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ORIGINAL ARTICLE

Properties of fucoidans beneficial to oral healthcare

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Abstract
Fucoidans are sulfated polysaccharides that are found in marine algae and have many useful activities, including antitumor effects, promotion of apoptosis of cancer cells, and antiviral, anti-inflammatory, and antiallergic actions. In oral medicine, several case reports have shown that fucoidan-containing creams and tablets markedly improved recurrent aphthous stomatitis, symptomatic inflamed tongue, and recurrent oral herpes labialis. The aim of this study was to examine the properties of fucoidans for use in oral healthcare. The antimicrobial, anti-adhesion, endotoxin-neutralizing, and cyclooxygenase (COX)-1 and COX-2 inhibitory activities of fucoidans were examined. Four key results were obtained: fucoidans showed strong antimicrobial activity against *Candida albicans*, *Streptococcus mutans*, and *Porphyromonas gingivalis*; significantly inhibited the adhesion of *S. mutans* to bovine teeth and porcelain; were suggested to bind to and neutralize endotoxin (lipopolysaccharide) in an LAL assay; and showed COX-1 and/or COX-2 inhibitory activity. These results suggested that fucoidans may be useful in the field of oral healthcare.

Keywords Fucoidan · Antimicrobial · Endotoxin-neutralizing · Anti-inflammatory · Oral healthcare

Introduction

Fucoidans are sulfated polysaccharides contained in seaweeds that were first identified in brown algae in 1913 [1]. In fucoidans, sulfate groups or uronic acids are bonded to L-fucose, with hundreds to thousands of L-fucose units linked by α-1-2 and α-1-4 bonds [2]. The structures of several fucoidans from various marine algae have been determined, and these are classified depending on the kind of sugar: for example U-fucoidan contains glucuronic acid, F-fucoidan includes sulfated fucose only, and G-fucoidan contains galactose [3]. Fucoidans are the main components in the well-known pharmacological effects of seaweed. Many useful activities of fucoidans have been described, including an antitumor effect [4], promotion of apoptosis of cancer cells [5], anti-HIV and other antiviral activities [6], and anti-inflammatory [7], antiallergic, and hypotensive effects [8]. The potential use of fucoidans as antitumor agents has been examined in vitro, with fucoidan extracts with reduced molecular weight by enzyme digestion shown to have a higher antitumor effect [9] and to enhance the action of other anticancer drugs [10]. Thus, fucoidans are used as supplements in cancer patients, and there is also evidence of anti-inflammatory effects in patients with advanced cancer [11].

We are working on the use of fucoidans in oral healthcare. We have described cases in oral medicine that showed marked improvement with use of fucoidan-containing cream or tablets for treatment of recurrent aphthous stomatitis [12], symptomatic inflamed tongue [13], and oral herpes labialis [14]. This cream was also useful for leukoplakia, precancerous lesions such as plaque disease, lichen planus, and trauma (unpublished data), but its mechanism of action is unknown. Therefore, the current study was performed to examine this mechanism from a basic science perspective, with the goal of promoting application of fucoidans for oral cancer treatment, early repair of lesion sites, and improvement of

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hygiene in the oral environment. The data obtained in the study support our hypothesis that fucoidans are useful for oral healthcare.

Materials and methods

Fucoidans and oral pathogens

Four kinds of fucoidans were used in this study. (1) Crude fucoidan from Bladder wrack, *Fucus vesiculosus* (*Fv*) [15] (F5631, Sigma-Aldrich, St. Louis, MO, USA), contains fucose (33%), uronic acid (8%), sulfate (23%) and minor amounts of amino sugar and protein with a molecular weight of 20–200 kDa, as assessed using multi-angle laser light scattering (Sigma-Aldrich Customer/Technical Service). (2) Purified (> 95%) fucoidan from Bladder wrack, *F. vesiculosus* (*Fv*) (F8190, Sigma-Aldrich), with a peak molecular weight of 68.6 KDa (Sigma-Aldrich Customer/Technical Service). (3) Sulfated α-L-fucan (9072-19-9, Cayman Chemical, Ann Arbor, MI, USA); the distributor was not able to provide an average molecular weight (Funakoshi Technical Support). (4) Fucoidan extract with a low molecular weight (FE) prepared by glycosidase digestion and used as a mixture of digested and nondigested fractions (brown seaweed Mozuku, *Cladosiphon novae-caledoniae*) [16]; and 4% FE cream (Cream). FE and Cream, which are commercially available as "Power fucoidan" and "Power fucoidan cream", respectively, were generously donated by Daiichi Sangyo Corporation, (Osaka, Japan). FE was prepared by Daiichi Sangyo Corporation as described previously [16]. Briefly, a fucoidan extract with high molecular weight was isolated from Mozuku seaweed, *C. novae-caledoniae*, purified to 85%, and digested with glycosidases. FE consisted of a digested low-molecular-weight fraction (72% < 500 kDa) and non-digested fraction (> 28%, 800 kDa peak). FE consisted of mostly fucose (73%), xylose (12%), and mannose (7%), and the ratio of sulfation was 14.5%.

