PHARMACIA

Pharmacia 71: 1–8
DOI 10.3897/pharmacia.71.e118021

Research Article

Physicochemical characterization of novel toothpaste from *Caulerpa racemosa* and *Thunnus* fish bone: Antibacterial potency against colonization of selected cariogenic-periodontal bacteria

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Received 30 December 2023 ♦ **Accepted** 10 January 2024 ♦ **Published** 24 January 2024

Citation: Theodorea CF, Nurkolis F, Idrus E, Yusuf TW, Vivo CD, Subali D, Taslim NA, Nugraha AP (2024) Physicochemical characterization of novel toothpaste from *Caulerpa racemosa* and *Thunnus* fish bone: Antibacterial potency against colonization of selected cariogenic-periodontal bacteria. Pharmacia 71: 1–8. https://doi.org/10.3897/pharmacia.71.e118021

Abstract

This study evaluated the physicochemical properties of toothpaste from combined *Caulerpa racemosa* and *Thunnus* fish bone (Toothpaste *Caulerpa* and *Thunnus* or TCT) and its antibacterial activity towards the colonization of selected cariogenic and periodontal bacterias. Four forms of toothpaste which contained *C. racemosa* extract and calcium carbonate or bone isolates of tuna and control (F1 (1.5:45); F2 (3:45); F3 (4.5:45); F4 (0:45)) were compared and analyzed for antioxidant activity (DPPH assay), organoleptic (sensory), homogeneity, viscosity, pH, and foamability. Antibacterial activity tests were conducted on *Streptococcus mutans*, *Staphylococcus aureus*, and *Porphyromonas gingivalis*. The antioxidant activity of the group's (F1, F2, F3, F4) p=0.0001 differed considerably (CI 95%). F3 was the most antioxidant-active formula, with 27.46 ± 3.09%. F3 also had good sensory tests, adequate homogeneity, optimal pH 7.64 ± 0.68, an increased viscosity level of 443.07 ± 0.12, and the least foam formations of 19.28 ± 0.07, all of which are significantly different (p<0.05) from other variations of TCT formulas. Interestingly, F3 has greater inhibition against the activity of selected bacterias. In conclusion, formula 3 (F3) is a recommended toothpaste, made from combined *C. racemosa* and *Thunnus* fish bone, and has promising physicochemical and antibacterial properties. A further clinical study is urgently needed.

Keywords

antibacterial, antioxidant, *Caulerpa racemosa*, medicine, natural toothpaste, oral hygiene

Introduction

According to the World Health Organization (WHO) (2017), caries, gingivitis, and periodontitis are the most common oral diseases and are caused by the formation of plaque (a thin layer consisting of a group of bacteria embedded in the extracellular matrix of the mucosa and the surface of the teeth in the oral cavity). Some microorganisms are found in the plaque that cause several dental and oral diseases, for example, *Streptococcus mutans*, *Porphyromonas gingivalis* and *Staphylococcus aureus* (Lien et al. 2014; Parija 2014). Inflammation in the oral and dental area produces reactive oxygen species (ROS) that worsen dental caries (Miricescu et al. 2014; Pandey et al. 2015). Hence, some antibacterial and antioxidant agents might help to control dental caries.

Sea grapes (*Caulerpa racemosa*) have the potential to be harvested intensively and can be found in the waters around Indonesia (Pakki et al. 2020; Manoppo et al. 2022). Several types of research have shown that *C. racemosa* contains bioactive compounds, such as polyphenols, flavonoids, and antioxidants (Yang et al. 2015; Yap et al. 2019; Manoppo et al. 2022; Permatasari et al. 2022). Furthermore, *C. racemosa* contains a caulerpin which potentially acts as an antibacterial agent (Lunagariya et al. 2019). Previous findings explained that antioxidants and polyphenols contained in *C. racemosa* had prevented dental caries (Delimont and Carlson 2020). Accordingly, *C. racemosa* extract has the potential to be used as an active agent of dental care products such as toothpaste.

