Chemical Properties and Toxicology Studies of Fucoidan Extracted from Malaysian *Sargassum binderi*

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Abstract Fucoidan is a sulfated polysaccharide that consists mainly of fucose and is found in brown seaweeds. In this study, fucoidan was extracted from *Sargassum binderi* (F_{sar}) from Malaysia and subsequently characterized in terms of composition, structure and toxicology. It was found that the molecular weight, polydispersity index, monosaccharide profile and degree of sulfation of F_{sar} differed from those of commercial food-grade fucoidan (F_{ysk}). NMR analysis suggested that the main structure of F_{sar} was $\rightarrow 3$ fuc-2-OSO₃⁻(1 $\rightarrow$ 3)fuc-2-OSO₃⁻(1 $\rightarrow$). A cytotoxicity study employing up to 200 mg/mL *Sargassum binderi* extract showed that cell inhibition was less than 50% (IC₅₀), while acute toxicity results classified *S. binderi* as category 5 (unclassified) according to the OECD Guideline 423, as no mortality was observed at the highest dosage (2,000 mg/kg). Both toxicity results showed that this material is safe to be consumed. The chemical characteristics and non-toxicity of F_{sar} demonstrate its potential in biological and food product applications.

Keywords: acute toxicity, characterization, cytotoxicity, fucoidan, *Sargassum binderi*

Introduction

Malaysia has an abundant supply of seaweeds, providing tremendous opportunities to produce and extract several functional ingredients, including fucoidan. According to the Food and Agricultural Organization of the United Nations (FAO), over the past few years, Malaysian aquatic plant production has developed at a rapid pace. In 2001, 18,863 metric tons, worth USD 1.99 million, were produced. This figure grew to 207,892 metric tons, worth USD 17.44 million, in 2010 (1,2). Despite the growth of the seaweed industry in Malaysia, the value of aquatic plants from Malaysia is still relatively low compared to that of aquatic plants from other major producers. Therefore, there remains considerable room for growth in the Malaysian seaweed industry. It is prudent that more focus be given to the extraction and production of various types of high-value functional ingredients from Malaysian seaweeds, including fucoidan, alginate, agar, and carrageenan.

Fucoidans are a type of glyconutrient and one of the main polysaccharides in brown seaweeds. They belong to the family of sulfated homo- and heteropolysaccharides composed mainly of α -(1 $\rightarrow$ 2)-, α -(1 $\rightarrow$ 3)- and/or α -(1 $\rightarrow$ 4)-linked L-fucose residues. The main monomer in fucoidan is fucose, one of the eight essential biological sugars. Fucoidans also contain galactose, mannose, xylose, and

glucuronic acid residues (3,4).

Fucoidans are reported to show varied biological activities, including, but not limited to, anticoagulant, antithrombotic, antiviral, antitumor, immunomodulatory, anti-inflammatory, blood lipids reducing, and antioxidant (4). Therefore, fucoidans have been developed as a specialized type of nutraceutical and food supplement (3,4). The non-toxicity of fucoidan is an important property, where application of fucoidan as functional ingredient needs the assurance that the product is safe to be consumed.

Studies by Rioux *et al.* (5) and Zvyagintseva *et al.* (6) reported that fucoidans have very diverse chemical compositions and structures, which vary significantly depending on the geographical location, species, season, and population age. A review by Li *et al.* (4) compared the compositions and structures of fucoidan from various species and locations showed that fucoidans have very diverse and complex structures, and they were very different between one another. Studies have shown that the structural properties of fucoidans have strong effects on their biological activities and medicinal impacts (7). Despite a great number of studies on the structural properties of fucoidans, no study has investigated the structure of fucoidans from Malaysian seaweeds.

In this study, fucoidan was extracted from *Sargassum binderi*, Malaysian brown seaweed. After extraction and isolation, the

fucoidan was subjected to physicochemical characterization. In order to ensure the safety of the fucoidan from *S. binderi* in food products, toxicology studies were also performed.

