Infiniderm® Daily Youth Lotion Executive Presentation

an Infinitum Health, LLC product

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FDA Disclaimer

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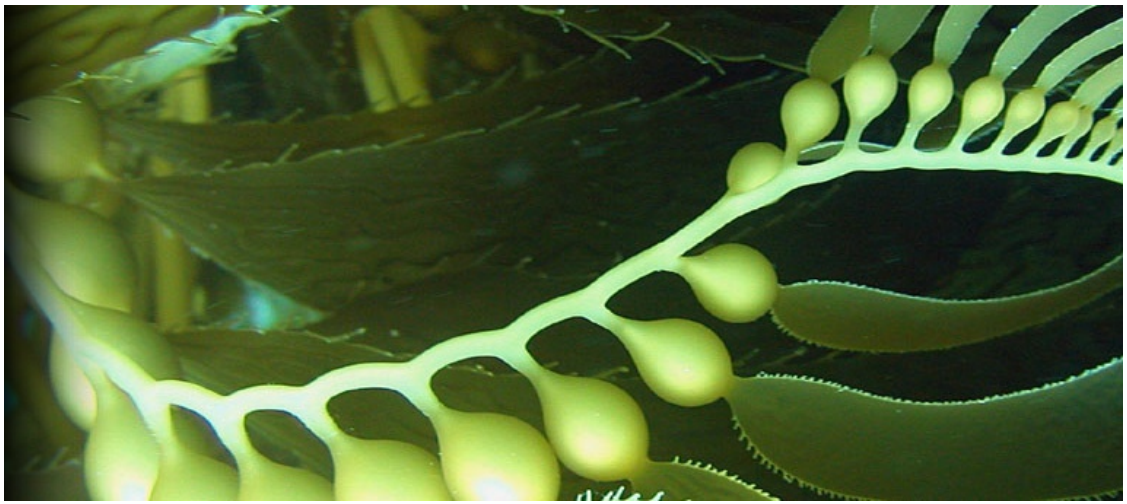
Infinitum Health, LLC Value Proposition

"To provide integrative medicine products, with evidence based research, and aid in providing a healthful existence, forever, without limits."



Executive Summary - Company

Infinitum Health, LLC was founded in March, 2013. Infinitum Health, LLC was created to deliver high quality natural holistic healthcare products to increase the overall health of the consumer.

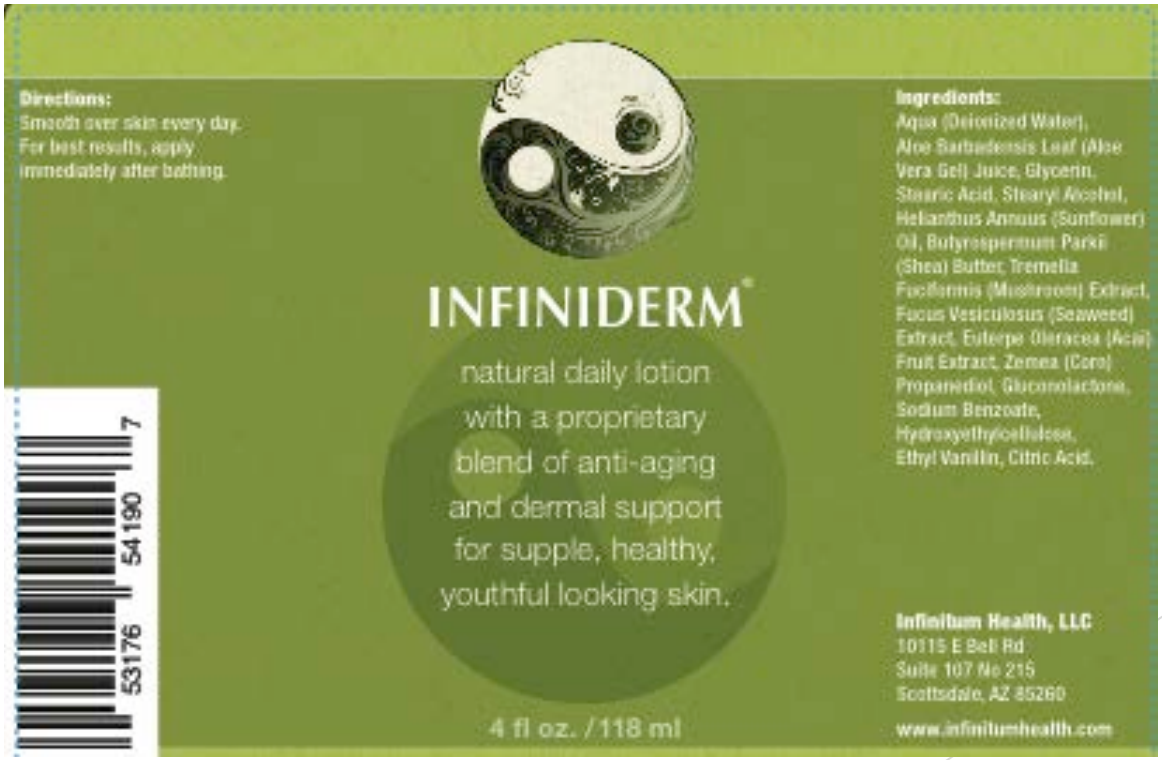
As Infinitum Health, LLC grows, we plan to have strong evidence-based research behind every product to help support its use, further its research, and expand its reach across the globe. We feel that by providing the evidence-based research to the consumer, they can be empowered and inform themselves on our products and their related health benefits. We look forward to collaborating with our consumer in providing a "healthful existence, forever, without limits."