Besides contributing antibacterial and antioxidant agents, *C. racemosa* also contains some minerals, including calcium and phosphorous. Calcium and phosphorous both play a vital role in the formation and maintenance of healthy teeth and gums in both children and adults. Ten calcium ions and six phosphate ions are required to form one unit cell of fluorapatite in teeth remineralization (Reynolds. 2008). Tuna fishbone (*Thunnus* sp.) is a waste from fish processing that is rich in calcium, phosphorus, and selenium (Hafsiyah 2018). The utilization of tuna bone as a source of calcium is an option to meet calcium needs while increasing the economic value of tuna bone waste (Nabil 2005).

Considering the potential of sea grapes and fishbone waste as an additive to dental and oral health products, this study aims to utilize the combination of *C. racemosa* with tuna fish bone waste to develop a herbal toothpaste (TCT). This study also aims to determine their physicochemical analysis and the antibacterial activity of *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Staphylococcus aureus*.

Materials and methods

This experimental study was conducted from January 2022–October 2022 in Oral Biology, Faculty of Dentistry, University of Indonesia, and UIN Sunan Kalijaga Yogyakarta. The tools utilized in this study were Pyrex glass, autoclave, stirring rods, maceration vessels, a blender (Cosmos), Petri dish, porcelain dish, cover glass, hot plate, incubator, ose needle, analytical scales (Sartorius), oven, ruler, microliter pipette (Socorex), drip pipette, pH-meter (SCHOTT Lab 860), vacuum rotary evaporator (RV 8 IKA), Brookfield viscometer, and toothpaste container. Aquadest, *Streptococcus mutans* bacteria, *Staphylococcus aureus* bacteria, Betel Nut (*Areca catechu* L.), ethanol 96%, glycerin, sterile cotton, calcium carbonate, menthol, sodium benzoate, sodium lauryl sulfate, sodium carboxymethyl cellulose, sodium saccharin, NaCl 0.9%, and nutrient agar (NA) were used as materials in this study.

Sampling and extraction of sea grapes

Fresh *Caulerpa racemosa* (10 kg) have been accumulated in the sea grapes cultivation pond in the region of Jepara, Indonesia (6°35'12.5"S, 110°38'36.0"E; Central Java).

Botanical identification and authentication were confirmed by Dian Aruni Kumalawati, M.Sc by using macroscopic and sensory (organoleptic) approaches (Nurkolis et al. 2022a, b), at the Integrated Laboratory of the Faculty of Sciences and Technology (Herbarium Laboratory), UIN Sunan Kalijaga, Yogyakarta-55281, Indonesia. Detailed instructions are provided by descriptions, including, (1) form; (2) shape and size; (3) color, exterior marks, and texture; (4) fracture and interior color; and (5) organoleptic features in materials (odor, taste, and mouth feel) (Upton et al. 2020). The existence of the predicted traits serves as important clues to the identification of the plant, while the intensity of the color, scent, or flavor gives clear indications as to the caliber of the plant. The result complies with National Center for Biotechnology Information (NCBI) Taxonomy ID 76317 (Eukaryota/Viridiplantae/Chlorophyta/Ulvophyceae/Bryopsidales/Caulerpaceae/*Caulerpa*). The researchers (authors) declare and confirm that all the methods performed in this study comply with the guidelines and regulations applicable to *in vitro* studies on dental materials (Faggion 2012).

The *C. racemosa* has been rinsed thoroughly with an aquadest, air-dried at room temperature for 30 minutes and in a 40 °C oven for 72 hours, then powdered using an electric-powered mill (BENSRA Laboratory Mills L120). The crushed powder (1 kg) was macerated for 72 hours in ethanol from Merck Millipore Germany (96%) and extracted in triple-time, yielding 34% crushed powder. The crude extract was filtered with Whatman 41 filter paper. The entire filtrate was concentrated and evaporated at 40 °C with a rotary evaporator (RV 8 IKA) beneath decreased pressure (100 mb) for 90 minutes. It was then evaporated in a 40 °C oven to provide a thick extract. Extracts were stored in refrigerators at 8 °C until they were used in research. This preparation method followed our previous research which showed the effective extraction method for Caulerpin (Nurkolis 2022; Permatasari et al. 2022) .

