FUCOSAN

Result Report
Fucoidan characterisation and database development

Interreg
Deutschland - Danmark

EUROPEAN UNION

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Imprint

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This result report presents a compilation on the key findings provided by the partners working in the work package:

WORK PACKAGE 1 — Project management

WORK PACKAGE 2 — Project communication & PR

WORK PACKAGE 3 — Algae sources, cultivation and collection

WORK PACKAGE 4 — Fucoidan characterisation and database development

WORK PACKAGE 5 — Pilot developments in medicine and cosmetics

WORK PACKAGE 6 — Organisation and business models

The FucoSan project

Algae from the North and Baltic Sea serve as an important but yet under-exploited marine bio resource. Brown algae contain fucoidan - a polysaccharide with highly health-promoting activities that could be used in medicine and cosmetics. Fucoidans are also valued for their positive influence on inflammation, vascular supply and tissue regeneration.
With their antimicrobial properties, infections in the bone could potentially be treated. However, fucoidan varies in structure, composition and modifications such as degree of sulfation or molecular weight - depending on the origin and other factors. This leads to different, sometimes even opposing effects.

The FucoSan project aimed at generating systematic knowledge of fucoidans and their modes of action. In various test systems, the project partners investigated on the optimal fucoidan for each particular application. Over the last three years, the project established a network in the German-Danish cross-border region pooling the expertise of companies and research institutions. They are active in the fields of extraction and purification as well as in chemical and biological characterisation of fucoidans.

March 2017 – August 2020

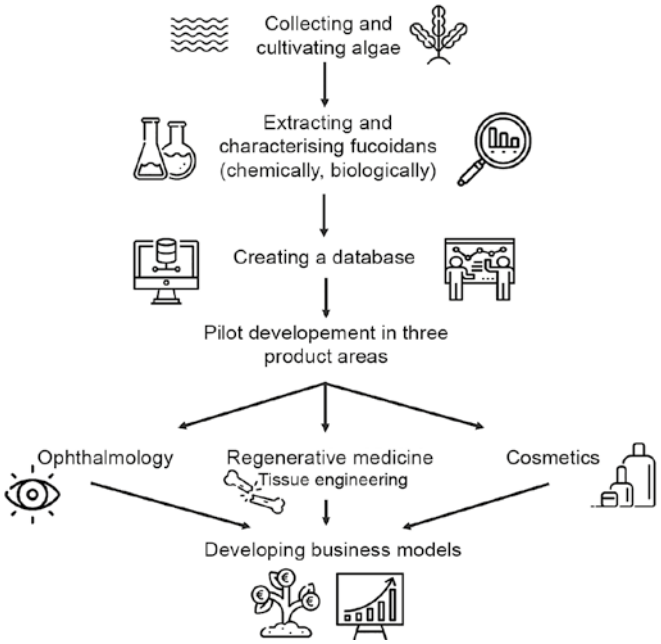
3.8 million Euros budget,
thereof 2.2 million Euros funds

8 partner organisations from
Denmark and Germany

Project aims

- ✔ Development of economically and ecologically sustainable processes to obtain brown algae from the Baltic Sea
- ✔ Setup of a database for the identification of suitable fucoidans
- ✔ Pilots for fucoidan-based applications in ophthalmology, regenerative medicine (tissue engineering) and cosmetics
- ✔ Establishment of a German-Danish value chain around the use of fucoidans

The FucoSan process chain



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Processes for fucoidan extraction, characterisation and data documentation

Fucoidans are a complex group of sulfur-containing sugars (sulfated polysaccharides) found in brown algae. As they are highly biologically active, they are widely considered to be promising candidates for applications in the fields of well-being, health and medicine.

Brown algae are common in coastal environments across the Northern hemisphere, including the Baltic Sea, but knowledge about the content and composition of their fucoidans is still limited. Furthermore, despite intensive international fucoidan research, there are still only a few well defined fucoidan products and no medical products available on the market. One reason for the absence of fucoidan-containing medical products from the market is the high complexity and variability of these marine polymers, which must be taken into consideration and defined for the specific applications.

International fucoidan research, which has greatly intensified over the last two decades, indicates pronounced differences in structure and activity between fucoidans derived from different brown algal species, leading to sometimes contradictory findings regarding their effects. A significant shortcoming with regard to any commercial use is, that although many biological activities of fucoidans are described, many reports lack necessary information on purity and structural composition of the investigated fucoidans.

In an application-oriented approach, the FucoSan project therefore has taken into account

- the extraction and fractionation of brown algae-sourced fucoidans,
- their chemical characterisation,
- the investigation of parameters influencing fucoidan yield and purity as well as quality in order to define the optimal sources and to establish protocols for standardised extraction, as well as
- bioassays for generating basic information on their activity and safety profile.

In order to provide a sustainable and expandable source of information for research and economic use of fucoidans, a database has been developed and published. It serves as a platform for documentation of the experimental results as well as for interactive integration of the project partners' findings. The joint analysis of the different data gained during the project allows the determinination of structure-effect relationships. Moreover, it allows the identification of fucoidan extracts suitable for further (application-oriented) testing.

Important prerequisites for any commercial application of fucoidans are, on one hand, the presence of reliable data, on the other hand the availability of suitable fucoidans in sufficient quantities, with a high and reproducible quality. The latter imposes a challenge, since the fucoidan composition may vary considerably, depending on the algal species and its environment, as well as the extraction process. The comprehensive dataset gained by the project consortium contributes to the identification of suitable algal sources, and the now established operating procedures can be useful for research as well as for the industrial development and quality control of fucoidan products. Furthermore, the experience gained and expertise generated in frame of the activities related to fucoidan production contribute to optimise the production and enable identifying further application possibilities and thus fuel both further research and product development.

Processes and Structures by Kiel University, Pharmaceutical Institute, Department of Pharmaceutical Biology (CAU-PHARMA)

CAU-PHARMA was centrally involved in the FucoSan project by leading WP4 and performing algae extraction, optimising the obtained fucoidans by degradation and fractionation, chemical characterisation as well as testing of the fucoidans in numerous in vitro and cell-based activity assays. Additionally, the Fucosan database was established.

Extraction und Purification

It is well-known that the structural composition of fucoidans and consequently their bioactivities vary largely. The structural characteristics dependent on the used algae material (i.e. algal species, harvest time and place, age, algal parts and reproductive stage of algae) as well as numerous environmental parameters (e.g. UV-light, salinity, temperature, tidal amplitude) and of course also on the process of extraction and purification [1-3].