Materials and Methods

Materials *Sargassum binderi* was obtained from Semporna, Sabah, Malaysia. Commercial food-grade fucoidan (F_{ysk}) was provided by Yaizu Suisankagaku Industry Co., Ltd. (Yaizu, Japan). The standard monosaccharides, fucose, galactose, xylose, mannose, glucose, and glucuronic acid, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extraction and isolation of fucoidan The extraction and isolation of fucoidan was conducted according to the previous study (2). Briefly, the *S. binderi* was ground and treated with a MeOH:CHCl₃:H₂O (4:2:1, v/v) solution, dried and sonicated in 2% CaCl₂. The extract was mixed with 10% hexadecyltrimethylammonium bromide solution (in excess), centrifuged (3,354*g*, 10 min), rinsed with distilled water, and stirred in 20% ethanolic sodium iodide. The extract was then rinsed with ethanol and dissolved in water and lyophilized to obtain fucoidan as the sodium salt in solid form (F_{sar}).

Molecular weight determination The molecular weight was determined using gel permeation chromatography (GPC) according to Rioux *et al.* (8). The mobile phase consisted of filtered (0.22 µm) 0.1 M sodium acetate prepared with ultrapure water. The flow rate was 0.5 mL/min at room temperature. Both the F_{sar} and F_{ysk} samples were dissolved in 0.1 M sodium acetate solution and filtered (0.45 µm) to remove dust particles. The MALLS instrument was placed directly after the GPC columns and before the refractive index detector (RI). Prior to measurements, a Dawn apparatus was calibrated using HPLC-grade toluene and normalized using a 20 nm polystyrene latex standard (Thermo Fisher Scientific, Waltham, MA, USA) in 0.1 M sodium acetate. The performance of the HPSEC-MALLS system was verified with monodisperse pullulan of various molecular weights. A dn/dc value of 0.129 for galactofucans was used (8). Data were collected from the RI and MALLS and evaluated with ASTRA software 5.3.4.14. Because galactofucans are polydisperse polysaccharides, only average weights were compared. The results were estimated using second-order Zimm fitting.

Fucoidan monosaccharide profiling The fucoidan (polysaccharide) was subjected to methanolysis according to the procedures by Dobruchowska *et al.* (9) and by Lawson (10). F_{sar} and F_{ysk} samples were prepared at 1.0, 5.0, 10.0, and 15.0 mg in sugar analysis tubes with screw caps. Standard monosaccharides (fucose, galactose, xylose, mannose, glucose, and glucuronic acid) were prepared individually at 1 mg (external standard) and as mixtures at 0.2, 0.4, 0.6, 0.8, and 1.0 mg (standard curve). All samples and standards

were added to 1 mg of mannitol as an internal standard and lyophilized before being treated with 0.5 mL of 1.0 M methanolic hydrochloric acid at 85°C for 24 h. The samples and standards were then cooled and neutralized using argentum carbonate. The samples were centrifuged three times (0.5 mL methanol, 200*g*, 2 min), and the supernatants were collected in new centrifuge tubes. These supernatants were then evaporated to dryness in a vacuum oven (VacuCell; MMM Group, Planegg, Germany) at 35°C for 18 h. Trimethylsilylation of the samples and standards was performed by adding 0.3 mL of silylation reagent (pyridine:hexamethyldisilazane:trimethylchlorosilane; 5:1:1, v/v) to the samples at room temperature and allowing to react for 30 min before injecting into a gas chromatograph equipped with a mass spectrometer (GC-MS). The elution was performed using a Bruker 436 GC gas chromatograph with a Bruker Scion SQ mass spectrometer (Scion Instruments, Fremont, CA, USA) and equipped with a CombiPAL.

Fucoidan elemental analysis Elemental analysis was performed using a Thermo Finnigan Flash EA1112 Elemental Analyzer for carbon, hydrogen, nitrogen, and sulfur (CHNS) equipped with an autosampler (Thermo Fisher Scientific). Both the lyophilized F_{sar} and F_{ysk} were weighed in tin crucibles (3.345 and 3.304 mg, respectively). Vanadium pentoxide was added to the samples as a catalyst, and sulfanilamide was used as a standard. Sample combustion was performed in a furnace at 1,800°C with an adequate oxygen supply, where the gas formed was eluted through a GC column with a helium gas mobile phase at a flow rate of 140 mL/min. The elemental analyzer was attached to a thermal conductivity detector. Data analysis was performed using the Eager 300 software, and the K-factors calibration method was employed (11,12).

Sulfate content and the degree of sulfation The sulfate contents in the sodium salts of F_{sar} and F_{ysk} were determined using the following equation (11,12):

$$\text{Sulfate content (\%)} = S\% \times 3.22 \quad (1)$$

where S% is the percentage of sulfur in the samples, and 3.22 is the conversion factor for the sulfur content to the sulfate content (as the sodium salt).