The production of tuna fishbone powder

Tuna fish bone was collected from the fish market in Manado, North Sulawesi, Indonesia. The production of tuna bone begins by boiling the fish bones and tuna fish heads until the fish meat and skin are separated from its bones and heads. After boiling, the bones are cleaned and washed to remove the remains of the meat that are still attached. After cleaning, the fish bones are softened and the bone size is reduced to 5–10 cm. The bone was then dried at a temperature of 55 °C and was milled and sieved with a 120-mesh sieve to obtain the powder. This method was modified from Trilaksani et al. 2006.

Toothpaste (TCT) formulation

The formula was modified from Afni et al. 2015 (Table 1) (Afni et al. 2015). The natrium carboxymethylcellulose (Na CMC) was developed in hot water at 100 °C for approximately 15 minutes and stirred homogeneously using the VELP Multi-HS 6/15 Digital Multi-Position Hot Plate Magnetic Stirrer (mass 1). The tuna bone powder and sodium lauryl sulfate were poured and stirred homogeneously into mass 1 (mass 2). Glycerin was then poured into mass 2 to produce a viscous and wet mass. Carbomer, sorbitol, and sodium benzoate were dissolved in the remaining water and sodium benzoate in the remaining water were mixed and stirred homogeneously to form a paste mass. Lastly, menthol was added to the paste mass and it was packed into a dry and clean container.

Table 1. Toothpaste (TCT) formulation with a variation of concentration of *C. racemosa* extract.

Ingredients (% w/w)	Function	Formula*			
		F1	F2	F3	F4
C. racemosa extract	Active Agent	1.5	3	4.5	0
Fishbone powder	Abrasive	45	45	45	45
Glycerin	Humectant	25	25	25	25
Natrium carboxymethylcellulose (Na CMC)	Binder	1.5	1.5	1.5	1.5
Sodium lauryl sulfate	Surfactant	1	1	1	1
Sodium benzoate	Preservative	0.1	0.1	0.1	0.1
Sodium saccharine	Sweetening	0.2	0.2	0.2	0.2
Menthol	Perfume	0.2	0.2	0.2	0.2
Aquadest	Solvent	ad 100	ad 100	ad 100	ad 100

*The toothpaste formula was modified from Afni et al. 2015. The active ingredient and abrasive agent were changed to C. racemosa extract and fishbone powder.

In vitro test of antioxidant activity of the toothpaste (TCT)

Antioxidant activity was determined using DPPH (2,2-diphenyl-1-picyrl-hydrazyl-hydrate). The stock solution was created by dissolving 24 mg of DPPH in 100 mL of methanol. Methanol was used to filter the DPPH stock solution, and the result was a useful combination with an absorbance of around 0.973 at 517 nm. 100 µL of TCT and 3 mL of DPPH working solutions were mixed in a test tube. As a standard, 3 mL of DPPH solution in 100 mL of methanol is frequently provided. The tubes were then left in full darkness for 30 minutes. Last, the absorbance was calculated at 517 nm with three replicates. Antioxidant activity was calculated by equation 1 as follows:

$$Inhibition\ (\%) = \frac{(A0 - A1)}{A0} \times 100\%$$

Note:
A0 = Blank absorbance.
A1 = Standard or sample absorbance.

Physicochemical evaluation of the toothpaste (TCT)

Organoleptic test

The sensory evaluation of TCT was tested for texture, smell, and taste using descriptive methods and open criteria (Agustina and Fadhil 2021). About 10 g of TCT was taken and observed objectively for its overall physical appearance by semi-trained panelists. This observation was carried out on day 1, day 7, day 14, and day 21 of storage (Afni et al. 2015).

