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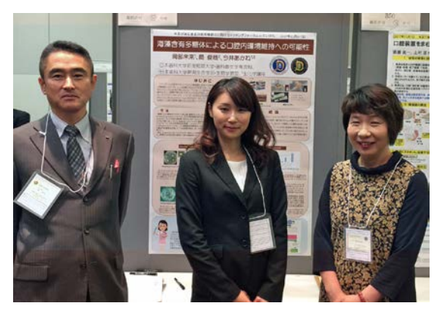
Effects of Fucoidan on Pathogens in Oral Cavity



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Objective: Fucoidan (***Fig. 1***), a mucus component of *Phaeophyta* seaweeds, has been reported to have antiulcer, cancer cell apoptotic, and immunostimulatory activities. The mechanisms of these fucoidan activities have not yet been completely elucidated. Fucoidan also has been reported to exhibit anti-*Helicobacter pylori* activity when ingested as a fucoidan-containing tea. In addition, aphthous and herpetic stomatitis have recovered with application of PF cream containing fucoidan. In the present study, we investigated whether fucoidan might inhibit the growth of oral cavity pathogens.

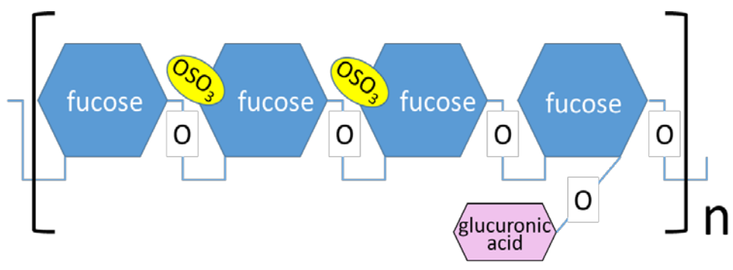

Fig. 1. The structure of fucoidan from *Nemacystus decipiens* (schematic diagram)

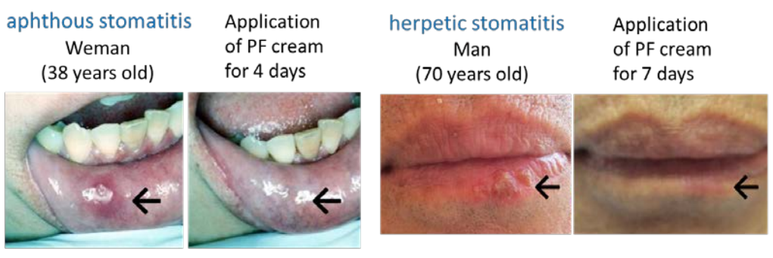

Fig. 2. Application with PF cream

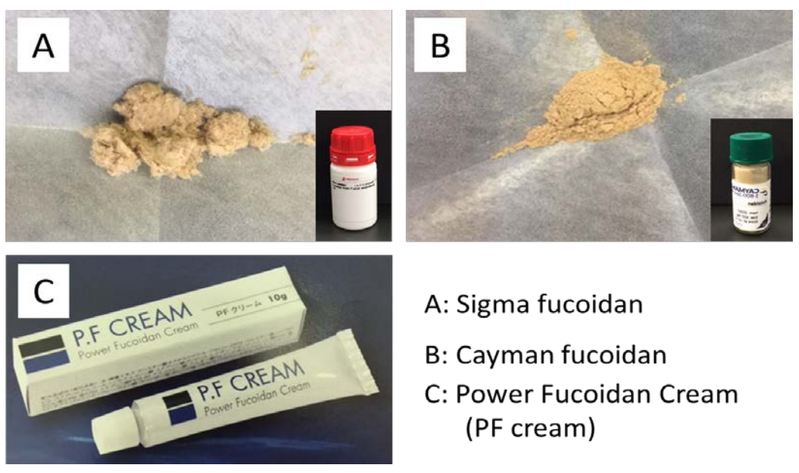

Fig. 3. Fucoidan used in this study

Methods: Disk diffusion assay: The antimicrobial activities of fucoidan against the oral pathogens *Candida albicans* (*C. albicans*. JCM1537), *Streptococcus mutans* (*S. mutans*. JCM5705), and *Porphyromonas gingivalis* (*P. gingivalis*. JCM8525) were assessed by the disk diffusion method. Five types of discs were prepared, using either an antibiotic with known activity against each pathogen, PBS, or three kinds of fucoidan (***Fig. 3***). The diameters of the inhibition zones were measured and analyzed by the one-way dispersion method and then subjected to multiple comparison using the Dunnett's test.