The degree of sulfation (DS) of fucoidan (mol of SO₃[−] per mol of monosaccharide unit) was calculated based on Synytsya *et al.* (7) using the following eq.:

$$DS = \frac{\%S}{\%C} \times (6 + 2[DAc]) \times \frac{A_C}{A_S} \quad (2)$$

where %S and %C are the sulfur and carbon percentages, respectively; Ac and As are the atomic masses of carbon and sulfur, respectively; and 6 and 2 are the numbers of carbons in the pyranoid ring and O-acetyl group, respectively. DAc is the degree of acetylation calculated from ¹H NMR.

Fucoidan structural analysis using nuclear magnetic resonance

(NMR) The dissolved F_{sar} and F_{ysk} samples in D_2O were used to perform NMR (Bruker Avance 600 equipped with a cryoprobe at 273 K; Bruker Corp., Billerica, MA, USA). The proton (1H) NMR and ^{13}C NMR (Attached Proton Test-APT) were scanned at 600.30 and 150.95 MHz, respectively. Two-dimensional (2D) TOCSY experiments were performed with 16 scans and a spectrum width of 5,400 Hz (9 ppm). HMQC experiments with 128 scans and 5,700 (9.5 ppm) and 25,661.5 Hz (170 ppm) spectrum widths for 1H and ^{13}C , respectively, were performed. HMBC experiments were carried out with 128 scans and 6,000 (10 ppm) and 33,510.9 Hz (222 ppm) spectrum widths for 1H and ^{13}C , respectively.

Degree of acetylation The degree of acetylation (DAC) (mol of *O*-acetyl per mol of monosaccharide unit) was determined based on Synnysya *et al.* (7), i.e. through 1H NMR using the ratio (A_1/A_0) of the integral area of the protons from the methyl groups of *O*-acetyl (A_1) to that of the C-6 of fucose (A_0), as shown in the following eq.:

$$DAC = \frac{A_1}{A_0} \times \frac{100}{\% \text{Fucose}} \quad (3)$$

Cytotoxicity of *Sargassum binderi* extract The cell viability or degree of cytotoxicity in a mammalian cell culture (ATCC V79-4 Chinese hamster lung cells) in response to *S. binderi* was determined using the MTT assay. The *S. binderi* sample was prepared by extraction in complete growth medium (DMEM) at a 200 mg/mL concentration. Positive (240 μ g/mL zinc sulfate) and negative (growth medium) controls were used in the study to validate the test system. Cell cultures were seeded into a 96-well plate and incubated at 37°C for 12 h or until 80% confluency was attained. The samples were added to the cell cultures in the 96-well plate in triplicates at concentrations of 6.25, 12.5, 25, 50, 100, and 200 mg/mL. The cultures containing the samples and controls were incubated in a 96-well plate at 37°C in a carbon dioxide incubator (5% carbon dioxide, 95% air) for 24 h. Incubation with 30 μ L of a 5 mg/mL MTT solution (in phosphate buffer, pH 7.2) was carried out for 4 h in the same conditions. After removing the medium, 150 μ L DMSO was added to terminate the reaction. The amount of purple formazan formed was determined by measuring the absorbance at 550 nm. The cell viability was calculated (13-15) using the following equation:

$$\text{Cell viability (\%)} = (A_1/A_0) \times 100 \quad (4)$$

where A_0 is the absorbance of the negative control and A_1 is the absorbance in the presence of the sample.

Acute toxicity study of *Sargassum binderi* towards rodents The acute toxicity of *S. binderi* was evaluated according to OECD Guidelines 423 (16). The toxicity study was conducted to determine the adverse effects following a single oral administration of *S. binderi* in Sprague-Dawley rats within a short period of time. According to OECD (16), when available information suggests that mortality is

unlikely, a limit test at 2,000 mg/kg body weight should be carried out to determine the acute toxicity of the test substance. The rodents used were Sprague-Dawley rats from the Animal Breeding Centre, Universiti Kebangsaan Malaysia (UKM). The rats were given a 6 day acclimation period prior to the study, in which they were kept one rat per cage in polycarbonate cages with solid bottoms and sides with stainless steel lids and with internal dimensions of 24.5 cm×17.5 cm×13.5 cm. The cages were filled with natural pine chip bedding. The rats were fed Barastoc animal feed and filtered water *ad libitum*. The environment was kept at a temperature of 20.9 to 25.1°C with 12 h light/dark cycles.