Homogeneity test

A homogeneity test was done by applying 10 g of toothpaste on a slide to observe its homogeneity. If no grains on the object glass are present, then the toothpaste being tested is considered homogeneous, while the presence of coarse grains indicates that the toothpaste is not homogeneous. Tests were carried out on day 1, day 7, day 14, and day 21 of storage (Afni et al. 2015).

Viscosity

The samples were put in a 250 ml beaker glass until the sensor on the spindle closed. The viscosity was evaluated using a Brookfield rotational viscometer and spindle by simulating the external forces through the set speed of rotation (50 rpm). The spindle was allowed to rotate, and the viscosity was calculated based on the reading of the number. Tests were carried out on day 1, day 7, day 14, and day 21 of storage (Afni et al. 2015).

pH test

The pH measurement was done by immersing the pH meter (SCHOTT Lab 860) into the toothpaste until it showed a constant number. The value shown was recorded as the pH value. Tests were carried out on day 1, day 7, day 14, and day 21 of storage (Afni et al. 2015).

Foam formation

The toothpaste foam formation test was carried out by making a 1% toothpaste solution from each TCT formula, which was achieved by diluting 0.25 g of TCT in 25 mL of aquadest. Then it was put in a 50 ml measuring cup and shaken vigorously for 1 minute using a GS-20 Orbital Shaker Lab. The height of the formed foam was measured using a ruler on the side of the measuring cups. Tests were carried out on day 1, day 7, day 14, and day 21 of storage (Afni et al. 2015).

Antibacterial activity assay of TCT

Sterilization of tools and materials

Sterilization was carried out in a way that is suitable for each tool. Sterilized tools must be clean and dry. Test tubes, measuring cups, and Petri dishes were covered with cotton and aluminum foil and then sterilized in the oven at 180 °C for 2 hours. The seed medium and NaCl solution were sterilized by autoclaving at 121 °C for 30 minutes using a Hirayama HVE-50 Autoclave. Tweezers and ose needles were sterilized by immersing them in a flame.

Preparation of nutrient agar (NA) medium

Nutrient agar (NA) medium was weighed out to 2.3 grams and dissolved in 100 ml of distilled water using a Pyrex Erlenmeyer. The medium was homogenized over a water bath in a Memmert WTB 6 until the NA medium was completely dissolved. The solution was then sterilized in an autoclave at 121 °C for 15 minutes, stored in the refrigerator, and reheated to 65 °C when used.

Test bacteria setup

Test bacteria *Staphylococcus aureus* ATCC® 6538™, *Streptococcus mutans* ATCC® 25175 ™, and *Porphyromonas gingivalis* ATCC® 33277 ™ were derived from pure cultures and 1 ose of each bacteria was taken. This was inoculated through streaking on inclined nutrient agar (NA) medium. After that, it was incubated at 37 °C for 24 hours in a Memmert IN55 Incubator. One ose of the bacterial cultures (0.5 ml) was taken with a sterile needle and then suspended in a test tube containing 10 ml of 0.9% NaCl solution until the turbidity of the bacterial suspension was obtained, which was the same as the standard Mc. Farland turbidity. This means the concentration of the bacterial suspension was 10^8 CFU/ml. The concentration of the bacterial suspension was 10^8 CFU/ml which was used in the antibacterial activity test.

Toothpaste antibacterial activity test

The antibacterial power test in this study was conducted with the diffusion method using wells. Nutrient agar (NA) medium was prepared, which was sterilized in an autoclave at 121 °C for 15 minutes. Then, while still warm, 15 ml of the nutrients was poured into 10 sterile Petri dishes, measuring 9 cm each, then allowed to stand until solid. A bacterial suspension of *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* ATCC 25175, and *Porphyromonas gingivalis* ATCC 33277 was prepared, which had been inoculated in 0.9% NaCl. A sterile cotton swab was then dipped into the bacterial suspension and smeared on the NA medium. A 7 mm diameter tip was used to make a hole in the nutrient medium, then a sample of 0.1 g of TCT at various concentrations of 1.5%, 3%, 4.5%, and control was prepared. The test was carried out by inserting toothpaste with various concentrations of 0.1 g each into the well, then the Petri dish was incubated for 24 hours at 37 °C. Measurements were made on the clear zone formed around the well, which indicates the zone of inhibition of bacterial growth. Measurements were done in triplicates.