Executive Summary - Product Infiniderm®

- Infiniderm®, is a premium natural daily lotion with a rare patented blend of unique extracts of Acai berry (antioxidant), Fucoidan extract (from Bladderwrack seaweed, *fucus vesiculosous*), and ß-glucans (*tremella fuciformis* mushroom) that are clinically proven for anti-aging and dermal support for supple, healthy, youthful looking skin.
- The extracts in the patented blend have over 1400 research articles illustrating their promising health benefits.
- The extract proprietary blend has a combination of over 5000 years of history of use with Traditional Chinese Medicine, Brazilian Medicine, and Ayurvedic Medicine and 100 years of efficient Western medicine use, bringing you a truly holistic daily lotion.

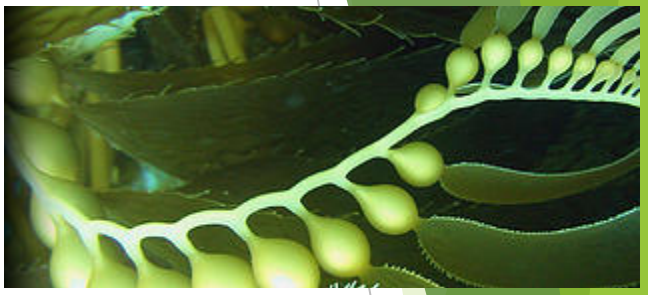
Infiniderm®- Label

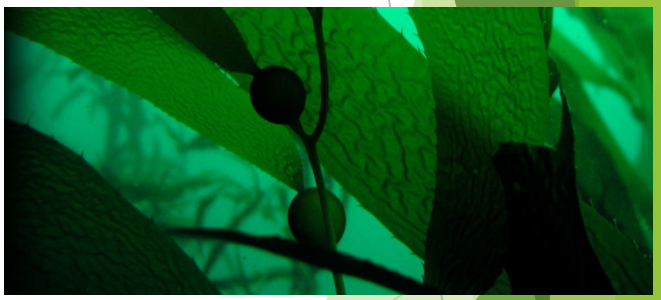
- Simple, elegant, and impactful labeling and formulation for “eye catching” consumers
- Ease of use instructions
- Simple tagline “… supple, health, youthful looking skin”



Patented Blend - Fucoidan

- **Fucoidan - from seaweed - becoming the most potent immunity stimulator on earth**
- In our product, this extract from brown seaweeds [from Bladderwack (***fucus vesiculosus***) shown significant promising immunostimulant and dermal support properties[1],[3]*.
- Fucoidan has blossomed the past 10 years in terms of evidence based research and is looking to be the most promising health support extract in the world from our ancient oceans.
- Having evolved and survived an intense environment of our oceans for over 3 billion years, seaweeds are one of our oldest and most beneficial plants to complement our overall daily diet and skin health.[2]





[1]Bo Li, Fei Lu, Xinjun Wei and Ruixiang Zhao; Fucoidan: Structure and Bioactivity. Molecules 2008, 13, 1671-1695

[2]Marcel Tutor Ale, Jørn D. Mikkelsen and Anne S. Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds. Mar. Drugs 2011, 9, 2106-2130

[3]. Treatment of human skin with an extract of Fucus vesiculosus changes its thickness and mechanical properties. Journal of Cosmetic Science, 2002.

Patented Blend – ß-glucans

- **ß-glucans - from mushrooms - broad spectrum anti-aging and immune enhancers**
- Extracts (ß-glucans) from specific mushrooms have been shown to have multiple immune supporting properties as well as improving skin health[3]*.
- Tremella Mushroom (***tremella fuciformis***,银耳), also known as silver ear mushroom, has been used for beauty regimes in China and Japan for over 2000 years[3].
- Tremella increases moisture retention in the skin and prevents senile degradation of micro-blood vessels in the skin, reducing wrinkles and smoothing fine lines, as well as providing a natural UV protectant[4].
- Other anti-aging effects come from increasing the presence of superoxide dismutase in the brain and liver; it is an enzyme that acts as a potent antioxidant throughout the body, particularly in the skin[4].

[3] Wasser, S. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology. March 2011, Volume 89, Issue 5, pp 1323-1332

4. Reshetnikov SV, Wasser SP, Duckman I, Tsukor K. (2000). "Medicinal value of the genus Tremella Pers. (Heterobasidiomycetes) (review)". International Journal of Medicinal Mushrooms 2 (3): 345-67.

Proprietary Blend – Acai Berry

- **Acai Berry - the most potent antioxidant in the world**
- The Acai berry (***euterpe oleracea***) from Brazil has shown to be the most potent anti-oxidant (anti-aging) in the world.
- In the lab, Acai has been found to possess remarkable antioxidant activity in human cells, even when diluted to one part per trillion†.
- Acai, among all antioxidant extracts, has the most significant impact and supports your cells to stay alive. This support is shown by what is know as the oxygen radical absorbance capacity, or ORAC. While other analytic methodologies may be used, ORAC is often considered preferable because of its biological relevance to antioxidant action in vivo (in living organisms). It measures both the degree and speed with which a certain food inhibits the action of an oxidizing agent, then integrates these two measurements into a single value, producing an accurate assessment of different types of antioxidants of different strengths.

