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Red dragon fruit extract as a natural disclosing agent with antioxidant, antibacterial, and antibiofilm properties against *Streptococcus mutans*

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Dental caries is a widespread health concern driven by poor oral hygiene and biofilm-forming bacteria *Streptococcus mutans*. While chemical disclosing agents effectively visualize dental plaque, their potential risks, such as mucosal irritation, highlight the need for safer, natural alternatives. Red dragon fruit (*Hylocereus polyrhizus*) contains bioactive compounds (betacyanin and phyllocactin) with antioxidant, antibacterial, and antibiofilm properties, making it a promising candidate. This study employed molecular docking, *in vitro* assays, and formulation development to evaluate its potential as a natural disclosing agent. Docking simulations assessed the binding of betanin and phyllocactin to *S. mutans* biofilm-associated proteins (SrtA and GbpC). The extract was prepared using 70% ethanol through maceration. Antioxidant activity was measured using the DPPH assay; antibacterial and antibiofilm effects were evaluated through disc diffusion, MIC, MBC, and biofilm inhibition tests. Molecular docking showed strong binding affinities (–8.8 to –12.2 kcal/mol). The extract demonstrated antioxidant activity (IC_{50}: 158.43 µg/mL), antibacterial activity at 1000 µg/mL with a 5.43 mm inhibition zone, and antibiofilm activity up to 91.57%. The chewable jelly formulation exhibited suitable physical characteristics, though limited colour intensity remains a key challenge. Red dragon fruit extract showed potential as a natural disclosing agent for caries prevention in children, supported by its bioactive properties and docking results. Further studies are recommended to enhance colour intensity and validate its efficacy through *in vivo* applications.

Keywords: Antibacterial, Antibiofilm, Antioxidant, Dental caries, Disclosing agent, Red dragon fruit, *Streptococcus mutans*

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Introduction

Tooth decay, or dental caries, remains a widespread chronic condition and a major global public health concern. The World Health Organization (WHO) reports that it impacts between 60% and 90% of school-aged children, as well as the majority of adults. The dental caries prevalence is elevating, especially in certain populations, due to factors such as poor eating habits, lack of oral hygiene, and limited access to dental care[1]. In Indonesia alone, a specific study conducted in East Jakarta found that around 71% 12-year-old school children had experienced caries incidence, on average, shows Decayed-Missing-Filled Teeth (DMFT) index of 2.278[2].

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Streptococcus mutans has long been identified as the main bacterium contributed in biofilm formation and the dental caries process. This bacterium metabolizes carbohydrates to produce acids and forms biofilms that adhere firmly to tooth surfaces. The acid produced causes enamel demineralization, thus increasing the risk of caries[3]. In addition, *S. mutans* interacts synergistically with other oral pathogens, further exacerbating the cariogenic process. Various studies have demonstrated that *S. mutans* biofilms are resistant to mechanical removal and host immune defences, making them a persistent source of infection unless adequately managed through preventive measures. Therefore, preventive measures through plaque detection and biofilm reduction are essential in controlling caries progression[4].

Biofilm formation in *S. mutans* is mediated by several virulence factors, notably Sortase A (SrtA) and Glucan-Binding Protein C (GbpC). SrtA is a membrane-anchored transpeptidase that attaches surface proteins, including GbpC, to the cell wall via the LPXTG motif[5]. GbpC contributes to biofilm stability by mediating binding to glucans and enhancing adherence to the tooth surface[6-7]. The interaction of these proteins facilitates initial colonization and biofilm maturation, increasing the cariogenic potential of *S. mutans*[8]. Targeting these proteins offers a promising approach for preventing dental plaque and caries.

Using toothpaste can reduce plaque formation, clean the tooth surface, provide a refreshing flavour, and eliminate bad breath. However, there is a lack of public awareness in controlling plaque, which can cause caries. Therefore, early detection of dental plaque using disclosing solution is essential to optimize plaque control and prevent caries. In modern dental practice, disclosing agents are commonly used visual aids to detect the presence of plaque on the tooth surface[9]. Disclosing agents allow dentists and patients to visually identify areas where plaque accumulates, enabling early intervention before conditions like dental caries[10]. However, currently available commercial disclosing agents generally contain synthetic chemicals such as erythrosine and basic fuchsin that can cause various side effects such as skin irritation, respiratory problems, thyroid dysfunction, carcinogenicity, and toxicity[11-12].

Plant-derived disclosing agents may serve as an effective alternative. Red dragon fruit (*Hylocereus polyrhizus*) is recognized for having betacyanin compounds which makes it a potential candidate as a disclosing agent. Betacyanin from red dragon fruit has potential as a natural colourant and a powerful antioxidant that can protect tissues from free radical damage and reduce oxidative stress that causes inflammation, and is often associated with various oral diseases[13]. In addition, studies show that red dragon fruit extract has antibacterial and antibiofilm properties that are effective against *S. mutans*, thus providing the potential to prevent biofilm formation and plaque development on teeth[14-15]. Betanin, the prominent betacyanin, is known for its capability to scavenge free radicals and has also been shown to improve lipid profiles and exhibit anti-inflammatory effects[11]. Phyllocactin, another bioactive compound present in red dragon fruit, has been associated with significant antioxidant activity that contributes to the fruit's potential as a natural preservative and health supplement[15].

Several studies have demonstrated the antibacterial, antioxidant, and antibiofilm properties of red dragon fruit extract. However, research focusing on its use as a plaque disclosing agent remains limited. Furthermore, no studies have evaluated incorporating this extract into a child-friendly, chewable jelly that can visualize plaque and deliver therapeutic benefits. We hypothesized red dragon fruit extract will exhibit notable antibacterial and antibiofilm activity while serving as an effective plaque disclosing agent. This study is expected to provide an alternative disclosing agent based on natural ingredients that is safe, acceptable to children, and effective in preventing dental caries.