This study was performed with two groups of rats (and a control group) using a stepwise procedure according to OECD Guidelines 423 (16). In the first step, 3 female rats (Group 1) were administered a single oral dose of *S. binderi* at 2,000 mg/kg body weight mixed with pre-filtered water at 2 mL/100 g of body weight. Three additional female rats used as the untreated group (control group) were administered drinking water (10 mL/kg). The rats were observed at 30 min interval for the first 4 h and every 24 h thereafter for clinical and toxicological effects up to Day 14. The rats were observed for morbidity, mortality, and clinical signs, as shown in Table 1. Their body weights were measured before treatment (Day 0) and at termination (Day 14). Gross necropsy was performed on both the treated and untreated animals following euthanasia on the termination day. The necropsy was conducted by examining the external features and external orifices of the carcass. The major internal organs were exposed by dissection, and a detailed gross examination of the cranial, thoracic, and abdominal cavities and their contents was performed. The brain, spleen, liver, heart, pancreas, lungs, kidneys, and stomach were appropriately trimmed of any adherent tissue and individually weighed. In the second step, following the no observable adverse effects level (NOAEL) findings from the previously treated group (Group 1), 3 female rats (Group 2) were dosed with 2,000 mg/kg body weight (in 2 mL/100 g body weight pre-filtered water) of *S. binderi*. The rats were observed for 24 h and sacrificed for necropsy as performed for the Group 1 rats. This study was approved by the Universiti Kebangsaan Malaysia's Animal Ethics Committee (UKMAEC).

Results and Discussion

Molecular weight of fucoidan The molecular weight determined was the average molecular weight (Mw), where the molecular weight is relative to the size/weight of every molecule present, as shown in Table 1. It was found that F_{sar} (47.87 kDa) had a higher Mw compared to that of F_{ysk} (27.98 kDa). However, F_{ysk} was more polydisperse, with Mw ranging from 11.15 to 611.80 kDa, compared to F_{sar} , which had Mw ranging from 18.35 to 96.78 kDa. The polydispersity indices of F_{sar} and F_{ysk} were 1.12 and 1.88, respectively. These findings proved that the Mw of fucoidan differs greatly depending on the species, season, and

geographical location, as reported by Rioux *et al.* (8).

Composition of fucoidan The monosaccharide profiles of F_{sar} and F_{ysk} are shown in Table 1. The results showed that both F_{sar} (34.50%) and F_{ysk} (43.30%) are composed mainly of fucose, which is the main characteristic of fucoidan (4,17). Apart from fucose, F_{sar} contains a substantial amount of galactose (12.14%). Other monosaccharides were found in F_{sar} ; namely, xylose, mannose, glucose, and glucuronic acid. However, apart from fucose, only xylose and glucuronic acid were found in F_{ysk} . This shows that the monosaccharide profiles of fucoidans vary depending on the source. Another characteristic of fucoidan is that it contains sulfate groups. It was determined that F_{sar} has a higher sulfate content (7.66%) and degree of sulfation (0.20) than does F_{ysk} (5.70% and 0.15, respectively), as shown in Table 1. It was also found that F_{sar} (0.33) has a lower degree of acetylation compared to that of F_{ysk} (0.53).

The variation of monosaccharide profile and sulfate content in both F_{sar} and F_{ysk} are the result of them both being extracted from different seaweed species from different location. Previous studies (4,17), which compared a series of fucoidan extracted from various species of brown seaweeds from different locations and seasons, showed that fucoidan do have different monosaccharide compositions and sulfate content. It was also reported that different extraction and purification techniques may influence the composition of fucoidan (17). The compositional differences between F_{sar} and F_{ysk} show that both of these fucoidans have their own characteristics, justifying more in-depth studies of F_{sar} and other fucoidans from Malaysian brown seaweeds.

