


Fucoidan Tsushin News Letter vol. 1

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The pioneer of mozuku sea algae foods Marine Products Kimuraya Co., Ltd.



**Suppressant function of
fucoidan on side-effects
caused by anticancer agents**

Obtained the Patent by the joint research with
Shimane University

Obtained the Patent on a suppressant agent of side-effects caused by anticancer agents. Intend to develop functional foods, accumulating clinical tests.

Marine Products Kimuraya publicized the research result that mozuku fucoidan has the effect of suppressing side-effects of anticancer agents and acquired a Patent (Title: Suppressant agent of side-effects of medical agents) on Nov. 2, 2007. This achievement is the result of many years of joint research with Shimane University. Let's review the reasons why Marine Products Kimuraya started conducting research on fucoidan.

Mass food-poisoning outbreak of E. coli O-157 was a trigger to start joint research on fucoidan with Shimane University

At first, Marine Products Kimuraya primarily produced and sold mozuku sea algae products all over Japan. Later, we pioneered the development of seasoned mozuku pack, and now seasoned mekabu, aji-nanban-zuke (deep-fried horse-mackerel fish marinated in sweet and sour sauce) and processed food of marine products and mozuku fucoidan.

In 1996, when a mass E. coli O-157 food-poisoning outbreak occurred in Osaka, many students suffered. This E. coli O-157 outbreak later spread to other cities, thus alerting all of Japan to the threat of food-poisoning and creating widespread awareness of the critical importance of careful hygiene management in the food industry.

At that time, we conducted joint research with Shimane University on microbiological controls to provide thorough scientific hygiene management.

Then to confirm the safety of our seasoned mozuku pack against E. coli O-157, we added experimentally O-157 to the seasoned mozuku pack and researched the affect this had on the product.

Generally, antibiotics as one of antimicrobials can kill E. coli O-157, at the same time verotoxin① is produced②, and it increases severity of the patients.

However, as a result of our research, we clarified that E. coli O-157 were killed without production of verotoxin in the seasoned mozuku products.

Not only did the vinegar contained in the liquid preparation show this effect, the



The certificate of patent which Marine Products Kimuraya Co., Ltd. acquired. (Title of the Invention: Suppressant agent of side-effects of medical agents. Patent No. 4034146)

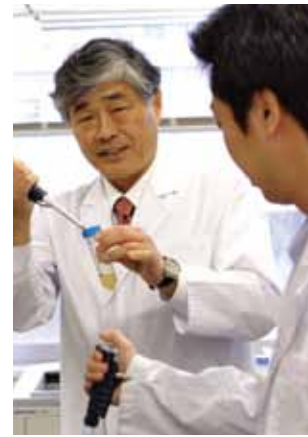
mozuku also showed more effective antibacterial action. Then we thought that mozuku might contain effective constitution.

As the result, it was clarified that the substance which was hydrothermally extracted from mozuku had antibacterial action, and further that high molecular fraction③ had antibacterial action.

Later it was clarified that the active substance included in this viscous constituent of mozuku was fucoidan.

This became a trigger for us to start researching fucoidan.

The patent “Suppression effect of side-effects of anticancer agents” was an achievement of many years of joint research with Shimane University.



Research scene of fucoidan together with our technical advisor, Prof. Emeritus Mr. Hideyuki Matsuda (Left)

Now that we are proceeding with joint research on the action experiment and clinical tests with Tottori University Faculty of Medicine, we have great expectations of the results of this new research.

We are addressing various research challenges of fucoidan, other than this patent, and achieving significant results.

Now that we are proceeding with joint research on the action experiment and clinical tests with Tottori University Faculty of Medicine, we have great expectations of the results of this new research.

Inhibition effect of proliferation of cancer cell & suppressant effect of side-effects of anticancer agents

In the experiment, we used high polymer fucoidan experimentally extracted from Okinawa mozuku (*Cladosiphon okamuranus*).

We researched effect of the extracted mozuku fucoidan against human stomach cells. Adding cell suspension of 2,000 pieces/well and fucoidan of 1mg/ml on 96 well plate ④, we cultured for 4 days.