To examine the antimicrobial activity of fucoidans, the following bacteria were obtained as oral pathogens from the RIKEN BioResource Center via the National BioResource Project of MEXT, Ibaraki, Japan: *Candida albicans* (JCM1537), *Streptococcus mutans* (JCM5705), and *Porphyromonas gingivalis* (JCM8525).

Antimicrobial activities

Antimicrobial activities of fucoidans against oral pathogens, *C. albicans*, *S. mutans*, and *P. gingivalis* were assessed using the disc diffusion method [17]. Using paper discs (8 mm diameter; Advantech Co., Ltd, Tokyo, Japan), five types of discs were prepared using an antibiotic (positive control) with inhibitory activity against each pathogen, PBS, or three kinds of fucoidan (100 mg/ml). The fucoidan discs were prepared by immersion of a 100 µl solution of *Fv* crude, sulfated α-L-fucan, PBS, or Cream. As the positive control, fluconazole (100 µg) was used for *C. albicans,* and antimicrobial discs of penicillin 10 U and tetracycline 30 µg (Sensi-Disc, Beckton-Dickinson, Franklin Lakes, NJ, USA) were used for *S. mutans* and *P. gingivalis*, respectively. The diameters of the inhibition zones were measured and analyzed using the one-way dispersion method and then subjected to multiple comparison using Dunnett's test. Experiments were performed five times each in duplicate.

Bacterial anti-adhesion test

The following three fucoidans were used for all subsequent experiments: *Fv* crude (F5631), *Fv* pure (F8190), and FE. The ability of each fucoidan to inhibit adhesion of *S. mutans* to bovine teeth (mandibular incisor, Yokohama Edible Meat Public, Yokohama, Japan) and porcelain (VITA VM®13, VITA Zahnfabrik H. Rauter, Bad Säckingen, Germany) was examined using an Alamar Blue assay [18, 19]. A real-time cell growth logger (BS-010160-A02, Biosan, Riga, Latvia) was used to monitor proliferation, and culturing was performed at 37 °C in 10 ml of a liquid-culture medium. This monitoring device measures absorbance at a wavelength of 850 nm (optical density 850; OD850) [17]. *S. mutans* was cultured in 1% glucose in brain–heart infusion medium (Becton–Dickinson) at 37 °C until the optical density at 850 nm reached 0.6 (about 8.0×10^8 cfu/ml). The culture of *S. mutans* was diluted with the same volume of PBS as the positive control or PBS containing fucoidan at 100 mg/ml (final concentration of fucoidan at 50 mg/ml). Two hundreds microliters (about 8.0×10^7 cfu/ml of *S. mutans*) of control or fucoidan sample solutions were placed on the surface of each material and cultured for 2 h at 37 °C for adhesion of *S. mutans* cells. After adhesion, the materials were washed twice with PBS to remove free bacteria and reacted with Alamar Blue solution (Thermo Fisher Scientific Inc., Waltham, MA, USA) for 2 h at 37 °C to evaluate the reducing activity of living bacteria adhering to each specimen. The reacted Alamar Blue solution was placed in 96-well plates and fluorescence was measured (excitation wavelength 540 nm; emission wavelength 590 nm) with a microplate reader (Powerscan MX, DS Pharma Biomedical Co. Ltd, Osaka, Japan). Experiments were performed three times each (eight samples) in duplicate for the experimental and control groups. Statistical analyses were performed by Student *t* test to compare differences between two groups.

Endotoxin-neutralizing activity of fucoidan

The ability of fucoidans to bind and neutralize smooth-type lipopolysaccharide (LPS; endotoxin) was assessed using

a quantitative chromogenic LAL assay kit (Endospecy ES-50 M, Seikagaku Corp., Tokyo, Japan) [20]. To exclude endotoxin contamination, fucoidan (0.1 µg/µl) in pyrogen-free water (Otsuka Distilled Water, Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) was pretreated with Detoxi-Gel Endotoxin Removing Gel (Thermo Fisher Scientific Inc.). The assay was conducted in duplicate for each experiment following the protocols recommended by the manufacturer. Fucoidan (0.1 µg/µl) in pyrogen-free water was incubated with LPS from *Escherichia coli* O55:B5 [0.5 endotoxin units (EU)/ml] in microtubes at 37 °C for 30 min to allow binding of fucoidan to LPS. This mixture (50 µl) was then added to an equal volume of LAL reagent containing a chromogenic substrate butyloxycarbonyl (Boc)-Leu-Gly-Arg-*p*-nitroanilide, and the mixture was further incubated for 10 min at 37 °C. The absorbance caused by cleavage of the substrate was monitored spectrophotometrically at 405 nm (A405) at 15-min intervals until 120 min at room temperature (23 °C) with a microplate reader (Viento ELx808, BioTek Inc., Winooski, VT, USA). Pyrogen-free water without endotoxin was used as a control. The A405 of the reaction mixture with endotoxin alone is expressed as 100%. The results are expressed as the mean ± SD of five individual experiments in duplicate. Polymyxin B sulfate (P1004, Sigma-Aldrich), a cationic, cyclic AMP produced by *Paenibacillus polymyxa* that is used for treatment of infectious Gram-negative bacteria was used as a positive control. Statistical analysis was performed by one-way analysis of variance followed by Dunnett's multiple comparison test to compare differences between two groups.