CAU-PHARMA extracted more than 50 fucoidan batches from nine different algal species overall resulting in 110 optimized fucoidan fractions. The extracted algae included four species from the order of Fucales (*Fucus vesiculosus* (FV), *Fucus serratus* (FS), *Fucus evanescens* (FE), *Ascophyllum nodosum* (AN)), four ones from the order of Laminariales (*Laminaria digitata* (LD), *Laminaria hyperborea* (LH), *Saccharina latissima* (SL), *Alaria esculenta* (AE)) as well as *Dictyosiphon foeniculaceus* (DF) (Ectocarpales).

One aim was to identify which algae species are suitable as sources of fucoidans worth for further research and development. Therefore, all the algae batches were submitted to the identical standardized extraction and purification procedure (Figure 1). This procedure is well-established in pharmaceutical industry and is simple, cheap and ecologically uncritical. There are no process-related contaminants like e.g. organic solvents, heavy metals, or enzymes, which have to be removed or impair the quality. The extraction was performed under non-degrading conditions to get information on the native molecular mass (Mw). Moreover, high Mw is unsuitable for applications of fucoidans as active agents, but it might be useful for their use as pharmaceutical excipients such as nanoparticles.

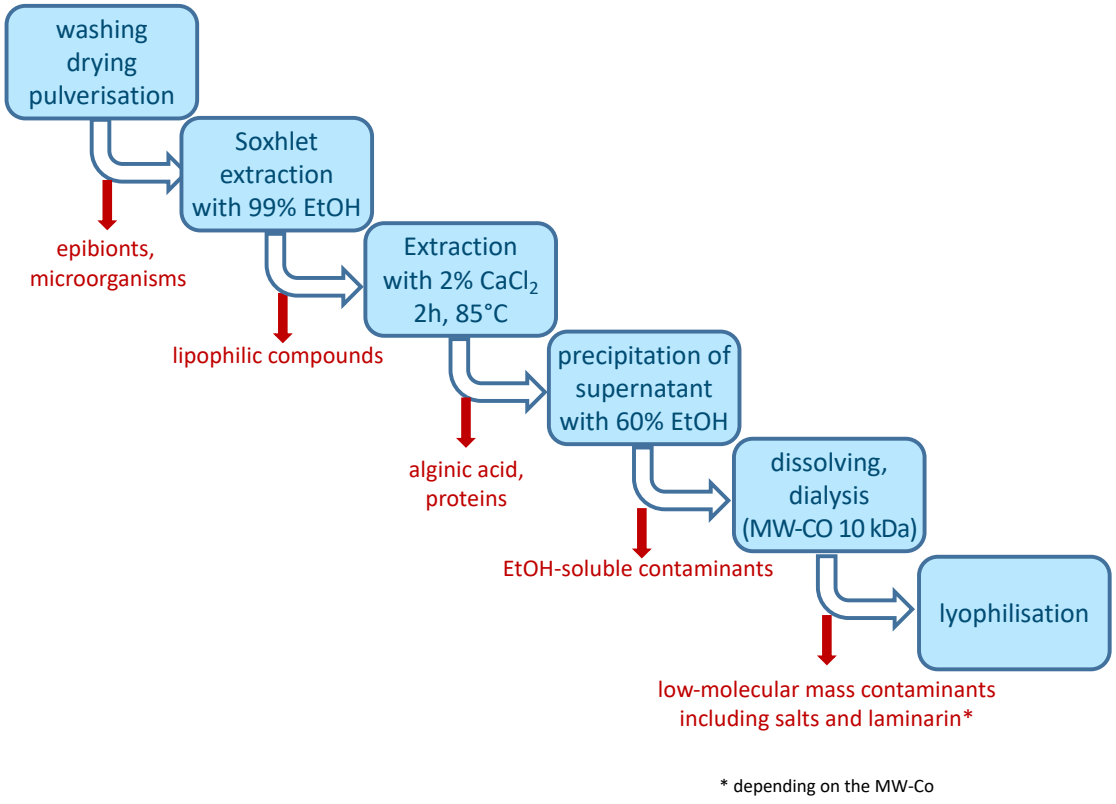


Figure 1: Fucoidan extraction and purification scheme.

The comparison of crude algae extracts demonstrated that fucoidans from Fucales are often rich in co-extracted polyphenols, whereas those from Laminariales often contain fractions tightly associated with proteins (i.e. proteoglycans). Concerning yield, purity, and biological activities, FE and SL, followed by LD, turned out as most promising fucoidan sources among the investigated algae species [2]. Additionally, the impact of origin, harvest time and the year of harvest were investigated with fucoidans from SL, FV and FE. Depending on the species, there are more or less algae batch-related differences [1, manuscript in preparation 1 (MiP1)], but these can be overcome by further processing of the extracted native fucoidans (see below).

Degradation and Fractionation

The usually high Mw (> 100 kDa) of native fucoidans is an obstacle to medical applications, as it is associated with unfavourable biopharmaceutical properties and possibly undesired effects. Therefore, it seems reasonable to develop fucoidan derivatives with reduced size, whereby it is essential that the degradation is not associated with any desulfation. Our recent studies revealed that treatment with hydrogen peroxide (H2O2) represents a simple, cheap and fast degradation method without any desulfation [4]. Further, it leads to microbial decontamination and is not associated with contamination by any reagents. Interestingly, it proved to have the additional advantage to improve the quality of fucoidans by eliminating co-extracted phenolic compounds and partly even increasing the DS (and thus also the activities) [4-6]. In line with previous observations, degradation of fucoidans from FV, FS, FE, LD, SL, and DF showed that the individual degradability depends on the structural composition. The detailed H2O2 treatment, therefore, has to be adapted to the individual fucoidan and the desired Mw (performed for FE, SL, and LD fucoidans) [MiP2]. But by using defined protocols, it is possible to reproducibly produce fucoidan fractions with improved quality and a desired Mw.

Fractionation of fucoidans by anion ion exchange chromatography (IEC) is widely used to reduce the complexity of the fucoidan composition and to isolate the high-sulfated, most active fractions. We also used this method, whereby we additionally intend to get more information about the degradation mechanisms.