Growth determination using nephelometry: The effect of fucoidan supplementation on *C. albicans* growth in liquid medium was assessed by hourly measurement of absorbance at 560 nm.

Results: Disk diffusion assays revealed that fucoidan generated inhibitory zones against *C. albicans* (***Fig. 4***), *S. mutans* (***Fig. 5***), and *P. gingivalis* (***Fig. 6***); this antimicrobial activity was significant compared to that of PBS. The *C. albicans* liquid culture assay revealed showed that growth was decreased to approximately 80 and 20% of control after 9 hours of growth in the presence of 10 and 100 mg/ml fucoidan, respectively. The antimycotic activity of 100 mg/ml fucoidan was comparable to that obtained with 0.2 mg/ml fluconazole (***Fig. 7***).

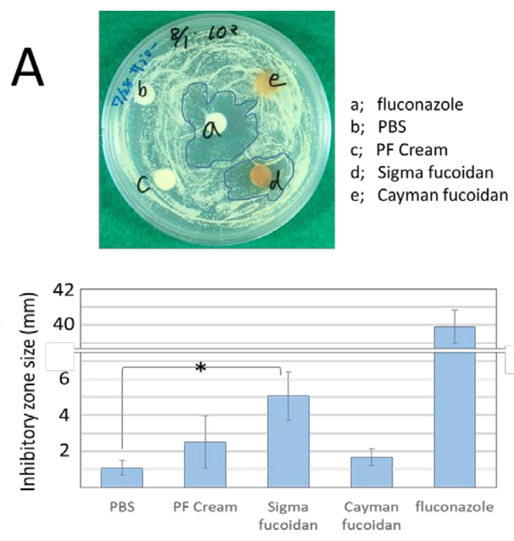

Fig. 4. Disk diffusion method was utilized to examine the antibacterial effects of Fucoidans on *C. albicans.*
A: Typical inhibitory zone against *C. albicans*. Fruconazole (a) is positive control. There was a inhibitory zone around Sigma fucoidan disc (d), though the zone was an unstructured circle.
B: Size of inhibitory zone against *C. albicans**, $P < 0.05$.

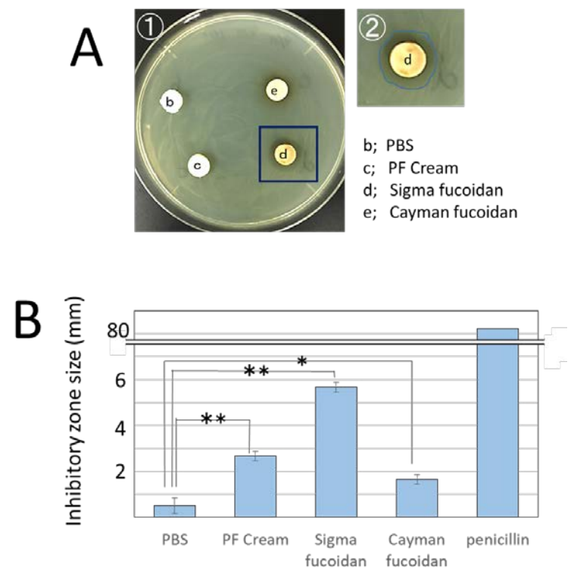

Fig. 5. Disk diffusion method was utilized to examine the antibacterial effects of Fucoidans on *S. mutans.*
A: Typical inhibitory zone against *S. mutans* . Penicillin is positive control (The inhibitory zone not shown). ② shows magnified view of the blue box in ①. There was a clear inhibitory zone around Sigma fucoidan disc (d).
B: Size of inhibitory zone against *S. mutans.* **, $P < 0.01$. *, $P < 0.05$.