Cyclooxygenase (COX) inhibitory activity

A Cyclooxygenase (Human) Inhibitor Screening Assay Kit (Cayman Chemical) was used for evaluation of the COX-1 and/or COX-2 inhibitory activity of fucoidans. COX activity was determined by measuring the synthesis of prostaglandin E_2 (PGE_2). The pre-incubation time of each reaction was set at 30 min at 37 °C. A standard curve with PGE_2 was generated at the same time and from the same plate and was used to quantify PGE_2 in the presence of fucoidans at various concentrations. ELISA (Competitive) Analysis Tools (Cayman Chemical) was used for data analysis. Data were plotted as $\%B/B_0$ vs. log concentration using a log–logit curve fit. Statistical analyses were performed by one-way analysis of variance followed by Dunnett's multiple comparison test to compare differences between control and fucoidan groups.

Results

Antimicrobial activity of fucoidans

In disc diffusion assays, fucoidan generated inhibitory zones against *C. albicans* (Fig. 1a, b), *S. mutans* (Fig. 2a, b), and *P. gingivalis* (Fig. 3a, b). These antimicrobial activities were significant compared with that of PBS.

Antimicrobial activity against *C. albicans*

Against *C. albicans* (Fig. 1a, b), large inhibition zones were present on *Fv* crude discs, with a long-axis diameter (mean ± SD) of 5.06 ± 1.34 mm that differed significantly

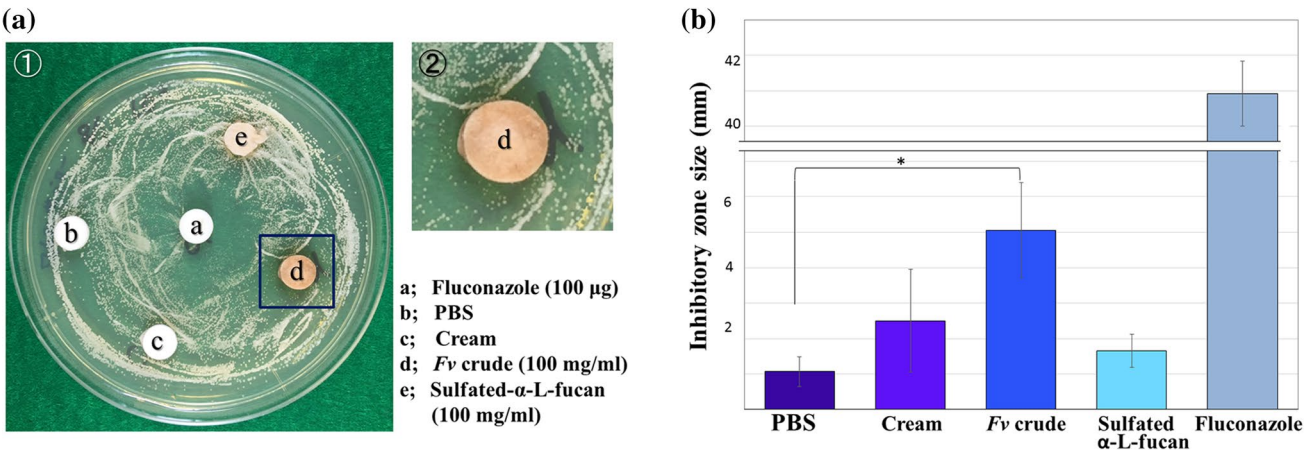

Fig. 1 Antimicrobial activities of fucoidans against *C. albicans* were assessed using the disc diffusion method. **a** Typical inhibitory zone against *C. albicans* (①), and enlarged view of the region in the blue square (②). Fluconazole (①-a) was the positive control. There was a inhibitory zone around *Fv* crude disc (①-d, ②), although the zone was an unstructured circle. **b** The inhibitory zone size was calculated by subtracting 8 mm, the diameter of paper disc, from the overall diameter. PBS, phosphate-buffered saline as a negative control; Cream, FE 4% cream; *Fv* crude,100 mg/ml *Fv* crude dissolved in PBS; sulfated α-*L*-Fucan, 100 mg/ml sulfated α-L-Fucan solution in PBS. $^{**}p < 0.01$. $^{*}p < 0.05$