Structural analysis of fucoidan The 1H NMR spectra of F_{sar} and F_{ysk} (Fig. 1) showed similar chemical shifts, with peaks in the 0.94–1.37 (F_{sar}) and 1.14–1.43 ppm (F_{ysk}) regions that were assigned to the methyl group (CH_3), which is the main characteristic of fucopyranose. This also enabled us to assign these peaks to H6 of the fucopyranose. There were peaks at 5.10–5.42 (F_{sar}) and 5.11–5.53 ppm (F_{ysk}), which were due to the anomeric protons (H1) of fucopyranose, while the peaks at 2.03–2.34 (F_{sar}) and 2.19–2.34 ppm (F_{ysk}) were assigned to the *O*-acetyl groups (7,18). F_{sar} (0.33) showed a lower degree of acetylation compared to that of F_{ysk} (0.53). The ^{13}C NMR spectra of F_{sar} and F_{ysk} showed similar chemical shifts as well, with peaks at 101.4–105.6 (F_{sar}) and 99.7–101.6 ppm (F_{ysk}) due to anomeric carbons (C1), while the intense signals at 16.7–17.3 (F_{sar}) and 16.4–17.5 ppm (F_{ysk}) were due to methyl groups (C6). The peaks at 22.2; 174.6–176.8 ppm for F_{sar} and 21.8; 175.1–177.9 ppm for F_{ysk} are assigned to *O*-

acetyl groups (7,18). To assign the other peaks in the spectrum, 2D NMR analyses were employed, namely TOCSY and HMQC for proton and carbon assignments, respectively. The chemical shifts of H1–H6 and C1–C6 of fucopyranose from both F_{sar} and F_{ysk} were compared with the chemical shifts of the α -fucose monomer from Daniel *et al.* (19). The fucoses present in both F_{sar} and F_{ysk} were confirmed to be in α -fucose configuration, as the chemical shifts of the anomeric protons were in the range of 5.20–5.49 ppm, as opposed to β -fucose, which displays chemical shifts at 4.55–4.69 ppm (19). Unfortunately, for F_{ysk} , the ^{13}C chemical shift for C3 and C4 could not be determined in the HMQC spectrum, most likely due to the uneven molecular size/weight distribution of F_{ysk} (high polydispersity index) which created interference in the spectrum.

The F_{sar} upfield signal of H3 (3.74 ppm) and downfield signal of C3 (86.21 ppm), compared to that of α -fucose (1H of H3: 3.86 ppm; ^{13}C of C3: 70.29 ppm), indicated that there are glycosidic bonds at the C3 position (3,18). A downfield signal in ^{13}C of a glycosidic bond is due to steric effects; carbons at the glycosidic bonds that do not have hydroxyl groups are deshielded compared to other carbons with hydroxyl groups (20). To confirm this configuration, HMBC analysis was done on F_{sar} . Correlations were found between C1 (104.07 ppm) and H3 (3.74 ppm), confirming that the glycosidic bonds between the fucoses were between C1 and C3, giving a configuration of $\rightarrow 3$) fuc(1 $\rightarrow$ 3)fuc(1 $\rightarrow$). The linkages could not be determined for F_{ysk} because the assignment of the ^{13}C spectrum was incomplete.

The positions of the sulfate groups were determined through the proton and ^{13}C NMR chemical shifts, where the downfield proton and ^{13}C chemical shifts of F_{sar} at H2 (0.90 ppm difference) and C2 (14.97 ppm difference) relative to those of α -fucose were due to the presence of sulfate group at the C2 position (18,19). This is consistent with the FTIR results reported in previous work by Lim *et al.* (2), where the sulfate group of F_{sar} was at the equatorial position (absorbance band at 822 cm^{-1}), *i.e.*, at C2 and/or C3. Therefore, it is proposed that the model structure of F_{sar} is $\rightarrow 3$)fuc-2-OSO₃[−](1 $\rightarrow$ 3) fuc-2-OSO₃[−](1 $\rightarrow$, as shown in Fig. 2. As a whole, the chemical characteristics of F_{sar} were found to be different from those of F_{ysk} and from those of previously studied fucoidans. This proposed structure showed that F_{sar} has similarity to the structures reviewed by Li *et al.* (4) and Ale *et al.* (17), where fucoidan is made up mainly of fucose and sulfated. It must be noted that there are some variations in the structures of fucoidan from different studies (3,4,6,17,18), where the glycosidic linkage position and sulfate group orientation differ from one another.

Table 1. Molecular weight and composition of fucoidan extracted from *Sargassum binderi* (F_{sar}) and commercial food grade fucoidan (F_{ysk})

	Molecular weight		Monosaccharide (%)						Sulfate content (%)	Degree of sulfation	Degree of acetylation
	Mw (kDa)	Polydispersity index	Fuc	Gal	Xyl	Man	Glu	GlcA			
F_{sar}	47.87	1.12	34.50	12.14	2.90	4.63	1.13	4.67	7.66	0.20	0.33
F_{ysk}	27.98	1.88	43.30	-	1.59	-	-	1.55	5.70	0.15	0.53

Fig. 1. Proton NMR spectrum (600.30 MHz) of fucoidan isolated from *Sargassum binderi* (F_{sar}) and commercial food grade fucoidan (F_{ysk}) (in D_2O).