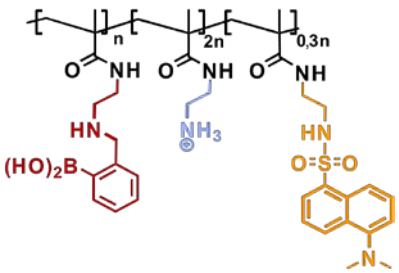
Chemical characterisation

The basic chemical characterisation of all the obtained fucoidans and fucoidan fractions included the following parameters:

- fucose content
- sulfate content and degree of sulfation
- monosaccharide composition
- molar mass characteristics (SEC with MALS-RI detection, i.e. determination of real Mw)
- uronic acid content
- protein content
- total phenolic content (TPC)
- laminarin, β-1,3-glucan

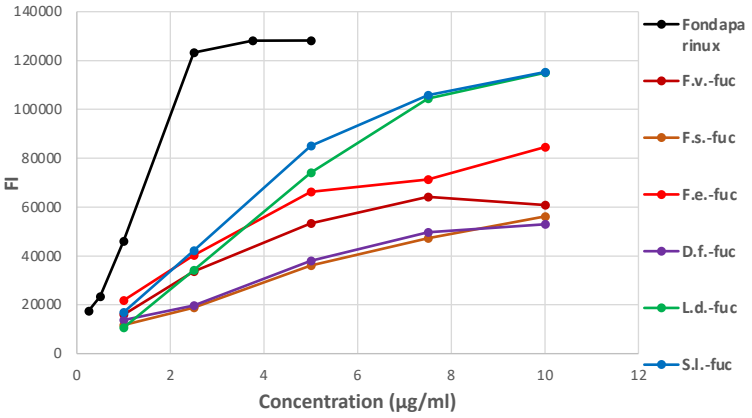
The biological activities of fucoidans depend on various chemical parameters (e.g. DS, Mw, basic glycan structure, branching, sulfation pattern), but it has to be considered that one crucial aspect is their purity. There are many products labelled as “fucoidans”, which are in fact crude algae extracts containing numerous co-extracted compounds. We showed that typical fucoidan activities of such extracts are accordingly rather weak [7]. In the course of the project, a very simple method, the so-called Polymer H assay, was identified to allow a first rough estimation of the quality and the bioactivity potential of any unknown fucoidan (Figure 2) [2, 8].

Polymer H



fluorescent sensor molecule for sulfated glycans

Concentration-depedendent increase of the fluorescence intensity (FI) of Polymer H by fucoidans



FI increase of Polymer H by fucoidans

➔ **positively correlated with degree of sulf. (DS) and fucose content**

➔ **negatively correlated with total phenolic and laminarin content**

	Ranking	Polymer H response	Polymer H response + DS	Mean of biological activities
SL	1			
FE	2			
LD	3			
FV	4			
FS	5			
DF	6			

Figure 2: The Polymer H assay as useful tool for an initial screening of algae extracts.

More detailed structural information was obtained by the following methods:

- SEC with multiple detection: UV, MALS, DLS, VIS, RI
- "methylation analysis", GC-MS
- FT-NMR (^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, TOCSY)
- FT-IR
- AFM with Raman imaging

Mass spectrometry (GC-MS, LC-MS) and NMR methods are powerful techniques for the elucidation of structural details of fucoidans (mostly of fractions and fragments). However, in this way, neither information about the structural composition of the often heterogeneously composed fucoidans nor about the structure of the whole macromolecules is obtained, even though the latter is critical for interactions with biomolecules and thus activities. Therefore, we established the analysis with SEC equipped with five different detectors, a technique so far not applied for fucoidan analysis [9]. In addition to the Mw, these detectors provide valuable data for the structural characterisation (Figure 3) [9, MIP2]. This includes information on the composition of crude fucoidans and thus for further expedient processing and fractionation as well as on the macromolecular structure of purified fractions. The latter is useful to establish macromolecular structure-activity relationships, which is supposed to be helpful for the targeted development of pharmacologically active fucoidans.

- **Molecular mass moments (Mw, Mn, Mz, Mv, Mp)**
- **Polydispersity**
- **Size (RMS radius, hydrodynamic radius)**
- **Cumulative mass and size distribution**
- **Differential mass and size distribution**

SEC-UV-MALS-DLS-VIS-RI

- **Molecular conformation (e.g. random coil, rod, sphere)**
- **Conformation of different fractions**
- **Detection of proteins, glycoproteins, phenolic compounds**
- **Branching (long-chain, short-chain)**
- **Conjugate analysis**

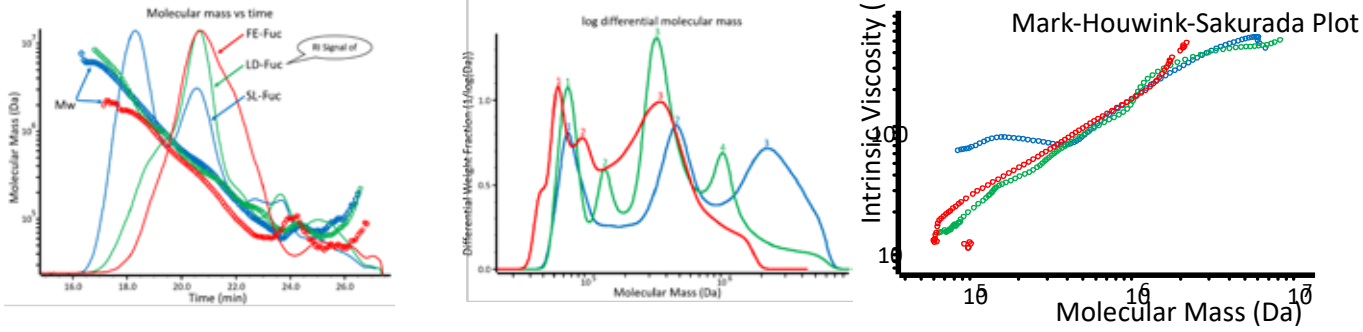


Figure 3: Size exclusion chromatography (SEC) coupled with five detectors as powerful technique for the structural characterisation of fucoidans.

Biological characterisation

The biological characterisation in WP4 aimed to identify promising fucoidans for further investigations in the pilots of WP5 and to establish structure-activity relationships.

A prerequisite for in vivo application of fucoidans is the absence of cytotoxicity, which was examined by the MTS assay on overall more than 9 cell lines by CAU-Pharma, Geomar, UKSH-Ophtha, UKSH-Trauma (see part of GEOMAR for detailed description of the published data) [10].

The basic testing of CAU-PHARMA further included typical activities of fucoidans and other sulfated polysaccharides. Compared to heparin, the crude fucoidans from the eight algae species (except for DF) inhibited the complement activation stronger than heparin, their elastase inhibitory activity was in the range of that of heparin, but their anticoagulants effects were much weaker [2]. The latter is considered an advantage, as heparins are known to exhibit pronounced anti-inflammatory and antimetastatic activity in vivo, which, however, cannot be utilized due to their strong anticoagulant activity and consequent bleeding risk.