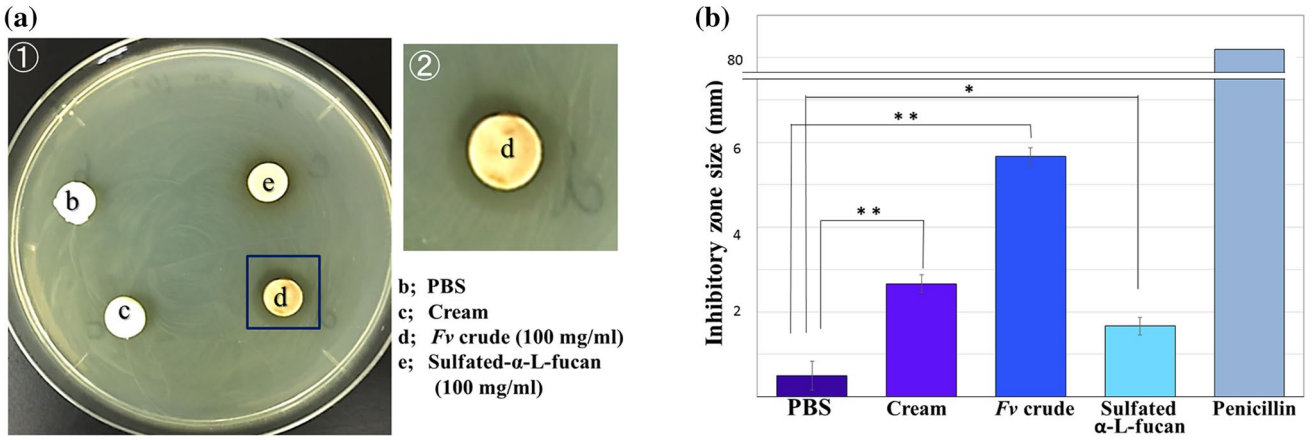

Fig. 2 Antimicrobial activities of fucoidans against *S. mutans*, assessed using the disc diffusion method. **a** Typical inhibitory zone against *S. mutans* (①), and enlarged image of the region in the blue square (②). There was a clear inhibitory zone around *Fv* crude disc (①-d, ②). For the positive control, Penicillin 10 U disc completely inhibited *S. mutans* on the plate medium, thus no inhibition circles were formed (data not shown). **b** Inhibitory zone size was calculated by subtracting 8 mm, the diameter of paper disc, from the overall diameter. $^{**}p<0.01$. $^{*}p<0.05$

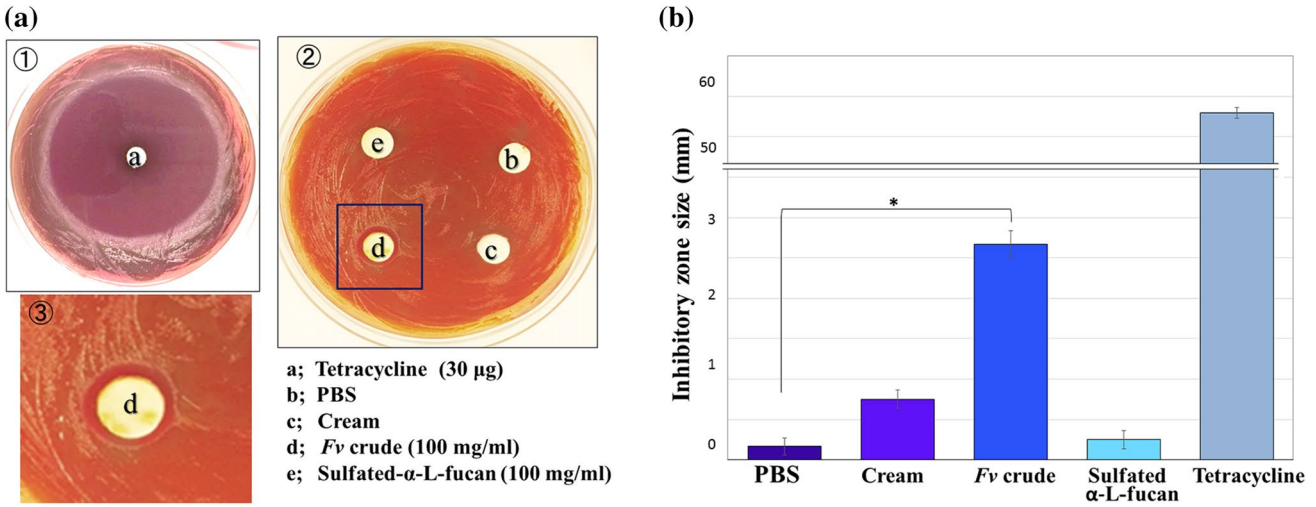

Fig. 3 Antimicrobial activities of fucoidans against *P. gingivalis*, assessed using the disc diffusion method. **a** Typical inhibitory zone against *P. gingivalis* (①, ②). Photograph "③" shows enlarged view of the region in the blue square "②". Tetracycline 30 µg discs were used as the positive control (①-a). There was a clear inhibitory zone around *Fv* crude disc (②-d, ③). **b** Inhibitory zone size was calculated by subtracting 8 mm, the diameter of paper disc, from the overall diameter. $^{**}p<0.01$. $^{*}p<0.05$

from that with the PBS control disc ($p<0.05$). Inhibition with Cream (2.51 ± 1.45 mm) and sulfated-α-L-fucan (1.66 ± 0.47 mm) showed no significant difference compared with the PBS control disc (Fig. 1b).

Antimicrobial activity against *S. mutans*

Against *S. mutans* (Fig. 2a, b), the *Fv* crude discs had the largest inhibition zones (5.67 ± 0.21 mm), followed by Cream (2.67 ± 0.21 mm), and sulfated-α-L-fucan (1.67 ± 0.21 mm). All of these zones were significantly larger than that on the PBS control disc (Fig. 2b; $p<0.01$, $p<0.01$, and $p<0.05$, respectively). As a positive control, penicillin 10 U discs completely inhibited the bacteria on the plate medium; therefore, the inhibition zones were not be able to seen (data not shown).