Statistical analysis

The data obtained in the physical and chemical quality tests were analyzed descriptively. Antioxidant and antibacterial activity data were statistically processed using the one-way ANOVA at a 95% (0.05) confidence level using the MacBook version of the GraphPad Prism 9 program. Then, for organoleptic (sensory), homogeneity, viscosity, pH, and foam formation data were analyzed descriptively. All tests were carried out in three repetitions (thrice).

Results

In vitro test of antioxidant activity in toothpaste (TCT)

The four formulations (F1, F2, F3, and F4) of *C. racemosa* extract toothpaste were statistically analyzed for their antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) *in vitro* using one-way ANOVA at 95% CI. The results of the test analysis are available in Fig. 1.

Antioxidant activity from the DPPH assay for all toothpaste formulas found a significant difference. There was a significant difference in antioxidant activity between F4, or control, with F1, F2, and F3 p=0.0001 (p<0.05) (Fig. 1). F3 is the formulation that has the highest antioxidant activity, namely 27.46 ± 3.09%.

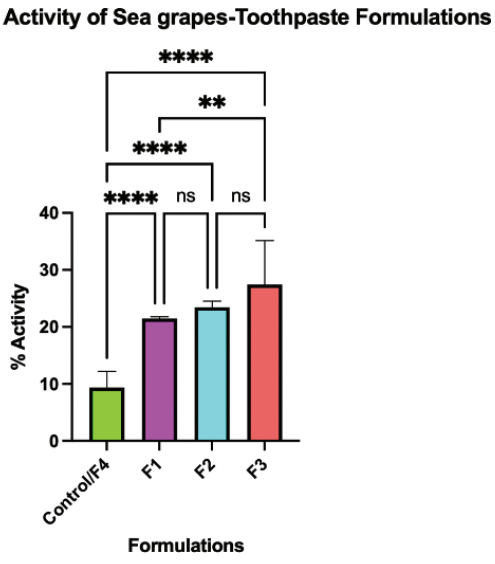

Figure 1. Antioxidant Activity of Toothpaste. (****)p=0.0001; (**)p=0.0099; (ns)=0.5126.

Results of quality evaluation of toothpaste (TCT)

In addition to the antioxidant activity test, an evaluation test of the quality of the toothpaste was also carried out, which included organoleptic, homogeneity, viscosity, pH, and foamability. The test results are presented in Tables 2–6 below.

Table 2. Organoleptic test of toothpaste formulas.

Formulas	Organoleptic Observations			
	Day 1	Day 7	Day 14	Day 21
F1	Beige, menthol scent, moderately viscous	Beige, menthol scent, moderately viscous	Beige, menthol scent, moderately viscous	Beige, menthol scent, moderately viscous
F2	Beige-brown, menthol scent, viscous	Beige-brown, menthol scent, viscous	Beige-brown, menthol scent, viscous	Beige-brown, menthol scent, viscous
F3	Brown, menthol scent, very viscous	Brown, menthol scent, very viscous	Brown, menthol scent, very viscous	Brown, menthol scent, very viscous
F4/control	White, menthol scent, moderately soft	White, menthol scent, moderately soft	White, menthol scent, moderately soft	White, menthol scent, moderately soft

The results of the organoleptic test in Table 2 showed the different characteristics of each toothpaste formula. Toothpaste characteristics were observed for color, scent, and viscosity. Each formula showed the same characteristics after 3 weeks of storage.

Table 3. Homogeneity test of toothpaste (TCT) formulas.

Formulas	Observations			
	Day 1	Day 7	Day 14	Day 21
F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F4/control	Homogeneous	Homogeneous	Homogeneous	Homogeneous

The results of the homogeneity test in Table 3 show that all formulations, from F1 to F4, have homogeneous properties, starting from the test on days 1, 7, and 14 until day 21.