Based on our previous findings on activities of fucoidans and other sulfated algae polysaccharides [11-15], optimized fucoidan fractions are currently tested for further effects elucidating their structure-dependent effects and modes of actions of their anti-inflammatory and anti-metastatic activities. Concerning the potential application of fucoidans in AMD, not only their anti-inflammatory effects, but also their potent anti-complementary activity and pronounced binding affinity to VEGF [16] are of special interest.

Although the crude fucoidans considerably differ in their structural composition, both their elastase inhibiting and anticoagulant activity well correlated with their DS and fucose content, whereas in case of complement inhibition other structural parameters turned out to be more important [2].

As fucoidans for medical applications should be as small as possible, it is important to investigate the Mw dependence of the activities. Whereas some activities showed to decrease with decreasing Mw (e.g. FXII activation (undesired activity) > anticoagulant activity (undesired activity) > anti-complementary activity), some turned out to be rather robust to degradation (e.g. elastase inhibition, hyaluronidase inhibition, C1-INH-activation), and some even improved with decreasing MW (heparanase inhibition) [MiP3].

According to literature, a claimed prominent bioactivity of fucoidans is radical scavenging potency (RSP). Accordingly, several in vitro assays (e.g. DPPH, ABTS, CUPRAC) were used to examine this activity of the fucoidans. Compared to reference compounds like Trolox, vitamin C, and quercetin, their RSP was only weak, but well correlated with their TPC [2, MiP4]. As already shown for FV fucoidan from Sigma [5, 12], both purification by IEC and treatment with H2O2 proved that the co-extracted polyphenols are mainly responsible for the RSP [MiP4]. But despite their poor RSP fucoidans showed to display indirect anti oxidative effects in cellular assays.

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Processes and Structures by the Technical University of Denmark, Department of Chemical and Biochemical Engineering (DTU)

DTU has developed an enzyme-assisted method for purifying fucoidan from brown seaweeds (Nguyen et al. 2020). Through the use of enzymes, the other cell wall components can be specifically degraded, leaving intact fucoidans behind. The chemical fine structure of fucoidans is probably very important for the bioactivity, and thus the enzyme-assisted method for purification might result in intact and highly bioactive fucoidans. The crude fucoidans obtained still contained a substantial amount of low molecular alginates, mainly mannuronic acids, and were therefore fractionated by ion-exchange chromatography resulting in three fractions. The first fraction F1 contained low amounts of fucoidan and all the contaminating alginates, while the F2 and F3 fractions were considered pure fucoidans, due to the high fucose and sulphate contents. The fucoidan from the enzyme-assisted purification was in the form of very large molecules, which were found to be optimal for use in the AMD bioactivity model (Dörschmann et al. 2020). However, the fucoidan did not seem optimal for the bone regeneration model. Therefore, we developed an additional method to produce specific fucoidan oligosaccharides, using endo-fucoidanase enzymes. The chemical fine structures of the oligosaccharides produced were determined by NMR. The low molecular oligosaccharides were purified and used for bone-regeneration bioactivity experiments, both in vitro and recently also in vivo in the sheep model.

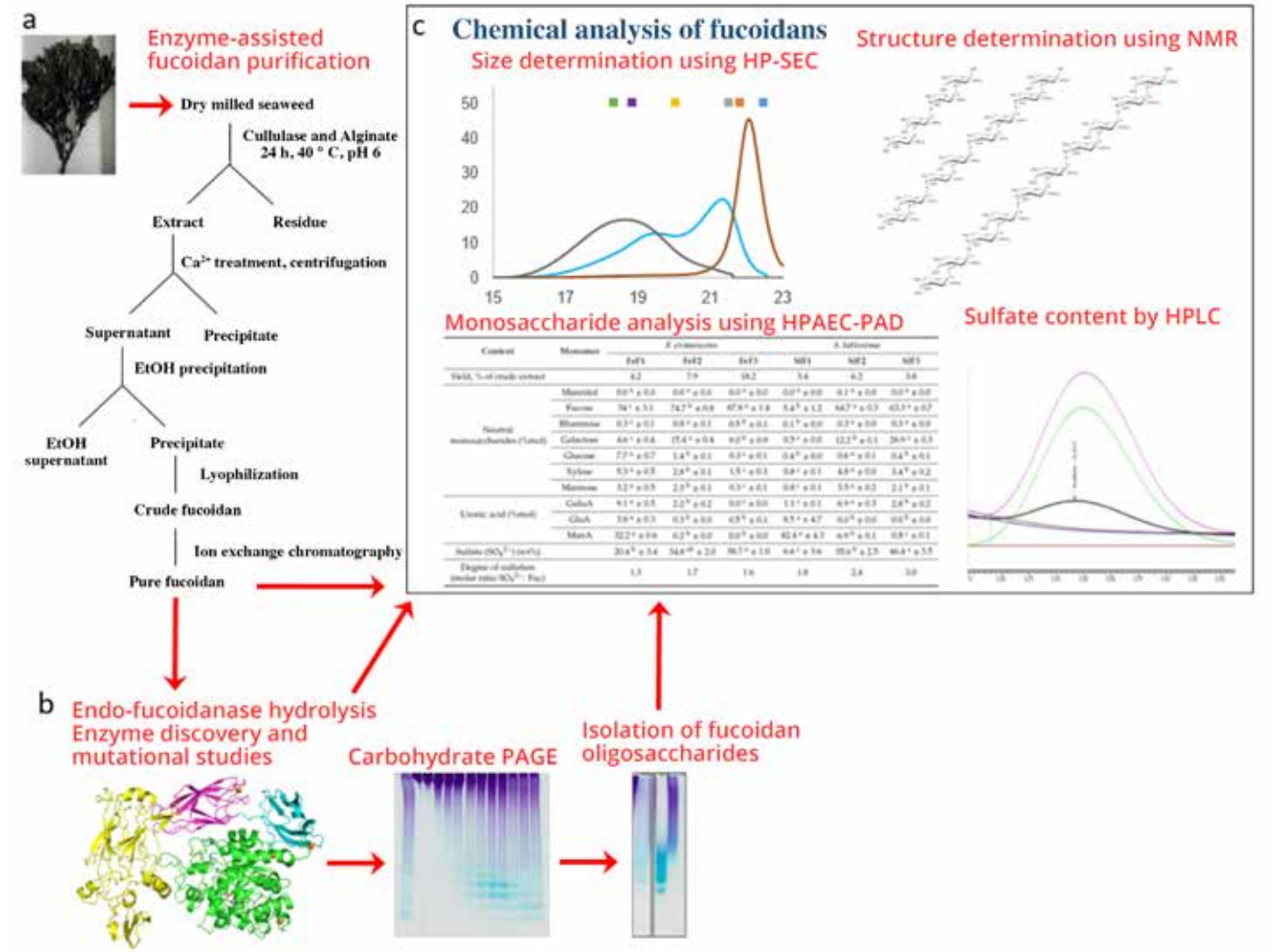

Figure 1: Overview of activities by DTU. a) Enzyme-assisted purification of fucoidans followed by ion exchange chromatographic fractionation. b) Endo-fucoidanase catalysed hydrolysis of fucoidan polysaccharides into oligosaccharides and separation by precipitation. c) Chemical analysis of fucoidan polysaccharides and oligosaccharides by HPEAC-PAD, HP-SEC, HPLC and NMR.