ORAC: TOP 5 - RANKED ANTIOXIDANT FOODS (ORAC units per 100 grams (about 3.5 oz))

Açaí berries	18,400
Pomegranates	10,500
Blackberries	5,100
Bilberry	4,200
Blueberries	3,200

†Schauss AG, Wu X, Prior RL, Ou B, Huang D, Owens J, Agarwal A, Jensen GS, Har, AN, Shanbrom E. Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, Euterpe oleraceae mart. (acai). Agric Food Chem. 2006 Nov 1;54(22):8604-10
Schauss AG, Wu X, Prior RL, et al. Phytochemical and nutrient composition of the freeze-dried Amazonian palm berry, Euterpe oleracea Mart. (Açai). J Agric Food Chem. 2006 Nov 1;54(22):8598-603.
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Lotion Complements / Foundation

- Aloe Vera – consistent base as well as long history of dermal health support
- Shea Butter – for consistency and smell
- Sunflower oil – for consistency
- Citric Acid – for skin tightening
- Vanillin – base smell of vanilla

Market Space

- Natural and Personal Care/HH
- >$27.1 Billion market
- Key Leaders (Daily Lotion)
  - Vaseline® Aloe Fresh (Unilever)
    - ~$250 Million
  - Aveeno® Daily Lotion ~$200 Million

By Product Category	2007 ($Billion)	2008($Billion)	2008Growth
Vitamins & Minerals	29,810	31,980	7%
Herbs & Botanicals	20,260	20,900	3%
SHM&S Supplements	22,160	23,670	7%
Total Supplements	**72,230**	**76,550**	**6%**
Natural & Organic Food	63,230	70,800	12%
N&O Personal Care/HH	24,310	27,100	11%
Functional Food	90,110	95,350	6%

"Global Nutrition Sales Expand 8% in 2008 Despite Economic Storm." Nutrition Business Journal. Nov/Dec, 2009 Issue

Marketing

- Highest ROI is word of mouth*
- Customer Testimonials
  - Whitney McLennan, Polycystic Ovarian Disease, IBD, Kidney Tumor, Gall Bladder Stones, Infertile
  - Polycystic Ovarian Disease cleared up, Tumor eliminated, and pregnant (4 month timeframe of taking Infinimin®)
  - http://www.infinitumhealth.com/our-story
- Research
  - Infinitum Health Preclinical Proof of Concept Anti-Cancer Study
  - *"A statistically significant reduction in the viability of all 4 cancer cell lines (glioblastoma, prostate, lung, and melanoma) was seen following treatment with Infinimin®."*
- Professional Athletics
  - Ginger Huber, sponsored professional high diver, currently competing in Red Bull® High Diving competitions, High Diving currently under review by International Olympic Committee for 2020 Olympics
- Social Media
  - Facebook, Instagram, Twitter
  - 10,000 likes on Facebook, active community sharing stories on health promotion from Infinitum Health, LLC

*95% of sales were sourced by word of mouth, referrals, and recommendations

Infiniderm® Price(s)

- Infiniderm® Suggested Retail Sale Price
  - $24.99
- Infiniderm® Wholesale Price
  - $11.99
- Margin: 52%
- Price is extremely competitive to Vaseline Aloe Fresh® and Aveeno Daily Lotion® with fucoidan, beta glucans, and acai raw material (source: Nutrition Business Journal, Jan, 2011)
  - ~$55

Audience / Distribution

- Informed, health conscious individuals
- Beauty and Cosmetic Stores
  - Ulta, MAC, Sephora
- Health/Nutrition stores
  - Hi-Health, GNC, VitaminShoppe, VitaminWorld
- Large Market Stores – Natural Focus – Future Market
  - Whole Foods, Sprouts, Fresh N Easy, Trader Joes
- Online
  - Amazon.com, Drugstore.com, Pharmaca.com
  - Smaller Sales Channels:
    - Health clubs, spas, dermatologist offices, naturopathic physician offices, chiropractic physician offices, allopathic physician offices

Additional Products

- Products to be added after due diligence research and safey concerns is concluded:
  - Infinimin® Ultravimtain
    - Natural premium multivitamin with a rare patented blend of Acai berry (antioxidant), Fucoidan extract (from seaweed), ß-glucans (from Reishi and Maitake mushrooms) that support anti-aging and dramatic immunostimulant properties - while providing 100% of Daily Value of 10 essential nutrients to help support your heart health, immunity, eye health and physical energy.
  - Releaf®
    - Unique natural pain reliever proprietary blend supporting muscle aches, headaches, and chronic pain.
  - Ancient Cleanse®
    - Natural face and body soap comprised of a proprietary blend for cleaning and exfoliating skin for a rejuvenating and healtful look.

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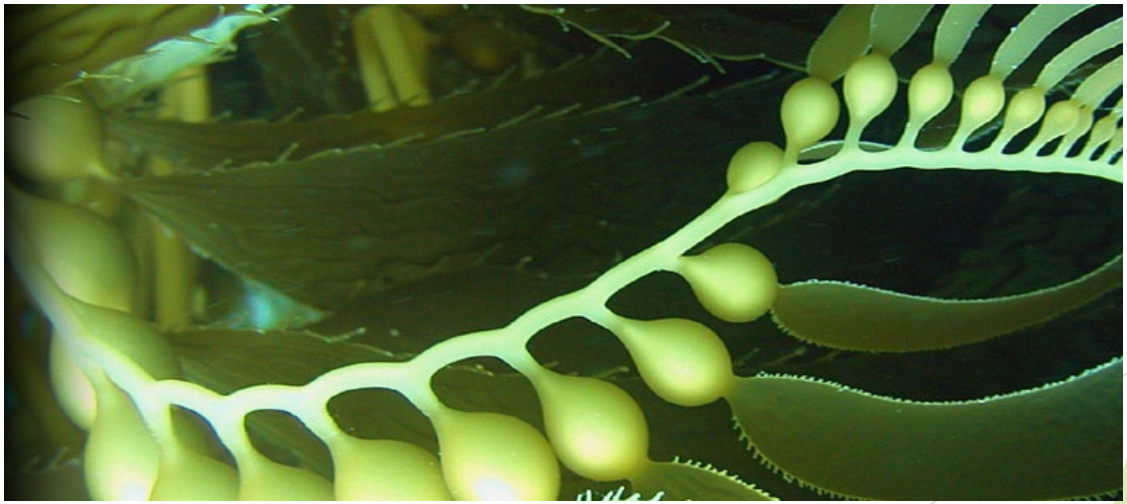