Materials and Methods

Molecular docking

A molecular docking study was conducted on two bioactive compounds from red dragon fruit extract—betanin and phyllocactin. The compounds were identified through the PubChem database. The target proteins, Sortase A (SrtA) and Glucan-Binding Protein C (GbpC), were retrieved from the RCSB Protein Data Bank. Afterwards, the proteins were prepared using AutoDock Tools, in which water molecules were eliminated and polar hydrogens were added. This resulted in the creation of AutoDock Vina-compatible PDBQT files. Docking simulations were performed in AutoDock Vina, based on the gridbox references in previous SrtA[16] and GbpC[17] studies. An exhaustiveness parameter of 16 and the number of output binding modes set to 5 were used in the docking simulation. For GbpC, the center of the grid box was positioned at x = 240.549, y = –25.160, and z = 8.572, with a grid box size of 26.00 × 18.00 × 23.25 Å. For SrtA, the center point of the grid was configured at x = –37.000, y = –18.200, and z = 4.300, with the same grid box size. The molecular docking results were then visualised using Discovery Studio to display the binding interactions, including hydrogen bonds and hydrophobic contacts.

Red dragon fruit extraction

Maceration was used as the extraction method. The red dragon fruit (*Hylocereus polyrhizus*) extract powder was obtained from PT. Fathonah Amanah Shidiq Tabligh (PT. FAST), Indonesia. The extraction

process involved macerating the powder in 70% ethanol. After the first 24 hours, the liquid extract (filtrate) was collected, and fresh 70% ethanol was added to the remaining material (residue) for another 24 hours. The filtered extract was then mixed with lactose to form a powder. This extraction process continued until the filtrate became colourless and the solvent had fully evaporated[18].

DPPH scavenging activity

The test was conducted by first adding 50 µL of the sample into a well of a 96-well plate, followed by 200 µL of 0.077 mmol DPPH solution. A separate blank well was filled with 250 µL of 10% DMSO, while another well, used as a control, contained 250 µL of 0.077 mmol DPPH. The samples were incubated for 30 minutes in the dark at room temperature, followed by absorbance measurements at 517 nm. The percentage of free radical scavenging was calculated using the appropriate formula[19]:

$$\text{Scavenging activity (\%)} = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \times 100$$

Antimicrobial assay by disc diffusion methods

Disc diffusion was used to assess antimicrobial effects. The process began by using a sterile cotton swab to spread bacterial suspensions of *S. mutans* onto Mueller-Hinton Agar plates. The plates were then left to settle at room temperature for 30 minutes. Paper discs (6 mm in diameter) were infused with either the test sample, positive control, or negative control solutions and placed on the agar surface. The plates were incubated for 24 hours at 37°C aerobically. The inhibition zones around each disc were measured using a vernier caliper, and the average was calculated from three measurements[20,21].

Antimicrobial assay by MIC and MBC

The antimicrobial test began by determining the minimum inhibitory concentration (MIC) level utilizing the broth microdilution strategy. For MIC and minimum bactericidal concentration (MBC) levels, each bacterial inoculum was prepared using the standard colony suspension method. Inoculum was obtained by transferring colonies of *S. mutans* that had been incubated for 24 hours on MHA media into Mueller-Hinton Broth (MHB) (Himedia, M391). Freshly cultured colonies were used to ensure bacterial viability before preparing the inoculum. Turbidity was adjusted to the McFarland 0.5 standard, corresponding to approximately 1.5×10^8 CFU/mL. MIC values were determined using 96-well plates containing 100 µL of red dragon fruit extract (RDFE) at concentrations of 1000, 750, 500, 250, 125, 62.5, and 31.25 µg/mL, supplemented with 100 µL of each microbe. A positive control was used 0.2% chlorhexidine, whereas the negative control was 10% DMSO. The plate was put in a incubator and kept at 37°C for 24 hours. Using spectrophotometry, we evaluated the turbidity of the plates following the incubation period (Multiskan GO Thermo Scientific 51119300) in the wavelength range 500~600 nm. After measuring the uptake, we determined the MBC level by taking 100 µL and performing graded dilutions ranging from 10^2 to 10^5 in each MIC result well. Exactly 50 µL of the aqueous solution was cultured using the pour-plate technique on MHA agar. We incubated the plates at 37°C for 24 hours. The next day, we used a Funke Gerber 8500 colony counter to assess the number of bacterial colonies. Least bactericidal concentration (MBC) is characterized as the extract concentration that produces an inhibitory impact of 99%[21].

Biofilm assay

Biofilm assay was initiated by culturing bacteria in BHIB medium for 24 hours at 37°C until it reaches McFarland 0.5 standard turbidity (1.5×10^8 CFU/mL). About 200 µL bacterial suspension was administered to a 96-well microplate then incubated for biofilm formation at 37°C for 24 hours. After incubation, 100 µL of RDFE treatment (1000-31.25 µg/mL), negative control (DMSO 10%), and positive control (chlorhexidine 0.2%) were added and incubated at 37°C for 24 hours, then supernatant from the wells was discarded. The wells were washed thrice with sterile PBS to remove planktonic cells, then fixed for 15 minutes with absolute methanol and dried. Staining for 5 min was performed with 0.1% crystal violet, then washed with water, and dried. Finally, 33% glacial acetic acid 200 µL was administered to dissolve the dye, and the biofilm was determined for OD at 595 nm with a microplate reader[21].

Chewy jelly preparation

Extraction of red dragon fruit was carried out via maceration with 96% ethanol as the solvent. The liquid phase was evaporated under reduced pressure using a rotary evaporator, yielding a thickened

extract. Each necessary ingredient were prepared according to the formula to be made. Aquadest was heated to a boil, gelatin is added, and allowed to stand for about 15 minutes until the gelatin expands (mixture 1). Gelatin was chosen as the main gelling agent because it can create a soft, elastic texture that is perfect for children and works well with acidic ingredients. Its clear base enhances the visual appearance of colour, an important quality for a disclosing agent. Then glycerin was heated to almost boiling (mixture 2). Mixture 2 was added to mixture 1 and heated using a water bath for 45 minutes at 40°C. Then added sucralose, mannitol, citric acid, methylparaben, BHT, and heated on medium heat. Add the active substance in the form of extract and stir until homogeneous (mixture 3). Mixture 3 was removed from heat, sprinkled with lemon oil, and allowed to stand for 5 minutes before being poured into the mold. Mixture 3 was poured into the mold and stored for 24 hours at room temperature until it formed a stable gummy mass[22].

Moisture and Ash content assay

Ash content was analyzed by placing 2–3 g of the sample into a pre-weighed silicate crucible and gradually heated until all carbon was removed. After cooling, the sample was weighed. If carbon remained, the sample was mixed with hot water and subsequently passed through ash-free filter paper. The residue and filter paper were then returned to the same crucible, while the filtrate was added back to the crucible, evaporated, and heated until a constant weight was achieved. The percentage of ash (w/w) was determined based on the initial weight of the sample[23].