Table 4. pH test of toothpaste (TCT) formulas.

Formulas	pH of Toothpaste			
	Day 1	Day 7	Day 14	Day 21
F1	7.01 ± 0.01	6.85 ± 0.05	7.23 ± 0.03	5.62 ± 2.87
F2	6.78 ± 0.02	6.81 ± 0.01	6.12 ± 0.02	7.34 ± 0.05
F3	6.91 ± 0.07	7.06 ± 0.01	6.59 ± 0.02	7.64 ± 0.68
F4/control	7.05 ± 0.02	6.83 ± 0.06	6.84 ± 0.88	7.48 ± 0.07

The results of the pH test in Table 4 show each toothpaste formula has a pH value of around 6 to 7. After 3 weeks of storage, the pH value for each formula slightly increased.

Table 5. Viscosity test of toothpaste (TCT) formulas.

Formulas	Viscosity (Cp)			
	Day 1	Day 7	Day 14	Day 21
F1	221.33 ± 1.15	235.33 ± 0.57	221.24 ± 0.11	235.00 ± 4.00
F2	331.33 ± 1.15	312.99 ± 0.57	386.44 ± 0.50	370.03 ± 0.06
F3	414.14 ± 0.16	443.07 ± 0.12	433.44 ± 0.38	433.03 ± 0.06
F4/control	206.07 ± 0.12	208.44 ± 0.38	220.11 ± 0.11	242.22 ± 0.19

The results of the viscosity test in Table 5 show different viscosity levels for each toothpaste formula. Formula F3 has the highest viscosity level, and formula F4 has the lowest viscosity level. Each formula shows a slightly different viscosity level after 3 weeks of storage.

Table 6. Foam formation test of toothpaste (TCT) formulas.

Formulas	Storage Foaming			
	Day 1	Day 7	Day 14	Day 21
F1	29.10 ± 0.10	23.44 ± 0.38	27.22 ± 0.29	26.14 ± 0.16
F2	23.33 ± 0.30	21.22 ± 0.19	20.00 ± 0.10	20.63 ± 1.48
F3	19.40 ± 0.36	22.92 ± 0.12	26.16 ± 0.28	19.28 ± 0.07
F4/control	55.22 ± 0.30	60.17 ± 0.16	68.16 ± 0.29	57.47 ± 0.32

The results of the foam formation test in Table 6 show different foam formations for each toothpaste formula. Formula F3 has the lowest foam formation level and formula F4 has the highest foam formation level. Each formula shows a different foam formation level after 3 weeks of storage.

The third formulation (F3) is toothpaste (TCT) which has the potential to be further developed in subsequent research (Fig. 2). F3 has greater inhibition against the activity of *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* ATCC 25175, and *Porphyromonas gingivalis* ATCC 33277 (F3 > F1, F2, F4/control). Statistically, ANOVA showed that there was a significant difference from all samples in the inhibition of the activity of the three bacteria ($p<0.05$) (Fig. 2).

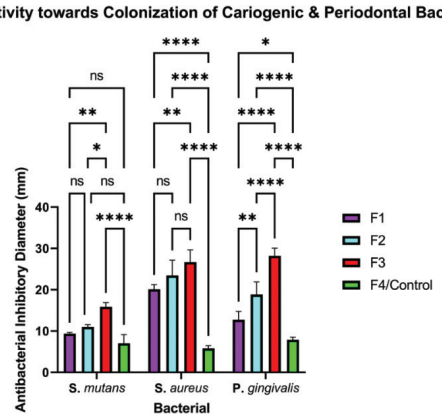

Figure 2. Antibacterial activity towards the colonization of cariogenic & periodontal bacterias. ns= Not Significant ($p>0.05$), *=0.0294, **=0.0026, ****<0.0001.