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References

Fucoidan: Structure and bioactivity

Fucoidan refers to a type of polysaccharide which contains substantial percentages of L-fucose and sulfate ester groups, mainly derived from brown seaweed. For the past decade fucoidan has been extensively studied due to its numerous interesting biological activities. Recently the search for new drugs has raised interest in fucoidans. In the past few years, several fucoidans' structures have been solved, and many aspects of their biological activity have been elucidated. This review summarizes the research progress on the structure and bioactivity of fucoidan and the relationships between structure and bioactivity.

Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds

Seaweeds—or marine macroalgae—notably brown seaweeds in the class Phaeophyceae, contain fucoidan. Fucoidan designates a group of certain fucose-containing sulfated polysaccharides (FCSPs) that have a backbone built of (1→3)-linked α-l-fucopyranosyl or of alternating (1→3)- and (1→4)-linked α-l-fucopyranosyl residues, but also include sulfated galactofucans with backbones built of (1→6)-ß-d-galacto- and/or (1→2)-ß-d-mannopyranosyl units with fucose or fuco-oligosaccharide branching, and/or glucuronic acid, xylose or glucose substitutions. These FCSPs offer several potentially beneficial bioactive functions for humans. The bioactive properties may vary depending on the source of seaweed, the compositional and structural traits, the content (charge density), distribution, and bonding of the sulfate substitutions, and the purity of the FCSP product. The preservation of the structural integrity of the FCSP molecules essentially depends on the extraction methodology which has a crucial, but partly overlooked, significance for obtaining the relevant structural features required for specific biological activities and for elucidating structure-function relations. The aim of this review is to provide information on the most recent developments in the chemistry of fucoidan/FCSPs emphasizing the significance of different extraction techniques for the structural composition and biological activity with particular focus on sulfate groups

Current findings, future trends, and unsolved problems in studies of medicinal mushrooms

The target of the present review is to draw attention to many critically important unsolved problems in the future development of medicinal mushroom science in the twenty-first century. Special attention is paid to mushroom polysaccharides. Many, if not all, higher Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and cultured broth. The data on mushroom polysaccharides are summarized for approximately 700 species of higher Hetero- and Homobasidiomycetes. The chemical structure of polysaccharides and its connection to antitumor activity, including possible ways of chemical modification, experimental testing and clinical use of antitumor or immunostimulating polysaccharides, and possible mechanisms of their biological action, are discussed. Numerous bioactive polysaccharides or polysaccharide–protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses and exhibit antitumor activities in animals and humans. Stimulation of host immune defense systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation, and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumor cells. While the mechanism of their antitumor actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Particularly and most importantly for modern medicine are polysaccharides with antitumor and immunostimulating properties. Several of the mushroom polysaccharide compounds have proceeded through phases I, II, and III clinical trials and are used extensively and successfully in Asia to treat various cancers and other diseases. A total of 126 medicinal functions are thought to be produced by medicinal mushrooms and fungi including antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, antiparasitic, antifungal, detoxification, hepatoprotective, and antidiabetic effects.

References

Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, Euterpe oleraceae mart. (acai).

The fruit of Euterpe oleraceae, commonly known as acai, has been demonstrated to exhibit significantly high antioxidant capacity in vitro, especially for superoxide and peroxyl scavenging, and, therefore, may have possible health benefits. In this study, the antioxidant capacities of freeze-dried acai fruit pulp/skin powder (OptiAcai) were evaluated by different assays with various free radical sources. It was found to have exceptional activity against superoxide in the superoxide scavenging (SOD) assay, the highest of any food reported to date against the peroxyl radical as measured by the oxygen radical absorbance capacity assay with fluorescein as the fluorescent probe (ORACFL), and mild activity against both the peroxynitrite and hydroxyl radical by the peroxynitrite averting capacity (NORAC) and hydroxyl radical averting capacity (HORAC) assays, respectively. The SOD of acai was 1614 units/g, an extremely high scavenging capacity for O2*-, by far the highest of any fruit or vegetable tested to date. Total phenolics were also tested as comparison. In the total antioxidant (TAO) assay, antioxidants in acai were differentiated into "slow-acting" and "fast-acting" components. An assay measuring inhibition of reactive oxygen species (ROS) formation in freshly purified human neutrophils showed that antioxidants in acai are able to enter human cells in a fully functional form and to perform an oxygen quenching function at very low doses. Furthermore, other bioactivities related to anti-inflammation and immune functions were also investigated. Acai was found to be a potential cyclooxygenase (COX)-1 and COX-2 inhibitor. It also showed a weak effect on lipopolysaccharide (LPS)-induced nitric oxide but no effect on either lymphocyte proliferation and phagocytic capacity.

Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, Euterpe oleraceae mart. (acai).