The moisture content measurement began by activating the moisture balance system. First, a 3 g extract sample was prepared. An aluminum plate was placed in the meter and zeroed by pressing the tare button. The sample was then transferred onto the aluminum plate. Upon pressing the start button, then the drying process began. The system automatically heated the sample until all moisture was removed, signaled by a beep and "test over" display. The final reading showed the water content as a percentage[23].

Texture profile analysis

A texture analyzer (Brookfield CT3) with a 50-kg load cell was used to analyze the texture of jelly cubes. With two compression cycles, jelly cubes were compressed to 40% of their initial height using a 5 cm diameter probe. The compression speed was maintained at 0.5 mm/s, with a 5-second interval between successive compressions. All measurements were performed at ambient room temperature. Key texture attributes were obtained by analyzing the resulting force-time curve, including hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness[24].

Physical stability test

Organoleptic test and weight uniformity test

Organoleptic tests evaluated the shape, aroma, taste, and colour of each gummy formulation. The colour appeared lighter than expected, which may indicate limited retention of red pigmentation from the dragon fruit extract. This outcome is likely due to pigment degradation during the heating process or interactions with other formulation components. A weight uniformity test was carried out by weighing 20 gummies one by one randomly from each formula made.

Homogeneity test

Two transparent glass objects were used under light to assess the homogeneity of a chewy jelly preparation. One glass slide was evenly coated with the chewy jelly sample, then a second glass slide was placed on top of it, aligning the two slides. A homogeneous chewy jelly formulation should not exhibit any visible coarse or clumped particles when viewed between the two glass slides[25].

pH test

The pH of the chewy jelly preparation was tested using a calibrated pH meter. The standard neutral (pH 7.00) and acidic (pH 4.00) buffer solutions were used to calibrate the pH meter. Electrodes were rinsed appropriately using distilled water then dried between measurements. The pH of the chewy jelly preparation was measured in real time until the readings stabilized at a constant value. The pH measurement was conducted in triplicate, and the average value was calculated[25].

Viscosity test

The viscosity of the chewy jelly preparation was measured using a Brookfield viscometer. The viscometer spindle was immersed in the chewy jelly sample placed in a glass beaker, and the speed was set

to 50 rpm. The viscosity value of the chewy jelly was monitored until a stable reading was displayed[25].

Statistical analysis

Statistical analysis was conducted with SPSS software version 20.0. One-way ANOVA then Tukey's HSD post hoc test was applied for data that showed normal distribution and homogeneity. Kruskal-Wallis and Mann-Whitney tests were used for data that did not meet these assumptions (abnormal data). Results with p-values < 0.05 was considered statistically significant.

Results

Molecular docking

Molecular docking analysis demonstrated that betanin and phyllocactin successfully interact with GbpC (Fig. 1) and SrtA (Fig. 2), as shown in Table 1. Betanin exhibited the highest binding affinity with GbpC (-12.2 kcal/mol), forming three hydrogen bonds and one hydrophobic interaction. Phyllocactin exhibited a lower binding affinity (-8.8 kcal/mol) with one hydrogen bond and two hydrophobic interactions. For SrtA, betanin demonstrated binding affinity of -9.5 kcal/mol with three hydrogen bonds and one hydrophobic interaction. In comparison, phyllocactin showed a slightly stronger binding affinity (-9.8 kcal/mol) with three hydrogen bonds. These results indicate that betanin and phyllocactin have strong potential as inhibitors of biofilm formation through their interactions with biofilm-associated proteins.

DPPH antioxidant activity of red dragon fruit extract

The DPPH free radical scavenging activity of red dragon fruit extract percentage as presented in Fig. 3. The results indicated that free radical scavenging activity enhanced proportionally with increasing sample concentrations. The 200 µg/mL concentration had the highest DPPH free radical scavenging activity in red dragon fruit extract, with a significant value (57.73±0.57 µg/mL). The red dragon fruit

