% of C-phycocyanin significantly decreased flexural strength (p<0.05).

In addition to investigating the mechanical properties, the antimicrobial activities of acrylic resin in combination with C-phycocyanin against S. mutans, L. acidophilus, and C. albicans were also evaluated. The reason for the selection of these microorganisms is due to their primary role in biofilm formation, caries, sometimes gingivitis, and prosthetic stomatitis in patients using acrylic devices. These microorganisms are abundant on the teeth and mucous surfaces of the oral cavity. Many strains are highly acidogenic and some are acid resistant. This group begins to grow and implant immediately after the first teeth appear in the children’s oral cavity and is found in abundance in dental plaque injuries and their plaque content increases with dietary sucrose intake. It has been found to play an important role in the development of dental and oral diseases. In the present study, the results of the DAD test showed the development of a growth inhibition zone, which means that C-phycocyanin was able to be transported directly to the adjacent acrylic regions by diffusion into the agar medium. Therefore, the direct release of C-phycocyanin can lead to antimicrobial activity from areas where there is direct contact between the acrylic plaque and the tooth. In this study, the maximum mean diameter of growth inhibition zone against all three microorganisms (S. mutans, L. acidophilus,
and *C. albicans*) was observed around discs containing 5% concentration of C-phycocyanin. The results of the DAD test in this study are in contrast to the results reported in a study by Sodagar et al.\textsuperscript{25} which investigated the antimicrobial properties of curcumin nanoparticles in orthodontic composite - the researchers observed no growth inhibition zone in the DAD test. In another study\textsuperscript{26} the growth inhibition zone was not displayed by adding 5% chitosan/zinc oxide nanoparticles, and growth inhibition zone was observed only by adding 10% concentration of these nanoparticles. The results of the DAD test in this study are in line with the results reported in a study by Gligorijević et al.\textsuperscript{30} which evaluated the antimicrobial activity of Ag nanoparticles on triplex acrylic self-cure. The researchers observed a growth inhibition zone in DAD test against *Staphylococcus aureus* at all concentrations of 2%, 5%, 10%, and 100%.\textsuperscript{30}

In the current study, the inhibitory effect of microbial biofilm formation was also investigated. Biofilms have been shown to play a major role in causing dental caries and periodontal diseases.\textsuperscript{28} Biofilms are defined as the concentrated accumulation of interconnected or surface-bound microorganisms encapsulated in self-produced extracellular polymeric material (EPS).\textsuperscript{25} The importance of biofilm inhibition testing is that it demonstrated that bacteria in the form of biofilms are four times more resistant to antimicrobial compounds than planktonic form at a similar density.\textsuperscript{31} Therefore, the bacteria in the form of biofilm on the tooth create a much more resistant microbial accumulation that can stimulate the onset of decay and accelerate its development. In the present study, inhibition testing of microbial biofilm formation on discs containing different concentrations of C-phycocyanin over 90 days of the study showed that the microbial biofilm formation ability of each microorganism was significantly increased by the addition of C-phycocyanin to acrylic resins, so that no biofilms were observed at 30 days on any acrylic disc containing 1%, 2%, and 5% concentrations of C-phycocyanin. But over time, microbial biofilm from *S. mutans* and *L. acidophilus* strains were prepared on acrylic discs at 1% concentration at 60 and 90 days and on acrylic discs at 2% concentration of C-phycocyanin at 60 days. However, *C. albicans* was able to form microbial biofilms on 60 days of study on acrylic discs containing 1%, 2%, and 5% concentration and at 90 days of study on acrylic discs containing 5% concentration of C-phycocyanin. The regular process of microbial biofilm reduction with increasing concentration of antimicrobial agents does not apply to the results of Sodagar et al.\textsuperscript{25} There was no significant inhibition of biofilm formation in their study by adding nano-curcumin particles to the composite with increasing concentrations from 1% to 2% and 10%.\textsuperscript{25} In a study by Marra et al.\textsuperscript{32}, the microbial biofilms of *S. mutans* and *S. aureus* were systematically attenuated with increasing concentration of poly (2-tert-butylaminoethyl) methacrylate. In this study, the formed microbial biofilms by *S. mutans* and *L. acidophilus* were inhibited for up to 90 days and *C. albicans* for up to 60 days with increasing concentration of C-phycocyanin. Additionally, as revealed by the results of this study, the expression of *gtfB*, *hsp16*, and ALS9 genes was downregulated (6.1-, 7.3-, and 3.9-fold, respectively) when exposed to 5% C-phycocyanin. Collectively, this observation clearly reveals that 5% concentration of C-phycocyanin is able to reduce the biofilm formation ability of *S. mutans*, *L. acidophilus*, and *C. albicans* strains.

**CONCLUSIONS**

Based on the results of flexural strength and antimicrobial and anti-biofilm activities of acrylic resin containing various concentrations of C-phycocyanin against *S. mutans*, *L. acidophilus*, and *C. albicans*, it can be concluded that the most acceptable concentration of C-phycocyanin is 5% in acrylic resins used in orthodontics.

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**Conflicts of Interest**

There are no conflicts of interest.

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Антимикробные свойства, антивирулентная активность и физико-механические характеристики ортодонтического клея, содержащего С-фіксоцианин: перспективное применение натуральных продуктов

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Антибиотико-резистентные ортодонтические адгезивы направлены на уменьшение деминерализации эмали, белих пятен и начального разрушения зубов вокруг несъёмных ортодонтических скоб, но они не должны ухудшать его механические свойства.

Цель: Оценить антимикробные и физико-механические свойства акрила, содержащего различные концентрации C-фіксоцианин, в отношении Streptococcus mutans, Lactobacillus acidophilus и Candida albicans.

Материалы и методы: Механические свойства акриловых смол были измерены с помощью испытания на прочность на изгиб после приготовления образцов акриловой смолы с концентрациями 1%, 2%, 5%, 7.5% и 10% C-фіксоцианин. Затем мы оценили антимикробный эффект акриловой смолы, содержащей максимальную концентрацию фіксоцианин, с клинически приемлемой прочностью на изгиб и изменениями в экспрессии фактора вирулентности.

Результаты: Самые высокие и самые низкие значения прочности на изгиб были достигнуты с акриловыми смолями, содержащими концентрации C-фіксоцианин 0% и 10% при 50.2±4.5 и 30.1±3.3 МПа, соответственно. Добавление 1%, 2% и 5% C-фіксоцианин не показало значительного снижения прочности на изгиб (p>0.05). Максимальный средний диаметр зоны задержки роста наблюдался вокруг дисков, содержащих 5% C-фіксоцианин. К 30 дню исследования микробные биоплёнки не образовывались ни на одном акриловом диске. Только микробные биоплёнки из C. albicans образовывались на дисках, содержащих 5% C-фіксоцианин, на 90-й день. 5% C-фіксоцианин может значительно снизить уровни экспрессии gtfB, hsp16 и ALS9 в 6.1-, 7.3- и 3.9-раза соответственно.

Заключение: Можно сделать вывод, что наиболее приемлемая концентрация C-фіксоцианин в акриловой смоле составляет 5% на основании результатов исследования прочности на изгиб и антимикробной активности акриловой смолы, содержащей различные концентрации C-фіксоцианин.

Ключевые слова
акриловые смолы, антимикробная активность, C-фіксоцианин, прочность на изгиб, экспрессия генов