Euterpe oleraceae is a large palm tree indigenous to the Amazon River and its tributaries and estuaries in South America. Its fruit, known as acai, is of great economic value to native people. In this study, a standardized freeze-dried acai fruit pulp/skin powder was used for all analyses and tests. Among many findings, anthocyanins (ACNs), proanthocyanidins (PACs), and other flavonoids were found to be the major phytochemicals. Two ACNs, cyandin 3-glucoside and cyanidin 3-rutinoside were found to be predominant ACNs; three others were also found as minor ACNs. The total content of ACNs was measured as 3.1919 mg/g dry weight (DW). Polymers were found to be the major PACs. The concentration of total PACs was calculated as 12.89 mg/g DW. Other flavonoids, namely, homoorientin, orientin, isovitexin, scoparin, and taxifolin deoxyhexose, along with several unknown flavonoids, were also detected. Resveratrol was found but at a very low concentration. In addition, components inluding fatty acids, amino acids, sterols, minerals, and other nutrients were analyzed and quantified. Total polyunsaturated fatty acid, total monounsaturated fatty acid, and total saturated fatty acids contributed to 11.1%, 60.2%, and 28.7% of total fatty acid. Oleic acid (53.9%) and palmitic acid (26.7%) were found to be the two dominant fatty acids. Nineteen amino acids were found; the total amino acid content was determined to be 7.59% of total weight. The total sterols accounted for 0.048% by weight of powder. The three sterols B-sitosterol, campesterol, and sigmasterol were identified. A complete nutrient analysis is also presented. Microbiological analysis was also performed.

References

A glycoprotein (Fucoidan)from Laminaria japonica induces apoptosis (cell death) in HT-29 colon cancer cells

We isolated a novel glycoprotein (Fucoidan) from the brown alga Laminaria japonica that has antiproliferative effects on HT-29 colon cancer cells. We also identified the mechanism by which this glycoprotein, named LJGP, induces apoptosis. MTS assays showed that LJGP inhibited the proliferation of several cancer cell lines (AGS, HepG2, HT-29) in a dose-dependent manner. Especially in HT-29 cells, proliferation was significantly decreased. LJGP treatment on HT-29 displayed several apoptotic features, such as DNA fragmentation, sub-G1 arrest, caspase-3 activation, and PARP degradation. Consistent with sub-G1 arrest, LJGP decreased the expression of Cdk2, cyclin E, cyclin D1, PCNA, E2F-1, and phosphorylated pRb. Furthermore, the increase of p27 expression was observed. We also determined that LJGP-induced apoptosis leads to the formation of a death-induced signaling complex of Fas, FADD, and procaspase-8. LJGP induced the reduction of mitochondrial membrane potential with activation of the Bcl-2 family of proteins and caspase-9. These findings suggest that LJGP inhibits HT-29 cell proliferation by inducing apoptosis, which may be mediated via multiple pathways, including the Fas signaling pathway, the mitochondrial pathway, and cell cycle arrest. **Therefore, LJGP can be a useful treatment option for colon cancer in humans.**

Apoptosis (cell death) induction by glycoprotein isolated from Laminaria japonica is associated with down-regulation of telomerase activity and prostaglandin E2 synthesis in AGS human gastric cancer cells.

Glycoprotein (Fucoidan) isolated from Laminaria japonica (LJGP) is known to exhibit significant cytotoxic activity against human cancer cells; however, the mechanisms of its cytoxicity are poorly understood. In this study, we investigated further possible mechanisms by which LJGP exerts its anti-cancer action in cultured human gastric carcinoma AGS cells. LJGP treatment of AGS cells resulted in inhibition of growth and induction of apoptosis in a time- and concentration-dependent manner, as determined by MTT assay, fluorescence microscopy, and flow cytometry analysis. The increase in apoptosis was associated with up-regulation of pro-apoptotic Bax expression, down-regulation of anti-apoptotic Bcl-2 and IAP family members, and activation of caspase-3 and -9. LJGP treatment markedly down-regulated the activity of telomerase and expression of human telomerase reverse transcriptase, a main determinant of telomerase enzymatic activity, with inhibition of Sp1 and c-Myc expression in a concentration-dependent manner. Furthermore, LJGP treatment also caused a progressive decrease in the expression levels of cyclooxygenase (COX)-2 without significant changes in the levels of COX-1, which was correlated with a decrease in prostaglandin E2 synthesis. **These results provide important new insights into the possible molecular mechanisms of the anti-cancer activity of LJGP.**

Fucoidan-Vitamin C complex suppresses tumor invasion through the basement membrane, with scarce injuries to normal or tumor cells, via decreases in oxidative stress and matrix metalloproteinases

Fucoidan (Fucdn) and vitamin C (VC) were saturatedly dissolved in water and lyophilized and thoroughly ethanol-rinsed until no detection for supernatant vitamin C to form the Fucdn-VC (1:0.23 wt/wt) inclusion body (Fucdn- VC-IB). Fucdn-VC-IB increased not only VC-stabilizing at 37˚C, but also hydroxyl-radical scavenging as shown by ESR/spin-trap method, more markedly than a mere mixture of Fucdn:VC (1:0.23 wt/wt). Invasion of human fibrosarcoma cells HT-1080 through the basement membrane was repressed by Fucdn-VC-IB of non-cytotoxic concentrations without significant inhibition to human skin dermal fibroblasts DUMS-16 cells. Fucdn-VC-IB suppressed the invasiveness-related gelatinases MMP-2/9, and diminished reactive oxygen species inside the cytoplasm around the nucleus, in HT-1080 cells as shown by electrophoretic zymography and the redox indicator NBT assay, respectively. Thus Fucdn-VC-IB markedly exhibits antioxidant and MMP-suppressing activities and preferentially inhibited tumor invasion without cytotoxicity to normal cells, and is suggested as a potent tumor-invasion suppressor. These findings indicate that combination of VC and fucoidan is expected to exert anti-cancer activity more marked than that by treatment with VC or fucoidan alone