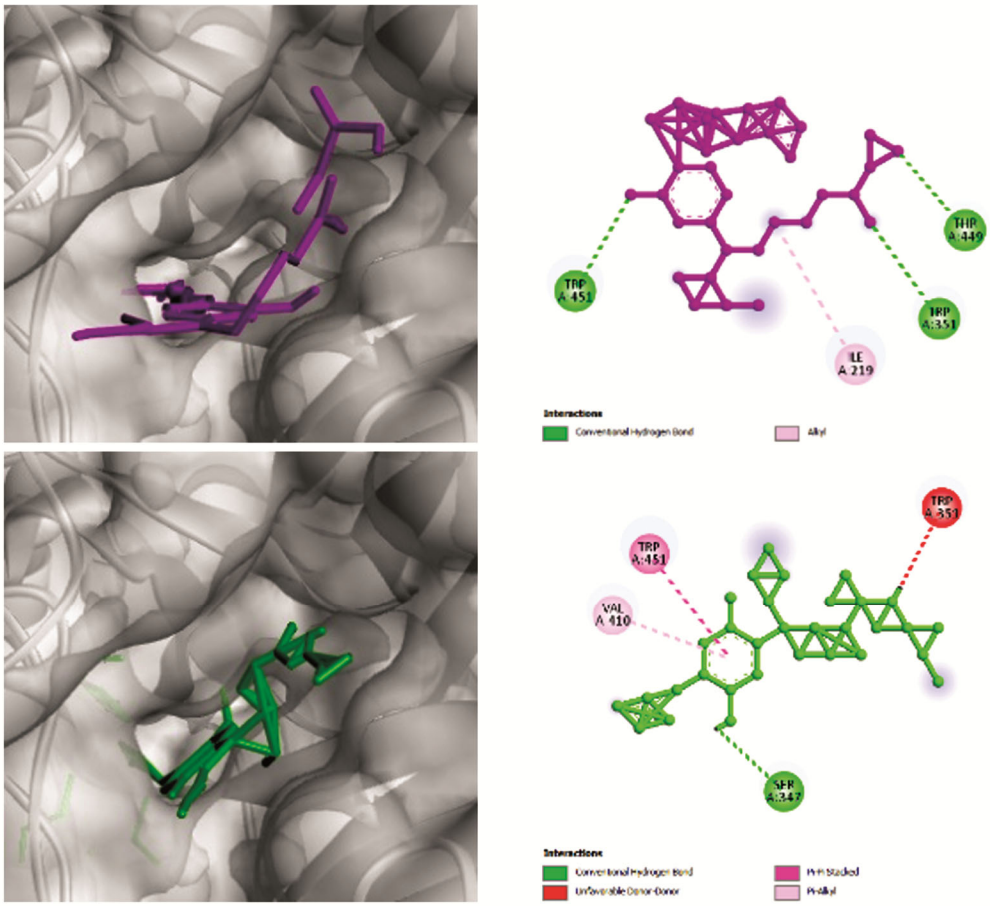

Fig. 1 — Molecular docking interactions of betanin (pink) and phyllocactin (green) with the GbpC protein. (Left) 3D visualization of the protein-ligand binding. (Right) 2D interaction diagram showing hydrogen bonds (green) and hydrophobic interactions (pink).

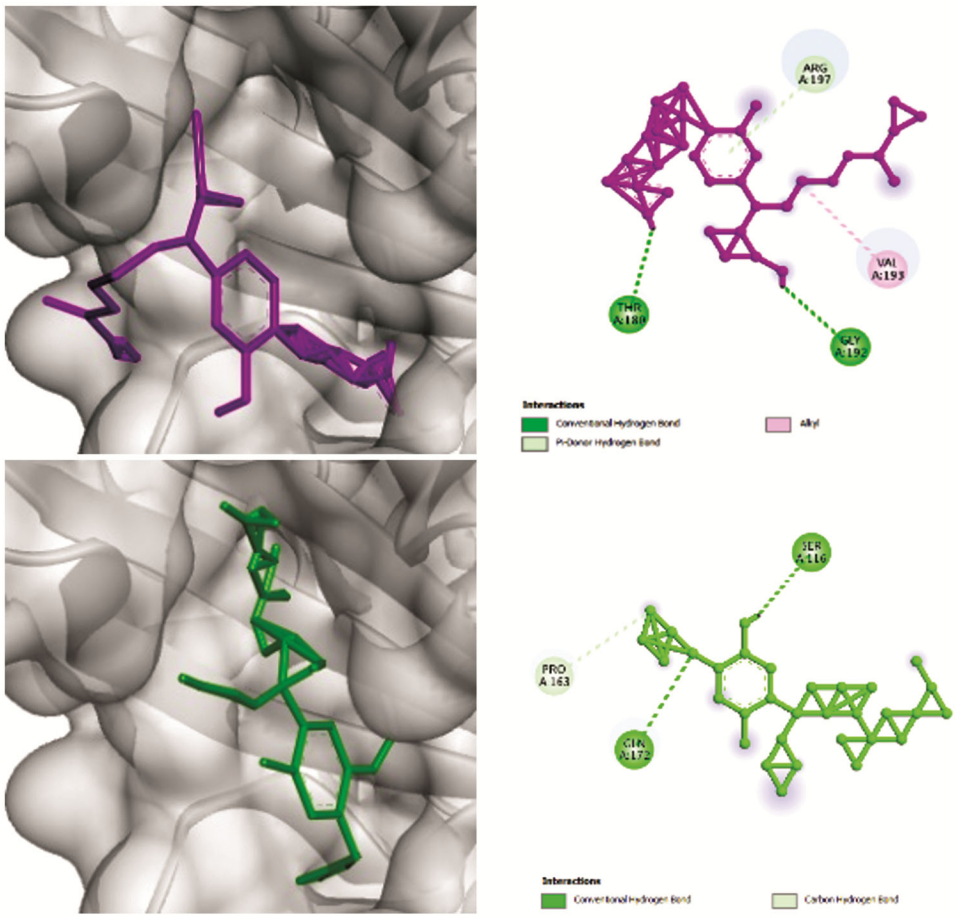

Fig. 2 — Molecular docking interactions of betanin (pink) and phyllocactin (green) with the SrtA protein. (Left) 3D visualization of the protein-ligand binding. (Right) 2D interaction diagram showing hydrogen bonds (green) and hydrophobic interactions (pink).

Table 1 — Binding affinity and amino acid interactions of betanin and phyllocactin with GbpC and SrtA proteins

Protein	Compound	BA (kcal/mol)	Hydrogen bonds	Hydrophobic interaction
GbpC	Betanin	-12.2	TRP351, THR449, TRP451	ILE219
	Phyllocactin	-8.8	SER347	TRP451, VAL410
SrtA	Betanin	-9.5	THR180, GLY192, ARG197	VAL193
	Phyllocactin	-9.8	GLN172, SER116, PRO163	

extract Inhibitory Concentration (IC_{50}) value is 158.43±3.67 µg/mL. This value is included in the weak category, but it can show that red dragon fruit has the potential as an antioxidant[26].

Antibacterial activity of red dragon fruit extract by the disc diffusion method

In antibacterial testing employing the disc diffusion method, it is known that RDFE has antimicrobial activity against *S. mutans* bacteria characterized by the presence of an inhibition zone or clear zone formed (Fig. 4). RDFE at 200 µg/mL (Treatment IX) showed the greatest antimicrobial activity among the extract groups, with an inhibition zone diameter of 5.43 mm, whereas RDFE at 25 µg/mL (Treatment VI) produced the smallest inhibition zone (1.14 mm). Statistical analysis indicated that the inhibition zone of Treatment VI was significantly different from that of the positive control (0.2% chlorhexidine) ($p < 0.05$).

Antibacterial activity by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods

The percentage of growth (viability) and inhibition of *S. mutans* bacteria with treatment using RDFE as

presented in Fig. 5. The minimum inhibitory level (MIC) was in treatment VII (RDFE 250 µg/mL) with a percentage of 60.36%. While the MBC is owned by treatment X (RDFE 1000 µg/mL), with a percentage value of 92.34%. RDFE treatment showed a concentration-dependent inhibitory effect on the growth of *S. mutans* bacteria are directly proportional to the concentration level. The higher the concentration given, the lower the growth activity of *S. mutans* and the higher the inhibition. This is in line with the results of the calculation of the number of colonies contained in Table 2.