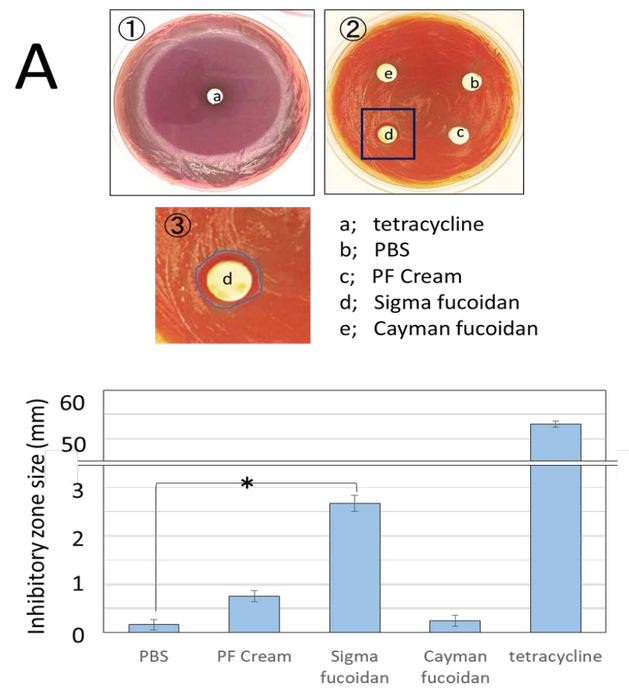

Fig. 6. Disk diffusion method was utilized to examine the antibacterial effects of Fucoidans on *P. gingivalis* .
A: Typical inhibitory zone against *P. gingivalis*. Tetracyclin is positive control. ③ shows magnified view of the blue box in ②. There was a clear inhibitory zone around Sigma fucoidan disc (d).
B: Size of inhibitory zone against *P. gingivalis.* **, $P < 0.01$. *, $P < 0.05$.

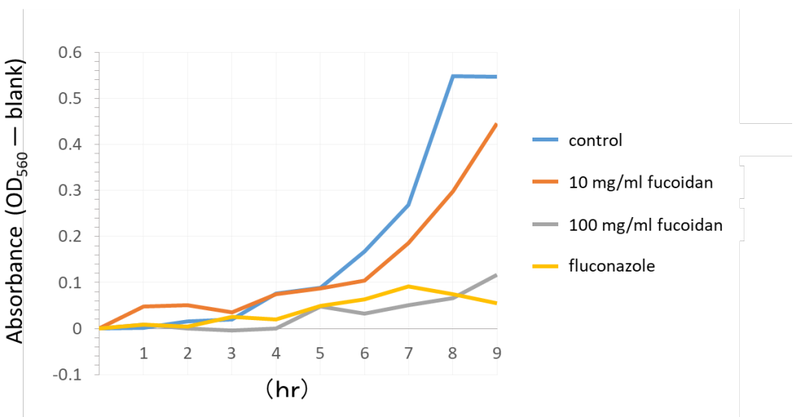

Fig. 7. Effect of Sigma fucoidan (F5631) on *C. albicans* growth curve. The effect of fucoidan supplementation on *C. albicans* growth in liquid medium was assessed by hourly measurement of absorbance at 560 nm. Inhibitory proliferation was comparable between 100 mg/ml fucoidan and fruconazole (positive control).

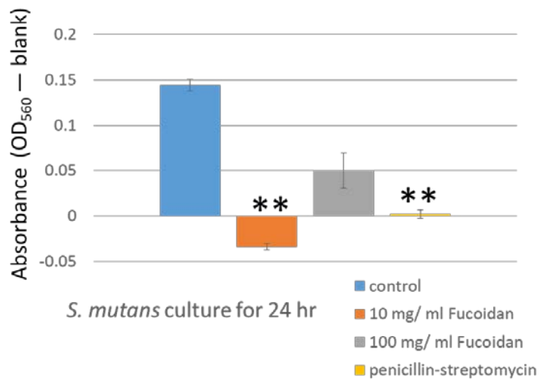

Fig. 8. Effect of Sigma fucoidan (F5631) on *S. mutans* growth. *S. mutans* was cultured for 24 hr in BHI liquid medium of fucoidan supplementation. *S. mutans* growth in was assessed by measurement of absorbance at 560 nm. Inhibitory proliferation was comparable between 100 mg/ml fucoidan and penicillin-streptomycin (positive control). The supplementation of 100 mg/ml fucoidan presented minus absorbance, because it was expected that the fucoidan of high concentration absorbed to pigmentary components in the medium. **, $P < 0.01$.