Then by MTT assay⑤, we checked the influence of cell proliferation.

MTT assay is a method to measure viable cell count, utilizing the shift of MTT which is tetrazolium-salt⑥, to blue formazan product⑩ by work of succinate dehydrogenase⑧⑨ in mitochondria⑦.

They calculated survival ratio assuming survival ratio 0% for the absorbance ⑪ in the culture media which contains neither cell nor specimen, survival ratio 100% for the absorbance of cell cultured in the proliferation culture media which doesn't contain specimen.

As for influence of fucoidan on human stomach cell lines⑫ MKN45, the survival ratio of cancer cell was 50%, it was clarified that mozuku fucoidan had a suppression effect on proliferation.

Mozuku fucoidan didn't affect proliferation of normal stomach cell Hs677.st. 1)

Generally, when using anticancer agents, like chemotherapy, to fight cancer, patients likely suffer from such side-effects as loss of appetite and vomiting.

As one of the causes of side-effects, the anticancer agents kill not only cancer cells which rapidly proliferate, but also normal cells.

Then we researched what kind of influence fucoidan exerts on the action of anticancer agents.

Using 5-fluorouracil (5-FU) 2) which is known as an anticancer agent, we added cell suspension of 2,000 pieces/well and fucoidan of 1mg/ml on 96-well-plate, we incubated for 4 hours and then added 5-FU of 50µg/ml, and we cultured for 4 days.

By MTT assay method we researched the influence on cell proliferation using normal stomach cell Hs677.st.

Commercially available fucoidan derived from fucus vesiculosus and alginate sodium didn't affect 5-FU action but mozuku fucoidan inhibited 5-FU action, and normal stomach cells survived.

Also 5-FU action against stomach cancer cells wasn't influenced, and the suppression effect of side-effects of anticancer agents were presented.

It was considered to utilize as side-effects agents 3). (Fig.)

This was the achievement of joint research with Shimane University.

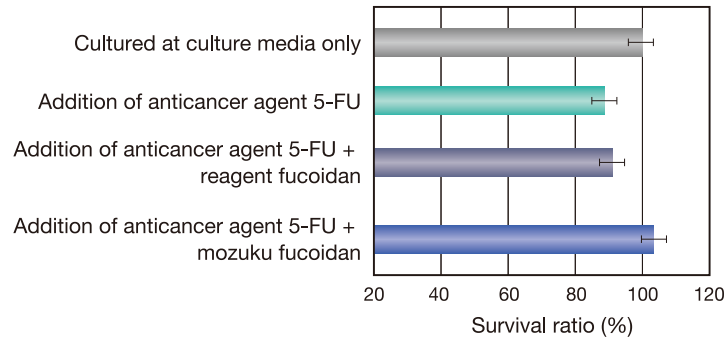
[Reference]

1)Hitoshi KAWAMOTO, Yasunari MIKI, Takayuki MIMURA, Tsuyoshi NAKAGAWA, Makoto KAWAMUKAI, Hideyuki MATSUDA. Effects of fucoidan from Mozuku on human stomach cell lines. Food Science and Technology Research 12(3):218-222(2006)

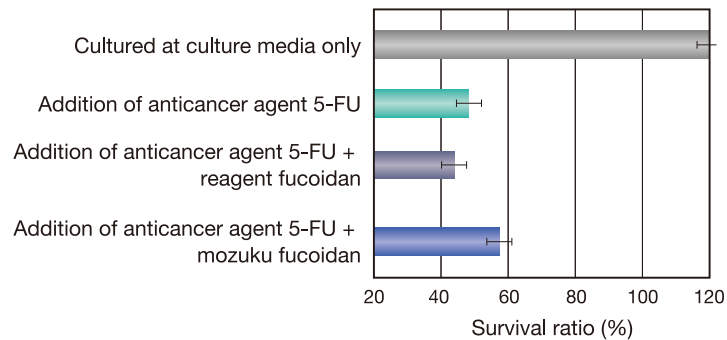
2)Masashi SASA, Yoshitsugu OKUMA. YAKURIGAKU Pharmacology, Kinpudo (1992)