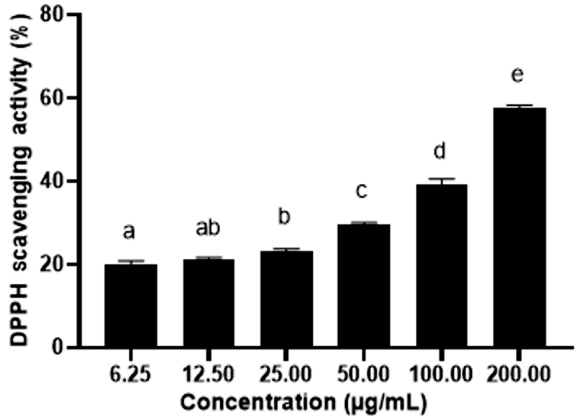

Fig. 3 — Effect of various concentrations of red dragon fruit extract on DPPH free radical scavenging activity.

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in three repetitions. Different superscripts (a,ab,b,c,d,e) mark significant differences between various concentrations of red dragon fruit extract (P <0.05, Tukey's HSD test). 6.25, 12.50, 25.00, 50.00, 100.00, and 200.00 µg/mL numbers on the x-axis indicate the variation of red dragon fruit extract concentration.

Antibacterial activity of *S. mutans* biofilm inhibition

The biofilm inhibition study in Fig. 6, RDFE has an effect on reducing biofilm. The highest antibiofilm effect was in treatment X (RDFE 1000 µg/mL; 91.57%), with the lowest anti-biofilm effect being treatment IV (RDFE 31.25 µg/mL; 17.60%) against the *S. mutans* bacteria growth. The effect of biofilm inhibition on RDFE exhibits a concentration-dependent effect; the higher the treatment concentration, the higher the biofilm inhibition produced.

Preparation of chewy jelly

Preparation of chewy jelly with the formulation used as in Table 3. Each necessary ingredient were prepared

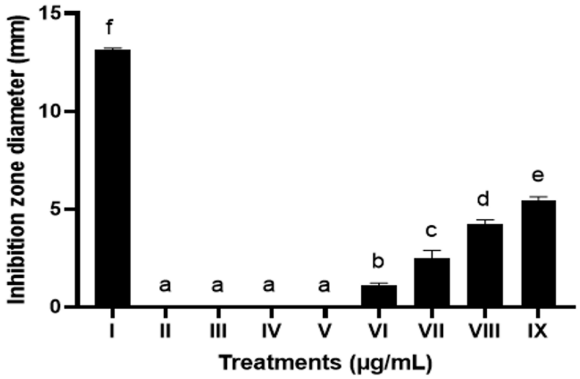

Fig. 4 — Effect of various concentrations of red dragon fruit extract on *S. mutans* bacteria.

*Data are presented as mean ± standard deviation. For each treatment, the test was performed in three repetitions. Different superscripts (a,b,c,d,e,f) mark significant differences between various concentrations of red dragon fruit extract (P <0.05, Tukey's HSD test). Treatment I: growth control, Treatment II: negative control (DMSO), Treatment III: positive control (0.2% chlorhexidine), and Treatments IV–X: RDFE at 6.25, 12.50, 25.00, 50.00, 100.00 and 200.00 µg/mL, respectively.

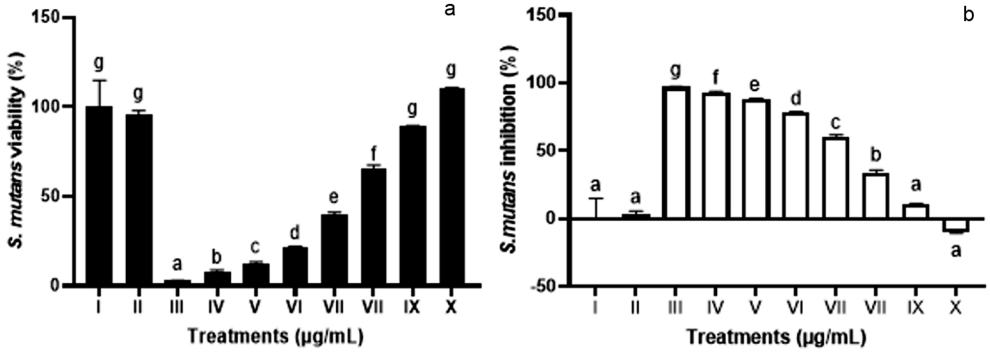

Fig. 5 — Effect of various concentrations of red dragon fruit extract by dilution method. a) Viability of *S. mutans*, and b) Inhibition of *S. mutans*.

*Data are presented as mean ± standard deviation. For each treatment, the test was performed in three repetitions. Different superscripts (a,b,c,d,e,f,g) mark significant differences between various concentrations of red dragon fruit extract (P <0.05, Dunnett T3 test). Roman numerals I–III represent the controls (I: growth control, II: negative control/DMSO, III: positive control/0.2% chlorhexidine), while IV–X indicate RDFE at concentrations of 1000, 750, 500, 250, 125, 62.5, and 31.25 µg/mL.

Table 2 — Colony counts of *S. mutans*

Sample	CFU/mL			Average
	P1	P2	P3	
Growth Control	TNTC	TNTC	TNTC	TNTC
Negative Control	TNTC	TNTC	TNTC	TNTC
Positive Control (Chlorhexidine 0.2%)	0	0	0	0
RDFE 1000 µg/mL	57 x 10^4	56 x 10^4	51 x 10^4	54.67 x 10^4
RDFE 750 µg/mL	65 x 10^4	68 x 10^4	75 x 10^4	69.33 x 10^4
RDFE 500 µg/mL	124 x 10^4	137 x 10^4	129 x 10^4	130.00 x 10^4
RDFE 250 µg/mL	192 x 10^4	163 x 10^4	187 x 10^4	180.67x 10^4
RDFE 125 µg/mL	243 x 10^4	248 x 10^4	201 x 10^4	230.67 x 10^4
RDFE 62.5 µg/mL	TNTC	TNTC	TNTC	TNTC
RDFE 31.25 µg/mL	TNTC	TNTC	TNTC	TNTC