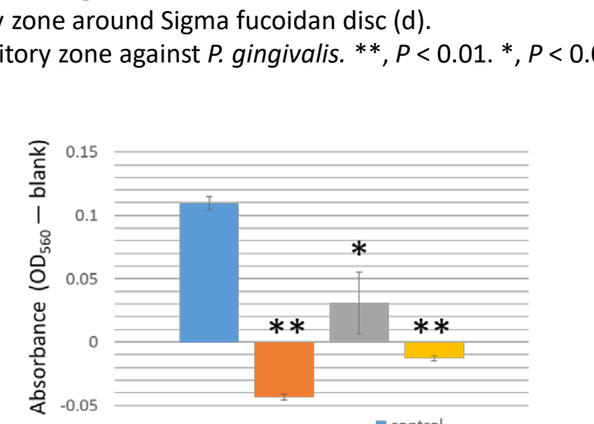

Fig. 9. Effect of Sigma fucoidan (F5631) on *P. gingivalis* growth. *P. gingivalis* was cultured for 2 days in GAM liquid medium of fucoidan supplementation. *P. gingivalis* growth was assessed by measurement of absorbance at 560 nm. Inhibitory proliferation was comparable between 100 mg/ml fucoidan and penicillin-streptomycin (positive control). The supplementation of 100 mg/ml fucoidan presented minus absorbance, because it was expected that the fucoidan of high concentration absorbed to pigmentary components in the medium. *, $P < 0.05$. **, $P < 0.01$.

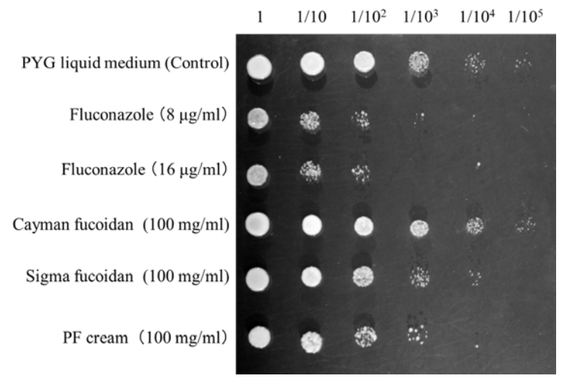

Fig. 10. Spot test of *C. albicans* growth by adding fucoidan and fluconazole. Each fucoidan and fluconazole (positive control) were added to *C. albicans* (500 cells/ml) overnight culture, and those diluents were spotted on the PYG agar plate. It was incubated at 37℃ overnight. PF cream and Sigma fucoidan inhibited *C. albicans* proliferation.

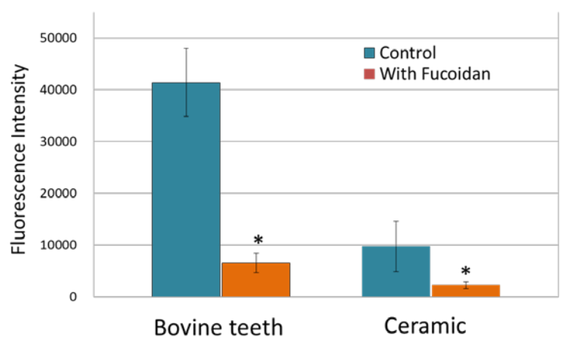

Fig. 11. Inhibition of *S. mutans* adhesion by Sigma fucoidan (50 mg/ml). The ability to inhibit adhesion of *S. mutans* to bovine teeth and porcelain was examined by applying MTT assay. The fucoidan significantly inhibited the adhesion of *S. mutans* to bovine teeth and porcelain. Student's *t* test. *, $P < 0.01$.

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Conflict of Interest: The authors declare no conflict of interests.

Conclusion: Fucoidan exhibited antimicrobial activity against *C. albicans*, *S. mutans*, and *P. gingivalis* growing both on solid medium and in liquid culture medium. These results suggested that fucoidan, currently considered a health food, might find additional application as an antimicrobial ingredient in materials used in the oral cavity, for instance, toothpaste, mouth wash, cream, *etc*.