Antimicrobial activity against *P. gingivalis*

Against *P. gingivalis* (Fig. 3a, b), the *Fv* crude discs had the largest inhibition zones (5.33 ± 0.33 mm), with a significant difference compared with the PBS control disc ($p<0.05$). Inhibition by Cream (1.5 mm) and

sulfated-α-L-fucan (0.5 mm) was not significant compared with the PBS control.

Inhibition of *S. mutans* adhesion by fucoidans

Fv crude (50 mg/ml) significantly inhibited ($p < 0.01$) the adhesion of *S. mutans* to bovine teeth and porcelain in an Alamar Blue assay (Fig. 4a), with about 84% inhibition for bovine teeth and 77% for porcelain. In contrast, *Fv* pure had inhibition rates of only 12% with bovine teeth, although this was still significant ($p < 0.05$), and 17% with porcelain [not significant (NS)] (Fig. 4b). The adhesion inhibition rates for the same concentration of FE were 28% with bovine teeth ($p < 0.01$) and 9% with porcelain (NS) (Fig. 4c).

Endotoxin-neutralizing activity of fucoidans

An LAL assay was used to assess the ability of fucoidans to neutralize smooth-type LPS from *E. coli* O55:B5. At 0.1 µg/µl, *Fv* crude and *Fv* pure and FE all gave a significantly lower A405 than samples with endotoxin only, indicating an endotoxin neutralization activity (Fig. 5). In our experimental conditions, 10 µU/µl polymyxin-B showed 23% neutralizing activity, compared with 6–12% for 0.1 µg/µl fucoidans. The high sensitivity of the LAL assay and the relatively low sample purity prevented determination of the 50% neutralizing concentration (EC_{50}) for fucoidans. Taking the positive control polymyxin-B (10 µU/µl) activity to be 100%, the endotoxin-neutralizing activities were 67% with *Fv* crude, 53% with *Fv* pure, and 33% with FE at 0.1 µg/µl.