Notes: *P= Repetition; TNTC = Too Numerous Too Count >250

Table 3 — Chewy jelly formulation design

Composition	Unit	Formulation 1	Formulation 2	Formulation 3
Red dragon fruit extract	Gram	0.5	1	2
Glycerin	G	16	20	25
Gelatin	G	7	5	6
Sucralose	G	0.015	0.015	0.015
Mannitol	G	1.5	1.5	1.5
Citric acid	G	0.15	0.15	0.15
BHT	G	0.006	0.006	0.006
Aquades	G	24.829	22.329	15.329
Corn oil	As needed	As needed	As needed	As needed

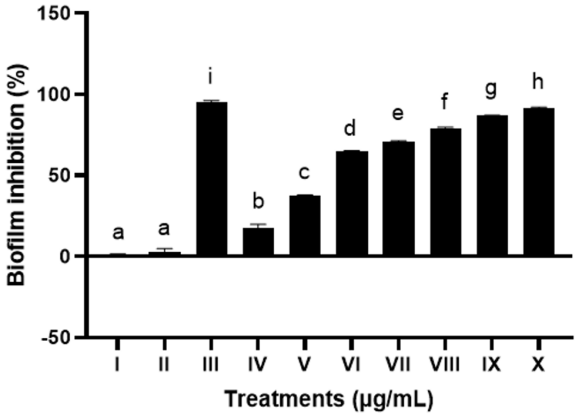

Fig. 6 — Effect of various concentrations of RDFE on biofilm growth of *S. mutans*.

*Data are presented as mean ± standard deviation. For each treatment, the assay was performed in three repetitions. Different superscripts (a,b,c,d,e,f,g,h,i) mark significant differences between various concentrations of RDFE (P <0.05, Independent t-test). Roman numerals I–III represent the controls (I: growth control, II: negative control/DMSO, III: positive control/0.2% chlorhexidine), while IV–X correspond to RDFE at concentrations of 31.25, 62.5, 125, 250, 500, and 1000 µg/mL.

according to the formula. Aquadest was heated to a boil, gelatin was added, and allowed to stand for about 15 minutes until the gelatin expands (mixture 1). Glycerin was heated to almost boiling (mixture 2). Mixture 2 was added to mixture 1 and heated using a water bath at 40°C for 45 minutes. Then, sucralose, mannitol, citric acid, and BHT were added and heated on medium heat. Add the active substance in the form of extract and stir until homogeneous (mixture 3). Mixture 3 was removed from heat, sprinkled with corn oil, and allowed to stand for 5 minutes before being poured into the mold. Mixture was poured on the mold tool and stored for 24 hours at room temperature until it forms a stable chewy jelly mass.

Evaluation of chewy jelly based on organoleptic test

The organoleptic test results were shown in Table 4. Chewy jelly had a consistency that was quite chewy. The aroma category of chewy jelly had a distinctive aroma but no flavour. This can be caused by the very small composition of sucralose. The physical appearance was a yellow colour for formulation I, yellow slightly pink in formulation II, and pink colour in formulation III The colour change occured because the composition of dragon fruit extract used differs.

Table 4 — Organoleptic test results

Organoleptic category	Formula I	Formula II	Formula III
Consistency	Quite chewy	Chewy	Chewy
Shape	Semisolid	Semisolid	Semisolid
Aroma	Dragon fruit aroma	Dragon fruit aroma	Dragon fruit aroma
Taste	Flavorless	Flavorless	Flavorless
Colour	Yellow	Slightly pinkish yellow	Light red

Table 5 — Texture analysis test results

Texture Profile Analyzer	Unit Value	Formula chewy jelly		
		I	II	III
Hardness	gForce	3,157,993	7,756,027	8,795,373
Fracturability	-	Undetected	Undetected	Undetected
Adhesiveness	g.sec	-0,0320	-95,975	-185,963
Springiness	%	38,303	0,9313	0,8730
Cohesiveness	%	0,8337	10,097	0,9950
Gumminess	gForce	1.094,2410	7,833,543	8,750,223
Chewiness	gForce	3.745,2050	7,351,033	7,656,663
Resilience	%	1.076	11,183	11,367

Evaluation of chewy jelly based on moisture and ash content tests

Evaluation of the moisture and ash content of chewy jelly was also carried out. Moisture and ash content were analyzed on all three RDFE-based chewy jelly formulations. The moisture content in formulation I was 1.79%, formulation II had the highest moisture level at 8.11%, and formulation III had the lowest at 1.08%. Moisture content is a crucial factor that affects the quality and freshness of food ingredients. Too high moisture content can cause the product to become moist, accelerate damage, and increase the risk of growth of microorganisms such as fungi and bacteria. Regarding ash content, formulation I recorded 0.10%, formulation II had 0.08%, and formulation III showed the highest ash content at 0.12%. This difference could indicate variations in the content of mineral ingredients or other inorganic components in each formulation. Ash content was analyzed because it can indicate the mineral content in a product. The higher the ash content, the poorer the food quality, as it can indicate contamination or poor-quality raw materials. From the results of the water and ash content tests carried out on all formulations, the water content values obtained are still in the safe category and meet the SNI requirements because they are not more than 20%. Additionaly, the ash content obtained was not more than 3% according to the provisions of the SNI 3547.2-2008 standard for the jelly candy category. Overall, the chewy jelly formula demonstrated potential for further analysis to ensure that chewy jelly products meet the expected quality standards and are safe for consumption.

Evaluation of chewy jelly based on texture analysis test

The texture profile analyzer results in Table 5, Formula I had the highest hardness value (3,157,993 gForce), while Formula II (7,756,027 gForce) had a lower hardness value and tends to be softer. The adhesive characteristic, namely the tendency for particles to stick together in formula I, had a very low adhesivity (-0.0320 g.sec), meaning that it was not too sticky and comfortable when consumed, and formula III was the formulation that had the stickiest adhesivity (-185,963 g.sec). In the Springiness category, or the ability to return to its original form, formula I had the highest springiness value (38,303%) compared to formulas II and III. Cohesion or attachment between particles in formula II had the highest cohesion (10,097%) and lower gumminess value (7,833,543 gForce), compared to formula I and III, that showed higher gumminess and chewiness values, thus making them chewier and requiring more effort to chew.

Overall, to be used as a disclosing agent, Formula II was a more suitable formula, as indicated by its hardness value (7,756,027 gForce), adhesivity (-95,975 g.sec), cohesion (10,097%), and gumminess (7,833,543 gForce) which were lower than formula III making it had a texture that was easily crushed and not too sticky so that it will not interfere with users.