References

Fucoidan Induces Apoptosis of Human HS-Sultan Cells Accompanied by Activation of Caspase-3 and Down-Regulation of ERK Pathways

Fucoidan, a sulfated polysaccharide in brown seaweed, was found to inhibit proliferation and induce apoptosis in human lymphoma HS-Sultan cell lines. Fucoidan-induced apoptosis was accompanied by the activation of caspase-3 and was partially prevented by pretreatment with a pan-caspase inhibitor, Z-VAD-FMK. The mitochondrial potential in HS-Sultan cells was decreased 24 hr after treatment with Fucoidan, indicating that Fucoidan induced apoptosis through a mitochondrial pathway. When HS-Sultan was treated with 100 mg/mL Fucoidan for 24 hr, phosphorylation of ERK and GSK markedly decreased. In contrast, phosphorylation of p38 and Akt was not altered by treatment with Fucoidan. L-Selectin and P-selectin are known to be receptors of Fucoidan; however, as HS-Sultan does not express either of these selectins, it is unlikely that Fucoidan induced apoptosis through them in HS-Sultan. The neutralizing antibody, Dreg56, against human L-selectin did not prevent the inhibitory effect of Fucoidan on the proliferation of IM9 and MOLT4 cells, both of which express L-selectin; thus it is possible Fucoidan induced apoptosis though different receptors. **These results demonstrate that Fucoidan has direct anticancer effects on human HS-Sultan cells through caspase and ERK pathways.**

PROMISING ANTIVIRAL (H1N1) GLYCO - MOLECULES FROM AN EDIBLE ALGA (Fucoidan)

From sporophyll of an edible alga Undaria pinnatifida , Fucoidan, a sulfated polysaccharide, was isolated and evaluated in vitro and in vivo as an inhibitor of influenza A virus replication. The Fucoidan showed
in vitro antiviral activity with selectivity index of more than 130. In the time - of - addition experiments, the most sensitive stage of viral replication to the Fucoidan was shown to be earlier than that to a neuraminidase inhibitor oseltamivir. **The binding of the virus to host cells and the penetration into host cells were inhibited by the Fucoidan.**

Fucoidan extracted from Cladosiphon okamuranus Tokida induces apoptosis of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells.

Adult T-cell leukemia (ATL) is caused by human T-cell leukemia virus type 1 (HTLV-1) and remains incurable. The highest endemic area of HTLV-1 carriers in Japan is located in Okinawa, and novel treatments are urgently needed in this area. We extracted Fucoidan, a sulfated polysaccharide, from the brown seaweed Cladosiphon okamuranus Tokida cultivated in Okinawa, Japan and examined its tumor-suppression activity against ATL. Fucoidan significantly inhibited the growth of peripheral blood mononuclear cells of ATL patients and HTLV-1-infected T-cell lines but not that of normal peripheral blood mononuclear cells. Fucoidan induced apoptosis of HTLV-1-infected T-cell lines mediated through downregulation of cellular inhibitor of apoptosis protein-2 and survivin and G1 phase accumulation through the downregulation of cyclin D2, c-myc, and hyperphosphorylated form of the retinoblastoma tumor suppressor protein. Further analysis showed that Fucoidan inactivated NF-kappaB and activator protein-1 and inhibited NF-kappaB-inducible chemokine, C-C chemokine ligand 5 (regulated on activation, normal T expressed and secreted) production, and homotypic cell-cell adhesion of HTLV-1-infected T-cell lines. **In vivo use of Fucoidan resulted in partial inhibition of growth of tumors of an HTLV-1-infected T-cell line transplanted subcutaneously in severe combined immune deficient mice. Our results indicate that Fucoidan is a potentially useful therapeutic agent for patients with ATL.**

References

Apoptosis Inducing Activity of Fucoidan in HCT-15 Colon Carcinoma Cells

The antitumor activity of Fucoidan from Fucus vesiculosus was investigated in human colon carcinoma cells. The crude Fucoidan, a polysaccharide composed predominantly of sulfated fucose, markedly inhibited the growth of HCT-15 cells (human colon carcinoma cells). After HCT-15 cells were treated with Fucoidan, several apoptotic events such as DNA fragmentation, chromatin condensation and increase of the population of sub-G1 hypodiploid cells were observed. In the mechanism of Fucoidan-induced apoptosis, we examined changes in Bcl-2 and Bax protein expression levels and activation of caspases. Fucoidan decreased Bcl-2 expression, whereas the expression of Bax was increased in a time-dependent manner compared to the control. In addition, the active forms of caspase-9 and caspase-3 were increased, and the cleavage of poly(ADP-ribose) polymerase (PARP), a vital substrate of effector caspase, was observed. Furthermore, the induction of apoptosis was also accompanied by a strong activation of extracellular signal-regulated kinase (ERK) and p38 kinase and an inactivation of phosphatidylinositol 3-kinase (PI3K)/Akt in a time-dependent manner. These findings provide evidence demonstrating that the pro-apoptotic effect of Fucoidan is mediated through the activation of ERK, p38 and the blocking of the PI3K/Akt signal pathway in HCT-15 cells. **These data support the hypothesis that Fucoidan may have potential in colon cancer treatment.**