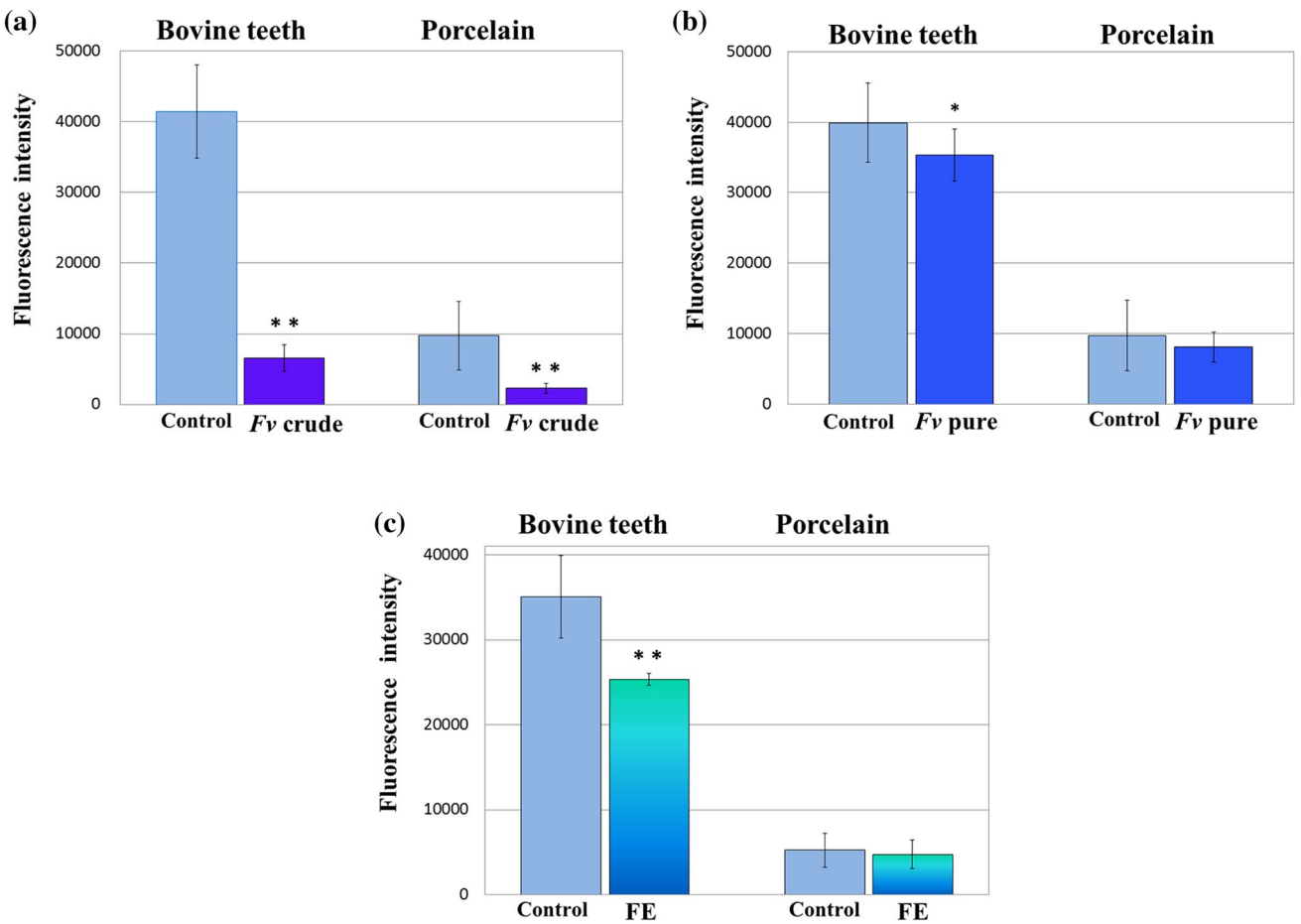

Fig. 4 Anti-adhesion activities of fucoidans for *S. mutans*. Anti-adhesion activities of fucoidans for *S. mutans* with bovine teeth and porcelain were evaluated using an Alamar Blue assay. *S. mutans* cultures were diluted with the same volume of PBS (control) or fucoidan at a final concentration of 50 mg/ml in PBS. Adhesion of *S. mutans* (about 8.0×10^7 cfu/ml) for 2 h at 37 °C is shown based on fluorescence intensity. Inhibition of adhesion of *S. mutans* by **a** *Fv* crude, **b** *Fv* pure, and **c** FE. $*p < 0.05$, $**p < 0.01$. Bars indicate mean values ± SD from three experiments each (eight samples) in duplicate for the experimental and control groups

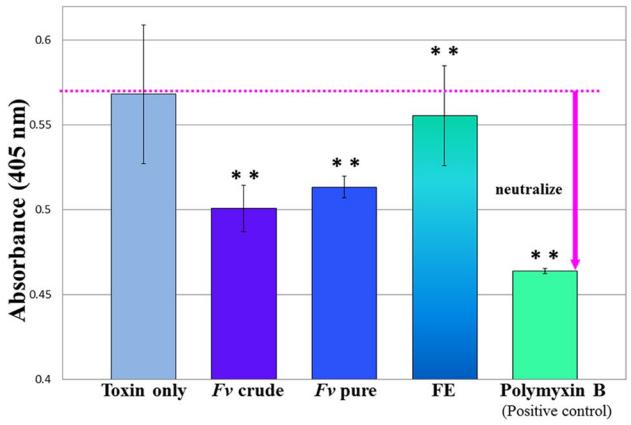

Fig. 5 Endotoxin-neutralizing activity of fucoidans. After pretreatment with Detoxi Gel, an endotoxin-removing gel, *Fv* crude, *Fv* pure and FE (all 0.1 µg/µl) were incubated with LPS from *E. coli* O55:B5 [0.5 endotoxin units (EU)/ml] at 37°C for 30 min to allow binding of fucoidans to LPS. Neutralization of the endotoxin by fucoidans was determined using a quantitative chromogenic LAL assay, based on the endotoxin absorbance at 405 nm (A405). *Fv* crude, *Fv* pure, and FE significantly reduced A405 compared with endotoxin-only samples, indicating endotoxin neutralization. The dotted line extending from "Toxin only" (LPS at 0.57 EU/ml in pyrogen-free water) indicates the total amount of toxin, and the downward arrow indicates neutralization. Polymyxin B (10 µU/µl) was used as a positive control. ** $p < 0.01$. Results are expressed as the mean ± SD of five individual experiments in duplicate

COX inhibitory activity

Fv crude significantly inhibited COX-1 activity (Fig. 6a), but did not inhibit COX-2 (Fig. 6b). The extent of COX-1 inhibition by *Fv* crude was 75, 59, and 31% at 1000, 100, and 10 ng/µl, respectively, and the inhibition constant (IC_{50}) was calculated to be 22.8 ng/µl. FE significantly inhibited both COX-1 (Fig. 6a) and COX-2 (Fig. 6b). The FE inhibition rates were 91, 67, and 16% for COX-1 at 1000, 100, and 10 ng/µl, respectively (IC_{50} 19.35 ng/µl); and 81% and 62% for COX-2 at 1000 and 100 ng/µl, respectively (IC_{50} 80.64 ng/µl). *Fv* pure showed significant inhibition at 1000 ng/µl and 100 ng/µl, but this did not exceed 50% and did not seem to be dose-dependent. Therefore, we concluded that the *Fv* pure did not have a COX inhibitory effect.