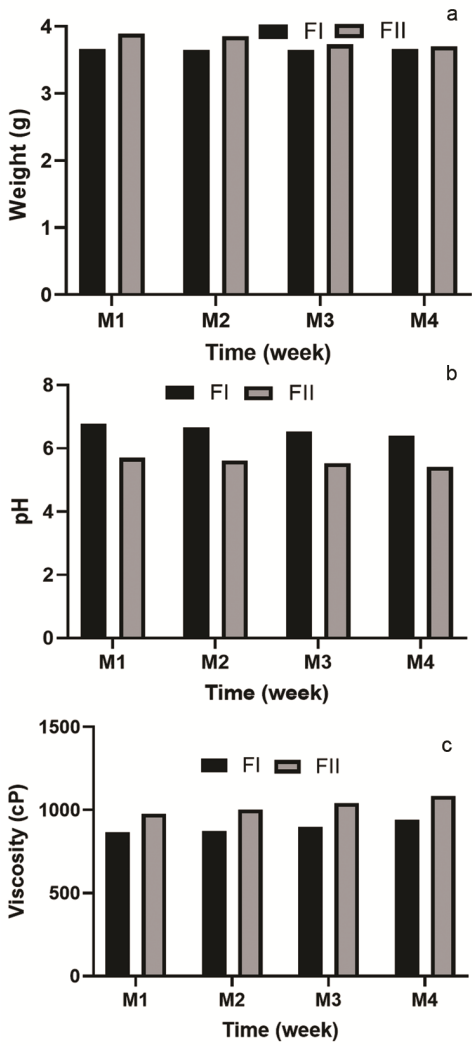

Fig. 7 — Visualization of the chewy jelly characteristics over four weeks of testing: a) weight uniformity, b) pH, and c) viscosity.

Evaluation of chewy jelly based on the physical stability test

Evaluation of the physical stability of chewy jelly was carried out for 4 weeks using formulas I and II based on the results of consideration of moisture content, ash, and characteristics according to TPA. The weight uniformity of the chewy jelly analyzed is shown in Fig. 7a, where formulation II had a weight loss over time. This can be due to the water content, which was higher than that of formula I, resulting in a decrease in the water content. The pH measurement of chewy jelly (Fig. 7b) tends not to be significantly different between formulas I and II. The texture analysis of the RDFE-based chewy jelly formulations included hardness, stickiness, and elasticity measurements. Formulation I exhibited a hardness value of 144.67 gF, stickiness of –6.00 gF, and elasticity of 2.11 mm. Formulation II demonstrated the highest hardness at 270.00 gF, with stickiness of –5.20 gF and elasticity of 2.07 mm.

Meanwhile, formulation III showed a hardness of 145.33 gF, the lowest stickiness at –10.30 gF, and the highest elasticity at 2.63 mm. These results indicated that formulation II had the firmest texture, while formulation III was the most elastic and sticky. While the viscosity results of chewy jelly formula II showed an increase over time, the viscosity, which reached 1000 cP showed the chewiness of chewy jelly (Fig. 7c). The colour of the chewy jelly produced tends not to change during the 4 weeks of testing, which remains yellow and remains homogeneous in the absence of lumps. No microbes were found in chewy jelly.

In this study, because it was expected to be a disclosing agent that was able to provide colour to dental plaque that sticks to the teeth surface, remained a challenge because the fruit of dragon used was a powder extract that was less effective in providing colour. To turn it into a disclosing agent that can colour, further research is needed related to the addition of more effective dyes in accordance with national standards related to the use of dyes in food products, for example, with Erythrosine dye (FD&C Red No. 3), Code E: E127 (SNI 01-2891-1992). Nevertheless, the chewy jelly formulation produced can be an illustration of the manufacture of chewy jelly as a disclosing agent, but only in the future will it need to be improved with more concentrated dyes, for example, by using Erythrosine.

Discussion

Molecular docking analyses of betanin and phyllocactin demonstrated their potential as an inhibitor of biofilm formation through their interactions with GbpC and SrtA, two proteins associated with biofilm development. Among the two compounds, betanin showed consistently stronger binding interactions than phyllocactin. This may be attributed to its ability to form more hydrogen bonds and favourable hydrophobic contacts, which likely contribute to a more stable ligand–protein complex. Differences in binding affinity and the interactions formed are important in the design of biofilm inhibitors, as compounds with lower binding affinity are often more effective in disrupting protein function[27]. Similar findings from other studies emphasise the importance of binding interactions in

the therapeutic efficacy of compounds targeting protein function[28]. Previous docking studies have shown that chlorhexidine, a widely used antibacterial agent in oral care, exhibits a binding affinity of approximately -6.29 kcal/mol against *S. mutans* SrtA[29]. In comparison, betanin and phyllocactin demonstrated stronger binding affinities (–8.8 to –12.2 kcal/mol). The ability of betanin and phyllocactin to effectively interact with key proteins associated with biofilms suggests they may act as biofilm inhibitors.

Red dragon fruit extract (RDFE) shows promising potential as a natural alternative for disclosing agents, attributed to its antioxidant, antibacterial, and antibiofilm properties. The DPPH assay results demonstrated moderate antioxidant activity, with an IC_{50} of 158.43±3.67 µg/mL. Although this falls into a lower efficacy category[26], RDFE's capacity to neutralize free radicals is still noteworthy, considering the oral cavity's susceptibility to oxidative stress, contributing to periodontal disease and caries. Research by Fidrianny *et al.*[30] also reported antioxidant activity in red dragon fruit extract with an IC_{50} of 94.17 µg/mL. In addition, other studies on antioxidants in dragon fruit peel extracts show more promising results. Wahdaningsih *et al.*[31] evaluated antioxidant activity through DPPH scavenging assay on red dragon fruit peel with two isolates and showed stronger antioxidant activity with IC_{50} of 2,952.14 and 25,635.95 µg/mL, respectively, while Fidrianny's *et al.*[32] study reported a DPPH scavenging activity IC_{50} value was 46.51±4.72 µg/mL on red dragon fruit peel extract. The higher antioxidant activity in the peel extract can be attributed to the higher concentration of phenolic compounds and betacyanin, especially in the fruit peel tissue. The antioxidant potential is likely linked to the high concentration of betacyanin compounds in RDFE, as previously established by Ramli *et al.*[13], suggesting its viability for supporting oral health through oxidative balance. The findings may thus contribute to broadening the antioxidant-focused approach in caries prevention.