Literature

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Extraction

Yield studies for upscale extraction were carried out using microwave assisted extraction on *Fucus vesiculosus* (Fig. 1). Water slightly acidified with sulfuric acid was a good overall extraction solvent, providing high fucoidan yields with moderate sulfation degrees. Similar yield studies were carried out on *Fucus serratus* and *Fucus evanescens*, showing a clear yield variation across solvents and species. These findings indicate that it may be worthwhile to optimise the extraction procedure for each seaweed species.

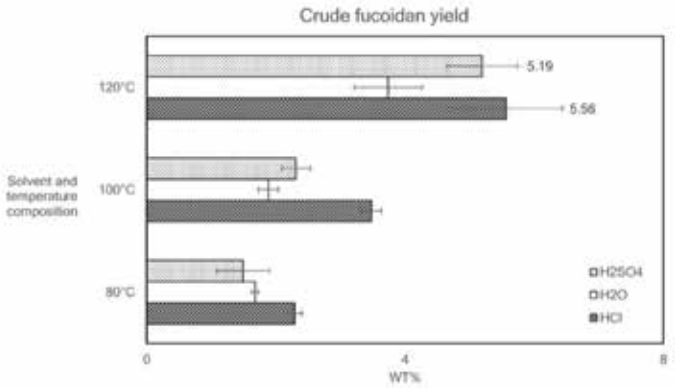

Figure 1: Yield (weight percent) of three Fucus Species.

Extraction studies on the variation in sulfate ester content

The sulfate ester content of fucoidans is generally regarded as a key parameter in bioactivity, and fucoidan producers are looking for patentable extraction methods which preserve these sulfate esters. Although the extraction method greatly impacts the fucoidan, other factors, such as species and seasonality are important considerations when upscaling the fucoidan production. Nine different algae species were freeze dried, defatted with ethanol and extracted with water acidified with sulfuric acid. After dialysing and freeze drying the fucoidan, it was combusted for elemental analysis. Figure 2 shows the influence of seaweed species on the sulphur content, which can be used as a measure of the sulfate ester content. *Ascophyllum nodosum* and *Fucus evanescens* had the highest sulphur content (7.5 %), while *Dictyosiphon foeniculaceus* had the lowest sulfur content (0.5 %). We could confirm that *Alaria esculenta, Fucus serratus, Laminaria digitatia* and *Laminaria hyperborea* had statistically similar sulfur contents (approx. 2-2.5 %), while *Saccharina latissima* and *Fucus vesiculosus* were statistically significantly different to the other sea-

weeds, with 4 % and 6 % sulfur contents, respectively. Based on the results of the extraction studies on sulfur content, we recommend using the seaweeds *Ascophyllum nodosum, Fucus evanescens,* and *Fucus vesiculosus* for extraction and production of high sulfate ester fucoidan.

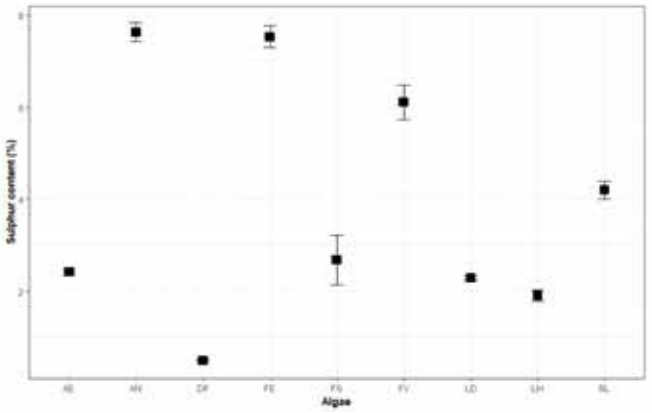

Figure 2: Variation in sulphur content across nine brown algae species. AE: Alaria esculenta, AN: Ascophyllum nodosum, DF: Dictyosiphon foeniculaceusae, FS: Fucus serratus, FV: Fucus vesiculosus, LD: Laminaria digitate, LH: Laminaria hyperborean, SL: Saccharina latissima.

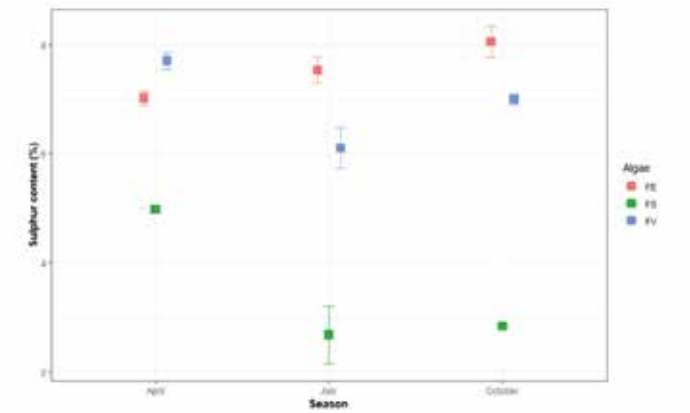

Figure 3: Monthly variation in sulphur content between three Fucus species, FE (Fucus evanescens), FS (Fucus serratus) and FV (Fucus vesiculosus).

It has been suggested that fucoidan functions as an anion exchanger[1], a structure stabilising cross-linker[2], and that it is somehow related to the reproduction cycle of the seaweed[3]. These theories would suggest that the fucoidan content and the sulfate ester content fluctuates with the seasons and across climates. We investigated the monthly variation in the sulfur content of three Fucus species (Fig. 3) and found a clear seasonal dependency for one species. The sulfur content of fucoidan extracted from *Fucus serratus* is generally lower than fucoidan extracted from the other *Fucus* species, but it shows a greater seasonal dependency. We recommend taking the seasonal variation into account when extracting fucoidan from *Fucus serratus*, particularly for upscale extractions.