Discussion

Fucoidans have many biological activities [8], including well-known antitumor effects and the induction of apoptosis in tumor cells, which have led to their use as a supplement for cancer patients [11]. However, application of fucoidans in oral healthcare has only just begun, and there are only a few case reports of the utility of fucoidans in this area [12–14] and almost no basic science studies of fucoidans for oral healthcare. For application of fucoidans in oral healthcare, antimicrobial properties were first examined. Fucoidans showed marked antimicrobial activity against *C. albicans, S. mutans*, and *P. gingivalis.* Such activity in seaweed extracts has been widely reported [21], but studies on the antimicrobial properties of purified and identified fucoidans are limited [22–25]. Fucoidans are thought to have various antimicrobial properties based on their structures, such as branching of sugar chains and the number of sulfate groups, and on the extraction method and the species of seaweed [26]. Antimicrobial activity of fucoidans against *S. mutans* and *P. gingivalis* has been reported, but for a fucoidan of unknown origin [22]. A fraction of seaweed extract from *Colpomenia sinuosa* was also shown to have activity against *C. albicans,* but the positive fraction was

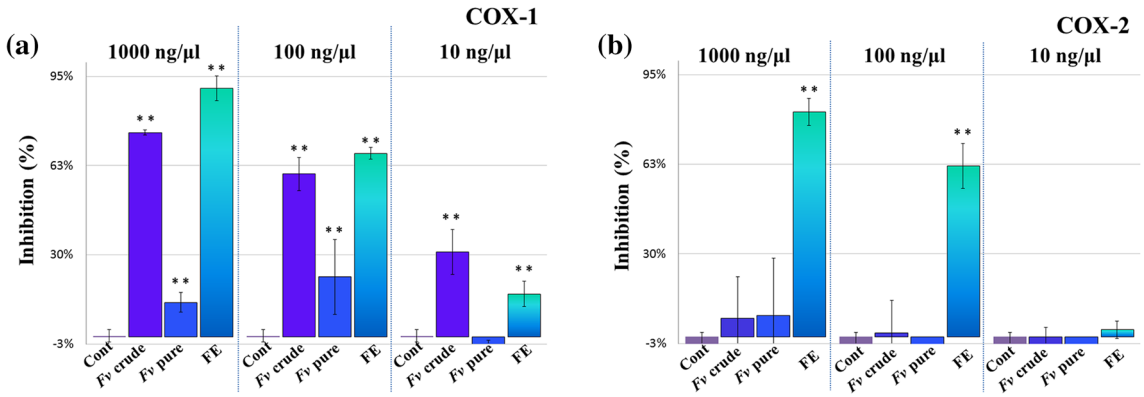

Fig. 6 Cyclooxygenase (COX) inhibitory activity of fucoidans. Inhibition of human COX-1 and COX-2 was examined using an AChE competitive ELISA. Fucoidans at three different concentrations (1000, 100, and 10 ng/µl) were preincubated with COX-1 or COX-2 and assayed. Cont, control with 0% inhibition of initial COX activity. **a** COX-1 inhibition. *Fv* crude and FE inhibited COX-1 activity, but inhibition by *Fv* pure was unclear. **b** COX-2 inhibition. Only FE significantly inhibited the COX-2 activity. Data are expressed as the mean ± SD (%) of three individual experiments in duplicate. ** $p < 0.01$

not distinctly identified as a fucoidan [27]. To avoid these problems, antimicrobial properties in the current study were examined for fucoidans with known purity and raw materials from particular seaweeds. Our results clearly showed that fucoidans have strong antimicrobial activity similar to that of fluconazole against *C. albicans.* Overall, the results suggested that fucoidans can reduce intraoral pathogens and are likely to be favorable for use in oral healthcare.

Based on the results for antimicrobial activity, three types of fucoidans were selected for use in further experiments: *Fv* crude and *Fv* pure from Bladder wrack, *F. vesiculosus* and FE from brown seaweed Mozuku, *C. novae-caledoniae*. In oral healthcare, prevention of oral biofilm formation is important, in addition to antimicrobial properties. It has been predicted that fucoidan, a sulfated polysaccharide, should have an effect when an insoluble glucan is involved in adhesion of *S. mutans* to teeth [28]. Adhesion inhibition was examined using a Alamar Blue assay for adhesion of *S. mutans* [18, 19]. All three fucoidans showed adhesion inhibition in this assay. *Fv* crude had a particularly strong inhibitory effect on adhesion, but this activity was greatly decreased for *Fv* pure. Many functions of acidic polysaccharides such as fucoidans depend on sulfate groups in the molecule [29], and fucoidans are known to lose their anticoagulant activity after defucosylation or desulfation [30]. The difference in adhesion inhibition between crude and pure fucoidan suggested that the structure of fucoidan responsible for the anti-adhesion activity was altered in the purification process. Thus, it is necessary to examine the source materials and purification methods. However, the *S. mutans* anti-adhesion property of fucoidans is likely to be valuable for the improvement of oral hygiene and dental caries prevention.