3)Patent 4034146 "Suppressant of Medical agent"

Human stomach normal cell Hs667.st



Human stomach cancer cell MKN45



Glossary

- ① **Verotoxin**: Toxin which a part of pathogenic fungus as pathogenic e. coli bacteria and a part of dysentery bacillus secretes
- ② **Produce**: Living organism produces substance and secretes. Nearly same meaning of production.
- ③ **Fraction**: Constituent separated from mixture of multiple substances.
- ④ **96-well-plate**: Culture vessel having 96 holes (wells)
- ⑤ **MTT assay**: Analytical method to measure number of alive cells by color change with a reagent called MTT.
- ⑥ **Tetrazolium-salt**: General term for yellow compound MTT in MTT assay.
- ⑦ **Mitochondria**: Organ in cell related to aerobic respiration.
- ⑧ **Enzyme**: Substance to accelerate chemical reaction in a living body. It doesn't change itself before and after the reaction
- ⑨ **Succinate dehydrogenase**: An enzyme in a living cell related to respiration.
- ⑩ **Formazan product**: General term for blue compound produced in MTT assay.
- ⑪ **Absorbance**: Density index of substance measured with utilization of light.
- ⑫ **Cell lines**: One kind of cell



Marine Products Kimuraya Co., Ltd.
Director, Head of R&D Division

Yasunari MIKI

Research of fucoidan by Marine Products Kimuraya

Marine Products Kimuraya Co., LTD. is a company that produces and sells seasoned mozuku. In order to enhance quality control management, we collaborated with Shimane University Faculty of Life and Environmental Science to jointly research the “Practical research related to quality maintenance and fermentation control of sea algae by biotechnology”.

On the extension line of this research, we discovered effective constituent of mozuku and later we learned that this had the same constituent as fucoidan substance. As we introduced in this news-letter, we made an epoch-making discovery that “fucoidan has suppressant effect of side-effects of anticancer agent”, in the experiment in which we used cultured cells, in 2001.

Further, Marine Products Kimuraya Co., LTD. started joint research on functional assessment of health activity by fucoidan with Tottori University Faculty of Medicine since 2005, and with Tottori University Faculty of Agriculture Dept. of Veterinary Medicine, and also with Tottori University Faculty of Engineering -- totally 17 researches over 5 years.

For next fiscal year, we plan to conduct new 4 research studies.

Now, total numbers of joint research of Tottori University, 160 in 2006, 168 in 2007, 165 in 2008, therefore for Tottori University, our company is a key partner that conducts a large number and variety of joint research studies.

In 2004-2008, Marine Products Kimuraya Co., LTD. participated in “City Area Industry, Academia, Government Cooperation Program” sponsored by “Ministry of Education, Culture, Sports, Science and Technology” (in the area of Yonago city and Sakaiminato

city, Tottori) (<http://www.toriton.or.jp/area/>) and carried out research on the functionality of fucoidan, supplying our extracted and refined fucoidan as research materials.

Among those researches, remarkable results included the following:

(1) As preventive and therapeutic effect of fucoidan for lifestyle related diseases, we discovered an improving effect of acidic urine.

This effect leads to prevention of gout.

(2) We discovered that fucoidan has improving effect of abdominal conditions.

(3) We discovered that fucoidan has the preventive activity of thrombosis.

(4) We confirmed in clinical tests that fucoidan has suppressant activity on the side-effects of anticancer agents.

Thus it was clarified that fucoidan has functions in various fields.

The reason why fucoidan shows those functional activities, is considered because the mechanism of health activity is to activate immunity power which humans originally have.

We would like to help your health, clarifying fucoidan's power, extracted from Okinawa mozuku, which is grown in a rich natural environment of Okinawa where the sun is shining brightly.

As for the functional activities of fucoidan, which were discovered in our research, we would be glad to introduce a series of "Fucoidan Tsushin" News Letters.

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