Fig. 2. Proposed model structure of fucoidan isolated from *Sargassum binderi* (F_{sar}).

Cytotoxicity of *Sargassum binderi* extract Toxicology studies were conducted on *S. binderi* and its extract to determine its suitability for the development of food products. The cytotoxicity of *S. binderi* was determined by MTT assay. The results in Fig. 3 show that the V79-4 cell viability was high at extract concentrations from 6.25 to 50 mg/

mL, with viability percentages near 100%. However, the cell viability decreased to 69.26 ± 1.55 and $55.68 \pm 2.54\%$ as the extract concentration increased to 100 and 200 mg/mL, respectively. However, these values were still high compared to that of the positive control ($29.38 \pm 0.48\%$), and it was lower than the IC_{50} value. This shows that *S. binderi* extract does not have harmful effects on the cells and thus is safe for consumption. This result is consistent with the work of Peng *et al.* (14), which reported that polysaccharide which contains high amount of fucose from brown algae showed no cytotoxicity on normal human cells.

Acute toxicity of *Sargassum binderi* in rodents This study was conducted to determine the adverse effects following a single oral administration of *S. binderi* towards the subject (Sprague-Dawley rats) within a short time period. In assessing and evaluating the toxic effect of a substance, determining the acute oral toxicity is usually the first step. Such studies provide information on the potential health hazards likely to arise from short-term exposure via an oral route (21). Oral gastric intubation was chosen as the route of administration as it is the representative route of exposure in humans and thus is the accepted route for assessing acute oral toxicity in animals (22).

Observations throughout the study showed that there was no mortality or adverse reaction in the group of rats dosed with 2,000 mg/kg body weight of *S. binderi*. All clinical observations were reported as normal/no changes for all treated (Group 1 and Group 2)

Fig. 3. V79-4 cell viability (%) at different concentrations of *Sargassum binderi* extracts through MTT assay ($n=3$).

and untreated (Control Group) animals throughout the monitoring period. The NOAEL findings on Group 1 prompted the Group 2 study. None of the animals demonstrated any clinical changes or abnormal behavior during the period of study. This enables *S. binderi* to be classified as category 5 or unclassified according to OECD Guideline 423; *i.e.*, the expected LD_{50} of *S. binderi* is at the range of 2,000–5,000 mg/kg body weight. Tests at 5,000 mg/kg body weight were not conducted because this test is discouraged by OECD Guideline 423 due to the necessity of protecting animal welfare (16).

The data on the body weight showed that the administration of *S. binderi* to Sprague-Dawley rats did not cause body weight loss in Group 1 (237.8 and 272.1 g-before and after administration) or Group 2 (229.2 and 233.1 g-before and after administration), with similar findings in the Control Group (264.6 and 290.3 g-before and after administration). The gross post-mortem examination of the

brain, spleen, liver, heart, pancreas, lungs, kidneys, and stomach did not present any abnormalities, where no lesions were found during close examination of the internal organs. There were also no remarkable differences in the mean percentages of organ to animal body weight between the treated (Group 1 and Group 2) and the untreated (Control Group) groups (Fig. 4). Therefore, it can be concluded that *S. binderi* does not demonstrate acute toxicity and thus may be safe for human consumption.

The toxicology studies on *S. binderi* and its extract showed that did not demonstrate any toxicity to the cells and animals. The cytotoxicity assessed through MTT assay showed that the cell inhibition was below IC_{50} , while the acute toxicity test did not reveal any mortality in rats, allowing *S. binderi* extract to be classified as category 5 or unclassified according to OECD Guideline 423. This research showed that the fucoidan extracted from Malaysian brown seaweed, *S. binderi*, has unique chemical characteristics and is non-toxic and safe for consumption.

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Disclosure The authors declare no conflict of interest.

Fig. 4. Acute toxicity study-comparison of the organ weight percentage in relation to the Sprague-Dawley rats body weight between Group 1, Group 2 (administered with *Sargassum binderi*), and Control Group (not administered with *Sargassum binderi*) ($n=3$).

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