To obtain further understanding of the detailed mechanism of the antimicrobial activity of fucoidans, the endotoxin-neutralizing activity was investigated. The activities of sulfated polysaccharides such as fucoidans are based on binding with proteins and other molecules [29]. Polymyxin B is an antibiotic that binds to LPS, an endotoxin that is the major component of the outer membrane of Gram-negative bacteria, with a clinical effect due to resultant neutralization of the endotoxin [31]. We hypothesized that the antimicrobial activity of fucoidans may be derived from a similar action to that of polymyxin B. Thus, specific binding of fucoidans with the endotoxin was examined by a LAL assay (Limulus test) [20]. The results showed that fucoidans can neutralize the endotoxin, which suggests that their antimicrobial activity is derived from specific binding to oral pathogens. However, antimicrobial activity of fucoidans was also observed against *S. mutans* and *C. albicans*, which are not Gram-negative bacteria. Therefore, further studies are needed to determine the detailed mechanism underlying the antimicrobial activity of fucoidans. Fucoidans have been suggested to have anti-inflammatory effects in terms of inhibition of expression of inflammatory mediators and pro-inflammatory cytokines [32–34], but this is the first report showing that fucoidans can directly neutralize an endotoxin. This is important because endotoxins are released from biofilms in the oral cavity, and then invade the bloodstream and cause systemic symptoms [35]. Neutralization of endotoxins in the oral cavity by fucoidans may be important in oral healthcare and may lead to improvement of general symptoms.

Finally, inhibition of COX-1 and COX-2 was investigated to examine the anti-inflammatory effect of fucoidans. Suppression of COX-2 gene expression by fucoidans has been reported [33], but direct inhibition of COX enzyme activity has not been shown, although inhibition of COX-1 and COX-2 was suggested using a computational (in silico) method [36]. In this study, we used a prostaglandin-acetylcholinesterase (AChE) competitive ELISA to measure inhibition of COX-1 and COX-2 by fucoidans in vitro, and the in silico prediction was confirmed. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce inflammation [37], and there are many reports of the therapeutic effects and side effects of NSAIDs due to inhibition of COX [38]. In this study, *Fv* crude inhibited COX-1, but not COX-2. The analgesic effect of NSAIDs depends on COX-1 inhibition, rather than COX-2 inhibition [39]. Selective inhibition of COX-1 or COX-2 does not elicit gastric damage in rats; rather, inhibition of both COX isoforms is required for NSAID-induced damage to develop [40]. Therefore, *Fv* crude, which inhibited COX-1 alone, may provide a basis for development of novel NSAIDs with excellent analgesic properties. In contrast, FE inhibited both COX-1 and COX-2. It is known that NSAIDs which inhibit both isoforms of COX can cause serious gastric damage, but fucoidans are extremely safe in humans with no reports of gastric damage [41]. It has also been suggested that fucoidans have antiulcer activity against ulcers induced by an NSAID (aspirin) [42]. Therefore, FE may also form the basis of development of novel NSAIDs without a side effect of gastric damage. COX-2 activity is directly related to the inflammatory response, and the results for FE are consistent with the case reports published previously [12, 14]. With regard to FE, this only showed COX-2 inhibition. We speculated one possibility that FE may have acquired COX-2 inhibition activity by being reduced in molecular weight. Low molecular treatment applied to fucoidans can result in higher antitumor effect [9] and enhance the action of other anticancer drugs [10]. Thus, elucidation of the detailed molecular mechanism of COX inhibition by fucoidans has potential for development of new NSAIDs.

Up to now, details of the reaction mechanisms of the activities of fucoidans have not been elucidated, but

discussions should be extended to cover the difference in activities between *Fv* crude, *Fv* pure, and FE.

Contrary to the purity of fucoidans, the activities of *Fv* crude and FE were higher than that of *Fv* pure. As discussed above on the antimicrobial and anti-adhesion activities, it was reported that the sulfate group plays an important role in the activity of fucoidans [2, 3, 29]. We think there is a possibility that sulfate groups in the molecule have been affected during the purification process of *Fv* pure. Although information on the purification method of *Fv* pure has not been released (Sigma-Aldrich Customer/Technical Service), it seems to be a reasonable speculation, because the activity was highest with *Fv* crude, which has the highest sulfation degree of 23%, the second highest is FE at 14.5%, and these were consistent with the degree of sulfation and activity. The other possibility was the presence of unknown activators for fucoidan activity. *Fv* crude was manufactured based on the method of Black et al. [15]. Although many years have passed, other active substances have not been found from these conjugates [26]. In addition, as the degree of purification increased, *Fv* crude (65%), FE (85%), and *Fv* pure (95%), the activity level decreased, which was consistent with the activator hypothesis. Concerning the activator hypothesis, several studies have been conducted on the synergistic effect of the combined use of fucoidan and various substances, and there had been a patent submitted for the combined use of fucoidan with jack bean lectin, as a sugar chain recognition protein, which may significantly enhance apoptosis-inducing ability against cancer cells [43].

Further studies are required, whether the results of this study differ depending on the raw materials and/or those obtained by molecular weight reduction.

Conclusion

The results in this study suggest that fucoidans can (1) reduce oral pathogens and improve oral hygiene; (2) inhibit oral biofilm formation by anti-adhesion activity to tooth surfaces; (3) prevent endotoxin-mediated systemic inflammation due to oral pathogens by neutralizing endotoxin released from oral biofilm; and (4) improve oral inflammation by COX inhibitory activity. Our results strongly support the application of fucoidans in oral healthcare. However, many aspects of the interesting pharmacological activities of fucoidans are still unclear, and further studies are needed to establish their use in oral healthcare.

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Author contributions SO, ST, and AI designed the study; SO, MO, MM, and AI performed experiments; SO, and AI analyzed data; and SO and AI wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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