Discussion

Toothpaste involves the use of *C. racemosa* extract as an antioxidant that reduces free radicals and reactive species at some stage in its kinetics process. Based on Fig. 1, the *in vitro* assay of antioxidant activity, performed by DPPH assay, showed that F3 has the greatest antioxidant activity, reducing 27.45 ± 3.09% of DPPH oxidants (Fig. 1). The F3 formula carried out has the best antioxidant activity due to its higher concentration of *C. racemosa*, proving that the higher the *C. racemosa* concentration, the better the antioxidant activity will be.

Based on organoleptic observation (Table 2), the toothpaste of F1, F2, and F3 had a brownish color due to the addition of *C. racemosa*, whereas F4 has a white color since it

does not contain *C. racemosa* extract. All the formula has a consistent organoleptic appearance as well as homogeneity until day 21. Table 3 showed that the formulated toothpaste has various pHs and the increase of *C. racemosa* extract led to the emerging number of pH values (F1= 7.27; F2= 7.32; F3= 7.97; F4= 7.55). All formulated toothpaste has met the pH requirement of the ISO IS0 11609:1995(E) for toothpaste characteristics (pH<10.5). In line with the study by Lugo-Flores *et al.* (2021), plant-derived substances have great potential for formulating oral healthcare products due to their promising antibacterial, antioxidant, and flavoring properties (Lugo et al. 2021).

Some of the substances contained in *C. racemosa* have a potent antibacterial, which inhibits the bacterial growth of *S. aureus*, *B. cereus*, and *P. aeruginosa* (de Gailande et al. 2016). A study by Yap (2019) estimates that Caulerpin, Caluerpa's distinctive alkaloid, is the reason for its potent antibacterial activity. This antibacterial effect is thought to be due to the secondary metabolite from the terpenoid group, such as squalene, carvacrol, and the functional group such as peptides, polysaccharides, sterol, ketone, etc. The polyphenol, flavonoid, and alkaloid components in *C. racemosa* may prevent oral diseases, including caries, gingivitis, and periodontitis, due to the secondary metabolites having anti-cariogenic activity as shown in Fig. 3 (Wells et al. 2017). This is because of the direct inhibitory effect on *S. mutans* from the secondary metabolite that prevents the attachment of bacterial cells to tooth surfaces and inhibits some enzymes, which include amylase and glucosyl transferase (Kakiuchi et al. 1986).

properties, called fluorapatite, it can be used as a remineralizing agent in oral care products (Abou et al. 2016). Another analyzed physical property is viscosity. The viscosity of the toothpaste increased as a result of the emerging number of extracts. This is the first research that succeeds in using *C. racemosa* extract and marine waste (tuna fish bone) for making toothpaste. The unexplored bioactive component of TCT is our limitation, and this concern will be explored in further studies that will be carried out. These include metabolomic profiling and looking at the potential of molecular docking metabolites that play a role in inhibiting several bacteria that cause toothache.

This study highlighted that TCT was formulated from novel ingredients, *Caulerpa racemosa* and *Thunnus* fish bone (Fig. 2). Moreover, aside from the antibacterial activity, this study performed the determination of the antioxidant potential of TCT (Fig. 2). Notably, this study has several limitations. First, considering that there are various kinds of cariogenic and periodontal bacteria, the TCT has not been evaluated on other cariogenic and periodontal bacteria, such as *Fusosbacterium nucleatum*, *Lactobacillus acidophilus*, *Aggregatibacter actinomycetemcomitans*, and others. Furthermore, a study on animal models regarding TCT has not been performed to support the antibacterial claim of TCT. Additionally, some methods utilized in this study were categorized as simple methods (sensory test, foamability, and homogeneity tests). These limitations can be addressed in future research to further solidify the evidence regarding the antibacterial potency of TCT against the colonization of cariogenic and periodontal bacteria.