Brown algae are widespread in saline and brackish, cool waters. Brown algae from brackish waters are reportedly almost entirely asexual[4] and they may potentially provide very consistent fucoidan yields and compositions. Since the environment of the brown algae may differ significantly and even affect their reproduction, we investigated how the region of origin affected the sulfur content of the fucoidan extracted (Fig. 4). From the extraction studies on fucoidan from seaweeds harvested in Denmark, France, Iceland and Kiel (Germany), we were unable to select a statistically significant, optimal location. However, fucoidans extracted from Kiel seaweed had consistently high levels of sulfur, with very low standard deviations. The water in the Kiel Canal and the Baltic Sea surrounding Denmark are both considered brackish, which does not explain the difference observed between the Danish and German seaweeds. Based on these observations, we recommend using Kiel seaweed for producing and extracting of consistently high sulfate ester fucoidan.

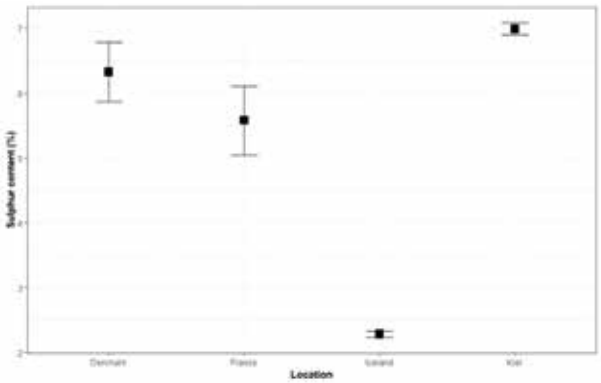

Figure 4: Sulphur content variation in fucoidans extracted from Denmark, France, Iceland and Kiel, Germany.

Fractionation and Purification

Scalable fractionation and purification options, including dialysis, precipitation and centrifugation and size-exclusion chromatography (Fig. 5) have been tested at lab scale. As displayed in table 1, the extraction methods tested produced both high and low molecular weight fucoidans, although high molecu-

lar weight fucoidans make up a significant fraction of each extract. The chemical analysis (see below) also showed that impurities were present in the extracts after precipitation. Thus, while these methods work, the membrane separation methods currently tested in our laboratory might be a more promising downstream process to be used between the extraction step and the final purification step using size exclusion chromatography. The advantage of a combination of membrane separation steps before size exclusion chromatography would reduce the use of additional chemicals in the fractionation steps and more concentrated fucoidan solutions for the final purification steps.

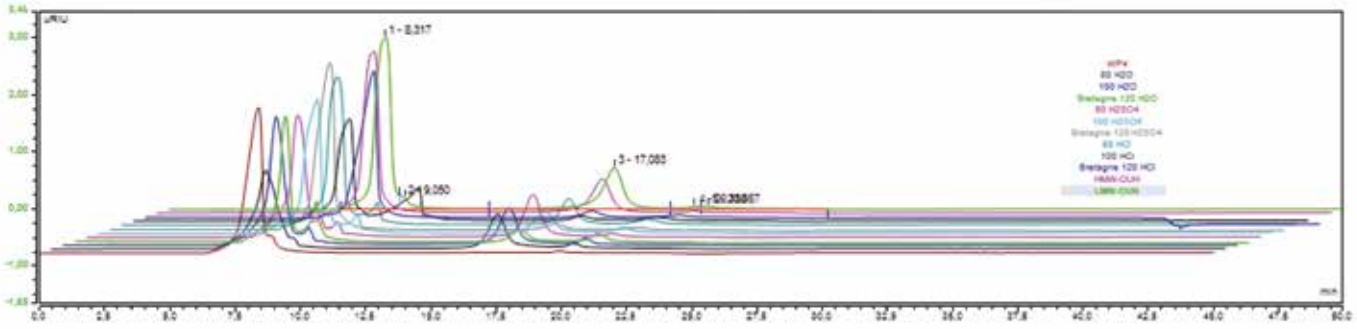

Figure 5: Size-Exclusion-Chromatography on Fucus serratus (171010) extracts, Fucus vesiculosus extracts (Brittany) and the internal work package 4 standard (WP4).

Extract	Mw [kDa]	Relative intensity (%)
WP4	1884 *809* *611* *4*	91.2 *2.8* *4.9* 1.1
FS_KB 171010 80 H2O	2533 *736* *470* *7* *4*	57.1 *12.8* *2.6* *24.6* 3.0
FS-KB_171010 80 H2SO4	3260 *718* *333* *7* *4*	64.6 *4.9* *4.9* *22.2* 1.5
FS-KB_171010 80 HCl	2313 *407* *271* *7* *4*	68.9 *6.0* *10.6* *11.9* 2.7
FS_KB 171010 100 H2O	2870 *1179* *424* *360* *7* *4*	55.9 *12.9* *7.7* *5.2* *15.8* 2.6
FS-KB_171010 100 H2SO4	4239 *1884* *226* *7* *4*	34.6 *50.8* *4.5* *8.3* *1.8*
FS-KB_171010 100 HCl	2313 *1037* *102* *91* *75* *7* *4.1* *3.9*	54.9 *1.3* *16.9* *18.4* *2.9* *2.3* *1.9* *1.4*

Table 1: Molecular weights of fucoidan fractions and their distribution (relative intensity) in Fucus Serratus extracts.

Chemical Characterisation

We extracted fucoidans from several seaweeds, which were characterised by elemental analysis and size-exclusion chromatography. Since these methods showed a clear variation across extraction methods, seasons, regions and species, we decided to also investigate non-destructive means of characterisation. In collaboration with Texas A&M University in the US, we found that Raman and infrared spectroscopy are complimentary techniques for a rapid analysis of crude extracts. Three *Fucus vesiculosus* fucoidans were analysed. The fucoidans proved to be somewhat poor Raman scatterers, but this was solved by improved sample preparation prior to scanning. The Raman spectra obtained showed a clear difference between extracts obtained by acid extraction and the extract from water extraction, as well as differences between the acid extracts (Fig. 6). From the Raman spectra, we observed several impurities in the fucoidan extracts, which confirmed that the extraction method produced crude extracts. We observed vibrational bands for alginate and glucose in the Raman spectrum, which suggested that precipitation with calcium chloride was insufficient for the removal of alginate, and that precipitation with ethanol was not enough to remove laminarin from the crude fucoidan extract. Although a precise molecular description is not possible based on Raman spectroscopy alone, we observed that the intensity of the carbohydrate stretches was different among the extracts, likely due to increased hydrolysis in the acid extracts. We propose that Raman spectroscopy can offer substantial insights into crude fucoidan extracts, provided that the extract contains no fluorescent compounds.