Our antibacterial assessments through the disc diffusion method showed RDFE's efficacy in inhibiting *Streptococcus mutans* growth, with a peak inhibition zone of 5.43 mm at a concentration of 1000 µg/mL. This effect aligns with prior studies by Rahayu *et al.*[14], which confirmed RDFE's antimicrobial capabilities against oral pathogens. Importantly, RDFE's concentration-dependent inhibition of *S. mutans* (60.36% MIC at 250 µg/mL and 92.34% MBC at 1000 µg/mL) suggest potent antimicrobial profile that can be harnessed in low concentrations for practical applications. The significance of this effect, given *S. mutans* role in cariogenic biofilms, underscores RDFE's potential as a therapeutic adjunct in dental products targeting oral microbiome modulation.

Comparing our findings with previous research, Amalia *et al.*[33] demonstrated that red dragon fruit extract at 40 mg/mL, produced a 12.80 mm inhibition zone against *Staphylococcus aureus*, indicating strong antibacterial potential, with inhibition percentages reaching approximately 85.1%. Similarly, Hendra *et al.*[34] demonstrated that extracts from red dragon fruit peel also effectively inhibited *Bacillus subtilis*, with inhibition percentages reaching 100% at 20 µg/mL concentration. While these studies focused on different bacterial strains, they support our findings regarding RDFE's broad-spectrum antimicrobial potential. The slightly lower inhibition zone observed in our study with *S. mutans* might be attributed to the different cell wall structures and resistance mechanisms between gram-negative and gram-positive bacteria. Nevertheless, the relatively small inhibition zone observed at the highest RDFE concentration (5.43 mm) compared to chlorhexidine, may suggest a need for further optimization. Future studies may consider using higher concentrations of RDFE or exploring synergistic combinations with other natural compounds to enhance its antibacterial effect against *S. mutans*.

Biofilm refers to a community of microorganisms enclosed by an extracellular polymer matrix produced by microorganisms such as *S. mutans*[35]. Most compelling was RDFE's antibiofilm activity, which achieved substantial inhibition (91.57%) at a concentration of 1000 µg/mL. This attribute is crucial since biofilm resilience and adherence are primary factors in caries progression[3]. RDFE's inhibition of biofilm formation suggests that it could effectively prevent *S. mutans* adherence to enamel surfaces, thereby reducing the initial stages of biofilm development. This finding holds significant implications for caries management, especially in pediatric populations where non-invasive, safe interventions are prioritized. Research by Yong *et al.*[36], which also evaluated the red dragon fruit extract antibiofilm activity, showed the percentage of inhibition against *Staphylococcus aureus* by 30-51%

and *Pseudomonas aeruginosa* by 23-42%. This shows that red dragon fruit extract has the potential to be an effective antibiofilm. Another comparison with other natural, such as green tea extract has been reported to inhibit *S. mutans* biofilms at concentrations around 3.1–12.5 mg/mL[37], while essential oils from clove, thyme, and cinnamon have shown reductions of biofilm ≥ 85–100% at 1–4× MIC[38]. Importantly, the 91.57% inhibition by RDFE is comparable to these agents and aligns with the efficacy of chlorhexidine in many assays.

The development of RDFE-based chewy jelly as a delivery vehicle for disclosing agents demonstrated promising physical and organoleptic properties. Among the formulations tested, Formula II exhibited optimal hardness (775.60 gForce) and adhesivity (-9.60 g.sec), making it suitable for oral application. However, the colour intensity of the jelly requires improvement, as the current formulation may not achieve sufficient visual contrast for effective plaque disclosure. Addressing this limitation could involve integrating approved food colorants, such as Erythrosine (E127) as per SNI 01-2891-1992 standards, to enhance the visual contrast without compromising safety. Future research might also explore alternative natural pigments to maintain the formulation's appeal as a natural product.

Stability tests revealed that Formula II maintained acceptable pH (5.56±0.12) and viscosity (1026.42±47.43) over four weeks, though minor weight loss due to moisture changes suggests a need for further optimization. Enhancements in the formulation's moisture retention could improve its long-term stability, which is critical for product viability. Improving viscosity enhances the structural integrity of the jelly matrix, minimizing syneresis and phase separation, while better moisture retention reduces evaporation-related weight loss and texture degradation during storage. This contributes significantly to product stability and shelf life. Moreover, the absence of microbial growth across the testing period indicates effective preservation, supporting RDFE's potential for stable product formulation.

In addressing broader implications, RDFE-based products could mitigate health concerns associated with synthetic disclosing agents, as noted by Silva *et al.*[11] and Britsun *et al.*[12]. RDFE's natural properties—combining colour, antioxidant, antibacterial, and antibiofilm activities—position it as a safer alternative for pediatric applications, where adverse reactions to synthetic agents are a significant concern. The potential for RDFE as a multifunctional additive in dental hygiene products aligns with the growing consumer and regulatory preference for natural product formulations, which may enhance compliance and safety in pediatric and general populations.

A limitation of the present study lies in RDFE's relatively weak colouring ability in powder form, which may limit its efficacy as a standalone visual indicator. Future research should explore optimizing the extraction process to concentrate natural pigments or evaluate biocompatible synthetic colourants that could supplement RDFE's properties without diminishing its health benefits. Additionally, investigating RDFE's impact on diverse oral microbiota could expand its application scope and efficacy insights, contributing to a more comprehensive understanding of its role in oral health maintenance.

Conclusion

Red dragon fruit extract exhibits promising antioxidant (IC_{50} 158.43±3.67 µg/mL), antibacterial (92.34% inhibition at an MBC of 1000 µg/mL), and antibiofilm activity (91.57% at 1000 µg/mL), supporting its potential as a natural base for dental disclosing agents. The development of a chewy jelly formulation was successful, with Formula II demonstrating acceptable physical characteristics and short-term stability over four weeks. However, the colour intensity was suboptimal, which may limit its effectiveness as a visual disclosing tool. Further optimization is needed to enhance pigment stability and moisture retention, and long-term storage studies are recommended to ensure product durability. Red dragon fruit extract exhibits promising antibacterial and antibiofilm properties, but optimization of colour intensity and *in vivo* validation are needed before clinical application.

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