Effect of Fucoidan on the Biotinidase Kinetics in Human Hepatocellular Carcinoma

Background: Hepatocellular carcinoma (HCC) is difficult to treat with anticancer drugs. Therefore, development of new drugs for HCC is required. Materials and Methods: The livers of 14 hepatoma patients accompanied by hepatitis B (2
cases) and hepatitis C (12 cases) were used. The biotinidase kinetics of HCC tissues were compared to those of the adjacent liver tissues of 13 liver cirrhosis (LC) and 1 chronic active hepatitis (CAH). Results: The Kip (the inhibition constant by
biotin) of HCC tissues were consistently higher than those of LC (plus CAH) tissues: the Kip was 450±231 μmol/l in HCC tissues and 240±111 μmol/l in LC (plus CAH) tissues, $p<0.001$. This increase of Kip is considered to be due to an increase of
biotin repulsion by biotinidase in the HCC tissues. Fucoidan, a sulfated poly-fucose, was found to decrease the Kip of biotinidase in HCC tissues, and conversely to increase it in LC tissues. Fucoidan was also found to decrease the Kip of the
hepatoma HuH-6 cells. **Conclusion: These findings suggest that Fucoidan has a potential therapeutic effect on HCC.**

Fucoidan inhibits parainfluenza virus type 2 infection to LLCMK2 cells

The effects of Fucoidan and L-fucose, a fundamental major component of Fucoidan, on the growth of human parainfluenza virus type 2 (hPIV-2) in LLCMK(2) cells were investigated. Fucoidan inhibited cell fusion and hemadsorption, but L-fucose only partly inhibited both. Virus RNA was not detected in the hPIV-2 infected cells cultured with Fucoidan. However, L-fucose did not inhibit virus RNA synthesis. Indirect immunofluorescence study showed that virus protein synthesis was inhibited by Fucoidan, but not by L-fucose. Furthermore, using a recombinant, green fluorescence protein-expressing hPIV-2, it was found that virus entry was inhibited by Fucoidan, but not by L-fucose. **These results suggested that Fucoidan inhibited virus adsorption to the surface of the cells by binding to the cell surface and prevented infection, indicating that the sulfated polysaccharide form was important for the inhibition by Fucoidan.**

References

Antiretroviral Activity of Fucoidans Extracted from the Brown Seaweed Adenocystis utricularis

Treatment of human immunodeficiency virus type 1 (HIV-1, causative agent of AIDS) infection represents a major challenge in antiviral therapeutics. Many difficulties are associated with the treatment, including toxicity, resistance and high costs. Taking this into account, research for novel compounds able to overcome these limitations is needed. Sulfated polysaccharides appear to be interesting, given their abundance as components of seaweeds. Herein, a series of fractions obtained from the brown seaweed Adenocystis utricularis was analysed for in vitro anti-HIV-1 activity. These fractions, which have anti-herpes simplex virus activity, were
determined previously to belong to the family of Fucoidans, sulfated polysaccharides obtained from the cell walls of brown seaweeds. Assays in human PBMC primary cell culture demonstrated that two of the five fractions analysed had potent anti-HIV-1 activity both against WT and drug-resistant HIV-1 strains. For active fractions,
it was also shown that the inhibitory effect was not due to an inactivating effect on the viral particle (i.e. no virucidal activity was detected) but rather to a blockade of early events of viral replication. **Given these encouraging results, these seaweed-derived fractions appear as good candidates for further studies on their potential for in vivo therapy and/or prophylaxis of HIV-1 infection.**

Immunosuppressive Activities of Fucoidan from Laminaria japonica

Effects of Fucoidan from Laminaria japonica on 2,4-dinitrochlorobenzene induced delayed-type hypersensitivity (DTH) reaction and the serum levels of IgG, IgM, complement C3 and C4 were investigated in the present study. Results showed that oral administration of Fucoidan at dose of 150 and 300 mg/(kg" d) for 9 days before the hapten challenge significantly inhibited 2,4-dinitrochlorobenzene induced delayed-type hypersensitivity reaction; and also inhibited the humoral immunity. Serum C3 and C4 levels were markedly reduced by Fucoidan at dose of 150 and 300 rag/kg; and serum IgG and IgM levels were reduced by Fucoidan at dose of 300 mg/kg. **The inhibitory effects of Fucoidan on delayed-type hypersensitivity suggested that it may be potential medication for chronic inflammatory diseases in the future.**

Anti-ulcer effects and biological activities of polysaccharides from marine algae

Fucoidan is a complex sulfated polysaccharide, derived from marine brown algae [17–19], the jelly coat from sea urchin eggs [20–23], and the sea cucumber body wall [23–25]. Most investigations into its biological activity have involved the Fucoidans from brown algae such as Fucus vesiculosus.The Fucoidan from F. vesiculosus mediates a variety of significant biological effects on mammalian cells. Fucus Fucoidan has anticoagulant activity [26–30] and is a potent activator of both anti-thrombin III and heparin cofactor II [30]. Fucoidan inhibits both the initial binding of sperm and subsequent recognition [31]. It also prevents the infection of human cell lines by several enveloped viruses [32,33]. Fucoidan blocks cell-cell binding mediated by P- or L-selectin but not E-selectin [34]. Furthermore, it demonstrates differential binding to interleukins lα and β, 2, and 6 [35] and hepatocyte growth factor [36]. Since this polysaccharide causes no toxicity or irritation, it may be useful as an anticoagulant, antiviral, anti-inflammatory and contraceptive agent [37–39]. The proposed structure of Fucus Fucoidan consists mainly of 4-sulfated and 2-linked α-fucopyranosyl units [17] and has recently been revised so that the α-fucopyranosyl units are now 1 → 3 linked (Fig. 5) [40]. This structure resembles that determined for a Fucoidan from Ecklonia kurome, another brown seaweed [29,41]. According to most authors, the sulfate groups are linked mainly to the 4-position of the fucose residue [41]. In our previous study, Fucoidan proved effective in healing and preventing of gastric ulcers in experimental animal models on oral administration [42]. Notably, Fucoidan from Cladosiphon okamuranus (Okinawa Mozuku) was more effective in healing ulcers than that from F. vesiculosus. **Furthermore, this Fucoidan blocks both Leb- and sulfatide-mediated adhesion of Helicobacter pylori to gastric cells [43]. Helicobacter pylori are a specific human pathogen. They colonize human gastric epithelium and are linked to serious diseases in the upper gastrointestinal tract, such as gastric and duodenal ulceration and gastric carcinoma [44].**