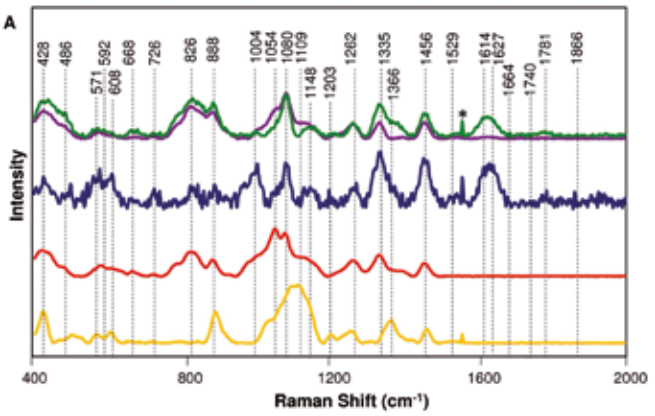

Figure 6: Raman spectra of fucoidan extracted from Fucus vesiculosus using microwave assisted extraction with hydrochloric acid (green), sulfuric acid (purple), and water (blue). A laminarin standard (gold) and a fucoidan standard from sigma-aldrich (red) were used for comparison.

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[1] Skriptsova, A. V., 2016, Seasonal Variations in the Fucoidan Content of Brown Algae from Peter the Great Bay, Sea of Japan, Russian Journal of Marine Biology,42 (4) 351-256.

[2] Deniaud-Bouët E., Kervarec N., Michel G., Tonon T., Kloareg B., and Hervé C, 2014, Chemical and enzymatic fractionation of cell walls from Fucales: insights into the structure of the extracellular matrix of brown algae, Annals of Botany,114, 1203-1216.

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Processes and Structures
by GEOMAR Helmholtz Centre for Ocean Research Kiel, Marine Natural Products Chemistry

GEOMAR's activities focused on biological characterisation of the fucoidans together with the other project partners Kiel University, Pharmaceutical Biology (CAU) and the Departments of Ophthalmology and of Orthopaedics and Trauma Surgery, University Medical Center Schleswig-Holstein (UKSH). In total, GEOMAR received 63 different extracts, which were tested for various biological activities (18 from the CAU, 19 from CRM, 18 from the University of Southern Denmark, 8 from the Technical University of Denmark (DTU)).

A requirement for the use of compounds in living tissue is to determine their influence on the viability of the cells. Therefore, GEOMAR's first test campaign was focused on the activity of fucoidans originating from 6 different brown algal species (*Fucus vesiculosus, F. serratus, F. distichus subsp. evanescens, Dictyosiphon foeniculaceus, Laminaria digitata, Saccharina latissima*) against selected tumour and non-tumour cell lines. In scientific literature, fucoidans are repeatedly reported to have considerable effects on the viability of various tumour cell lines; however, published data often suffers from a lack of comparability, especially due to differences in experimental set-ups. To overcome this, we applied a standardised approach together with our partners from the CAU and UKSH. This included a standard assay protocol using a commercial cell viability assay and standard reference compounds for determining cell viabilities in response to the application of differently sourced crude fucoidan extracts in 4 different test concentrations (1, 10, 50, 100 µg/mL). GEOMAR tested the viability of the liver cancer cell line Hep G2, the colon cancer cell line HCT-116, the skin cancer cell line A-375 as well as the non-tumour skin (keratinocyte) cell line HaCaT. Unexpectedly, fucoidan extracts from *F. vesiculosus, F. evanescens, D. foeniculaceus, L. digitata*, and *S. latissima* showed low but statistically significant increase in cell viability of the tumour cell lines, especially at low concentrations (Figure 1).

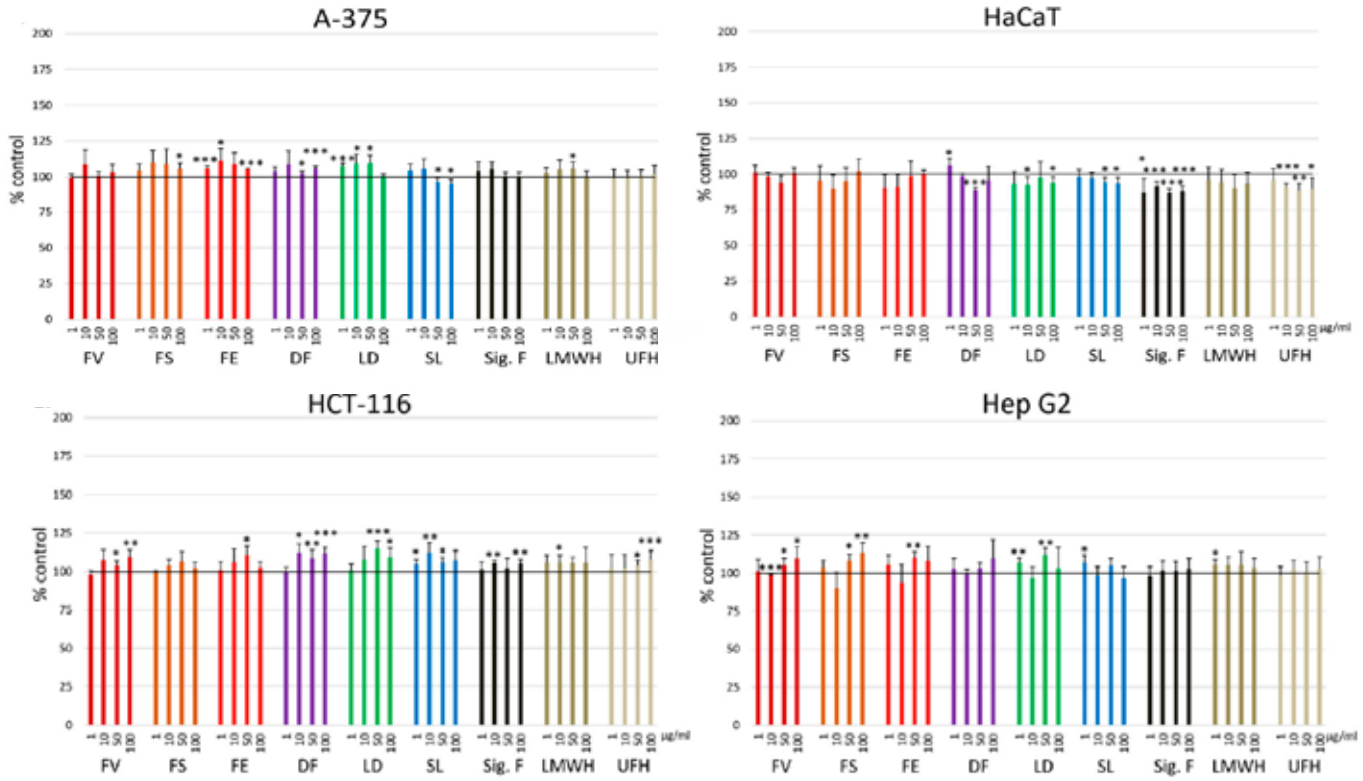

Figure 1: GEOMAR cell viability assay results of crude fucoidans from different algal species. FV: Fucus vesiculosus, FS: Fucus serratus, FE: Fucus evanescens, DF: Dictyosiphon foeniculaceus, LD: Laminaria digitata, SL: Saccharina latissima, SigF: Sigma fucoidan reference (origin F. vesiculosus), LMWH: enoxaparin reference, UFH: heparin reference. A-375: human skin tumor cell line, HaCaT: human non-tumour cell line, HCT-116: human colorectal tumour cell line, HepG2: human liver tumour cell line.: significant change in activity.*