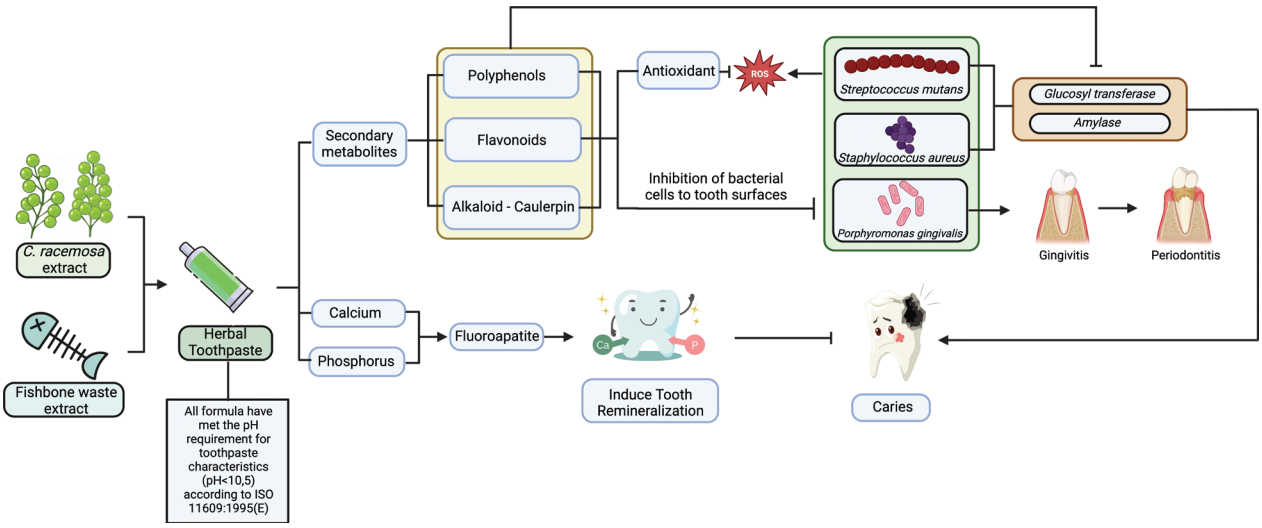

Figure 3. Possible mechanism of combined *C. racemosa* and *Thunnus* fish bone flour toothpaste to cariogenic & periodontal bacteria. This figure is an original figure produced by the author(s) for this article.

Fish bones contain 60–70% minerals with the components mostly consisting of bioapatite, including hydroxyapatite, carbonated apatite, and 30% collagen protein (Mutmainnah 2017). Hydroxyapatite is well-known to be biomimetic, or a bionic active ingredient, when used in oral care. Human enamel consists of approximately 97% hydroxyapatite. By synthesizing these hydroxyapatite-like

Conclusion

Caulerpa racemosa, or sea grapes, can be combined with tuna fish bone flour for making toothpaste (TCT). TCT with F3 formula (4.5:45), *C. racemosa* extract, calcium carbonate or bone isolates of tuna, has promising anti-

bacterial and antioxidants properties to inhibit the colonization of cariogenic and periodontal bacteria, such as *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Staphylococcus aureus*). A clinical study using animal (*in vivo*) and human clinical trials will be welcomed in future studies.

Acknowledgments

We offer a great thank you to the Chairman of the Indonesian Association of Clinical Nutrition Physicians, and Professor Hardinsyah, Ph.D. (as President of Federations of Asian Nutrition Societies), who has reviewed and provided suggestions with motivational support, as well as input on the draft of this critical review article.

This research was supported by PUTI Q2 2022 from Universitas Indonesia (contract number NKB-1269/UN2.RST/HKP.05.00/2022) to CFT. The datasets generated and/or analyzed during the current study are available in the [Figshare] repository, https://doi.org/10.6084/m9.figshare.21431925.v1.

FN and CFT: conduct experiments, analyzed data, write the manuscript, design research, and conceptualize ideas; while CFT, FN, EI, TWY, and CDV: contribute to data analysis, critiquing manuscript, interpret manuscript results, assisting in the processing of data, as well as helping to revise and graphical figure editing. DS, CFT, EI, FN, NAT, APN: critiquing, writing – review & editing manuscript. All authors have read and also approved this final manuscript.

The authors and/or contributors to the study stated that they had no conflict of interest.

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