References

Fucoidan present in brown algae induces apoptosis of human colon cancer cells

Algae induces apoptosis (cell death) of human colon cancer cells. Background: F sulfated polysaccharide found in brown algae; it has been shown to exhibit a numb effects, including anti-tumor effects. In this study, we evaluated the effects of Fucoidan on apoptosis in HT-29 and HCT116 human colon cancer cells. Methods: HT-29 and HCT116 cells were cultured with various concentrations of Fucoidan (0 - 20 µg/mL). Apoptosis was assayed via Hoechst staining and Annexin V staining followed by flow cytometric analysis. Western blot analyses and JC-1 staining were conducted to determine the levels of apoptosis-regulating proteins and mitochondrial membrane permeability, respectively. Results: Fucoidan induced substantial reductions in viable cell numbers and apoptosis of HT-29 and HCT116 cells in a dose-dependent manner. In HT-29 cells, Fucoidan also increased the levels of cleaved caspases-8, -9, -7, and -3, and cleaved poly (ADP-ribose) polymerase (PARP) levels. The levels of the X-linked inhibitor of apoptosis protein and survivin were attenuated in the Fucoidan-treated cells. Fucoidan was also shown to enhance mitochondrial membrane permeability, as well as the cytochrome c and Smac/Diablo release from the mitochondria. Fucoidan increased the levels of the Bak and truncated Bid proteins, but reduced the levels of Mcl-1. Additionally, Fucoidan increased the levels of the tumor necrosis factor-related apoptosis-inducing ligand, Fas and death receptor 5 proteins. The caspase-8 and -9 inhibitors Z-IETD-FMK and Z-LEHD-FMK induced a reduction in Fucoidan-mediated apoptosis. Caspase-8 inhibitor inhibited the Fucoidan-induced cleavage of Bid, caspases-9 and -3, and PARP.
Conclusion: The findings of this study indicate that Fucoidan induces apoptosis in HT-29 and HCT116 human colon cancer cells, and that this phenomenon is mediated via both the death receptor-mediated and mitochondria-mediated apoptotic pathways. **These results suggest that Fucoidan may prove useful in the development of a colon cancer-preventive protocol.**

Treatment of human skin with an extract of Fucus vesiculosus changes its thickness and mechanical properties

Recently the researchers found that an extract of Fucus vesiculosus, Fucoidan, which is a type of seaweed, promotes the contraction of fibroblast-populated collagen gels through increased expression of integrin molecules. In this study, they investigated the effects of topical application of an aqueous extract of this alga on the thickness and the mechanical properties of human skin. A gel formulation that included 1% of the extract was applied topically to human cheek skin twice daily for five weeks. A significant decrease in skin thickness measured by B-mode ultrasound was elicited, as was a significant improvement in elasticity measured with a Cutometer as compared with controls. In cheek skin, the thickness normally increases and the elasticity usually decreases with age. **These results suggest that the Fucus vesiculosus extract, Fucoidan, possesses anti-aging activities and should be useful for a variety of cosmetics.**

Fucoidan is the active component of fucus vesiculosus that promotes contraction of fibroblast-populated collagen gels

The fibroblast-populated collagen gel culture method has been evaluated as a dermal model of wound contraction and granulation in tissues during the wound healing process and as an in vitro model of dermal tissue. We previously reported that an extract of Fucus vesiculosus promoted fibroblast-populated collagen gel contraction and that the promotion of the gel contraction was due to the increased expression of integrin alpha2beta1 on the surface of the fibroblasts. In this study, we investigated the active component of the extract of this alga using extraction and fractionation techniques. Water extraction of the alga was followed by precipitation with excess ethanol and then gel filtration with the boundary molecular weight of 30,000. The high molecular weight fraction obtained from gel filtration was fractionated by ion exchange chromatography on diethylaminoethyl cellulose column to give active fractions that have more polar properties. These polar, high molecular weight fractions which contained molecules with fucose and sulfate groups showed significant gel contraction-promoting activity and integrin expression-enhancing activity, and were estimated to be the sulfated-polysaccharide Fucoidan. Commercially available Fucoidan showed similar activities to the above-described fraction of this alga. **Although it remains necessary to precisely identify the specific active component, the above results indicate that Fucoidan is the active component which promotes collagen gel contraction, and also indicate the possibility that it dose so by enhancing the integrin alpha2beta1 expression.**

A 4-week repeated oral dose toxicity study of Fucoidan from the Sporophyll of Undaria pinnatifida in Sprague–Dawley rats

Fucoidan is extracted from brown seaweeds, which can have anti-coagulant, antithrombotic, antitumor, and antiviral activities. However, detailed studies on the toxicology of Fucoidan have not been performed. Here we tested the toxicity of Fucoidan in Sprague–Dawley rats. Fucoidan (1350 mg/kg bw/day for 4 weeks) did not induce statistically significant differences in groups matched by gender with respect to body weight, ophthalmoscopy, urinalysis, hematology, and histopathology. Fucoidan did not change prothrombintime or activated partial thromboplastin time, indicating an inability to change blood clotting. **This study demonstrated that Fucoidan is not toxic under this administration paradigm.**