A slight (but statistically significant) decrease in cell viability of the non-tumour cell line HaCaT was only measured with fucoidan derive from *D. foeniculaceus* and *S. latissima* at the highest test concentrations. Together with data from our co-workers, we concluded from our experiments that none of the fucoidans tested has an anti-proliferative effect at the concentrations tested, which is in contrast to many other publications that report an antiproliferative effect. Results from this study were jointly published in the peer-reviewed scientific journal Marine Drugs (Bittkau et al. 2019)[1].

Interestingly, extracts from SDU (microwave-assisted extraction) tested against the same tumour and non-tumour cell lines did not show any impact on viability, hence we conclude that the mode of extraction, which results in differently-sized fucoidans, plays a major role in anticancer activity.

In scientific literature, many types of biological activity are attributed to fucoidans, including antioxidant, anti-coagulant, antithrombotic, immunoregulatory, antiviral and anti-inflammatory, rendering them almost "magic molecules". However, antibacterial activities are not widely reported for fucoidans. Notably, bacterial infections are of high relevance for all applications piloted in the project´s WP5, namely cosmetics, prevention of age-related macular degeneration (an eye disease) and also in orthopaedics/implant coatings. Hence, in further studies, GEOMAR focused on an assessment of antimicrobial activities.

Twelve fucoidan extracts initially received from the CAU showed inhibitory activities (60 – 80%) at a test concentration of 100 µg/mL against the gram-positive pathogen *Staphylococcus aureus*. This bacterium is not only involved in many chronic skin diseases, but is also a major cause of removal of surgical implants, making these results interesting for the WP5 pilot on regenerative medicine. A dose-dependent effect was observed as inhibitory activities increased at higher test concentrations. These results are relevant for a potential application in a cosmetic formulation. However, other bacterial or fungal skin pathogens tested showed no inhibition of growth, except for the dermatophyte fungus *Trichophyton rubrum* (>60% for selected extracts).

High activity against *S. aureus* was also found for extracts received from DTU generated by enzymatic extraction and also for several *Fucus sp.* extracts obtained by lactic acid fermentation and provided by the partner CRM, further indicating the high relevance of extraction mode for biological activity.

None of the extracts received and tested showed activity against any often multidrug-resistant and hospital infection causing bacteria (the so-called ESKAPE[2] panel), which contained a methicillin-resistant strain of *S. aureus*.

Literature

[1] Bittkau KS, Dörschmann P, Blümel M, Tasdemir D, Roider J, Klettner A, Alban S. Comparison of the Effects of Fucoidans on the Cell Viability of Tumor and Non-Tumor Cell Lines. Mar Drugs. 2019,17(8):441. doi: 10.3390/md17080441.

[2] E: *Enterococcus faecium*, S: *Staphylococcus aureus*, K: *Klebsiella pneumoniae*, A: *Acinetobacter baumannii*, P: *Pseudomonas aeruginosa*, E: *Enterobacteriaceae sp.*

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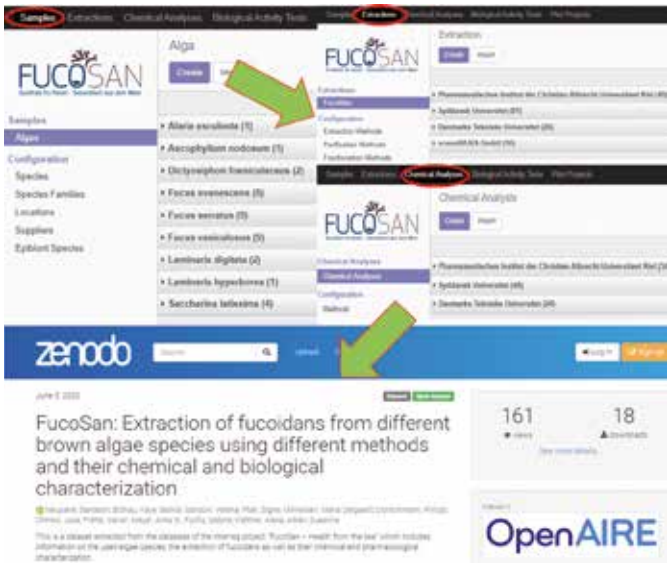
The fucoidan database

The available literature on fucoidans illustrates that there are pronounced differences in the structural composition as well as the activities of fucoidans. Sometimes even contradictory structure and / or activity data can be found on fucoidans from the same alga species. Regarding the development of fucoidans for specific applications, a shortcoming is that there are publications describing promising bioactivities, but not containing any information on the purity and structural composition of the tested fucoidan.

In line with the conception and the aim of the FucoSan project (combining the complete expertise for target-oriented fucoidan development), a database should be established collecting all the relevant data on the fucoidans produced within the project and thus providing systematic information from the used algae up to bioactivities of the extracted fucoidans. In this way, not only a structured overview of different fucoidans is given, but also the selection of suitable fucoidans for different applications areas is facilitated.

The FucoSan database was established in 2018. It currently comprises more than 200 entries on extracts from nine different brown algal species. It contains information of the used algal material, the applied methods of extraction and purification as well as basic chemical and pharmacological characteristics of the obtained fucoidans. They were tested in a collection of bioassays enabling the selection of the most promising candidates for further investigations targeting applications in ophthalmology (age-related macular degeneration), regenerative medicine (tissue engineering) and cosmetics.

Part of this database has recently been published on the EU-sponsored open science platform Zenodo (see zenodo.org). The database can be cited as source with a digital object identifier. Since it contains the characteristics of fucoidans from different brown algae sources extracted by three different methods, this database may serve as valuable virtual screening tool for stakeholders interested in fucoidans either for research targeting specific medical or cosmetic applications or for commercial exploitation.



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