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In Vitro Anticoagulant Effects of Fucoidan-Rich Extracts from South African Brown Macroalgae

Coleen E. Grobler [1], Bongile Mpetsheni [2], Blessing Mabate [1,2], Carminita L. Frost [2] and Brett I. Pletschke [1,*]

[1] Enzyme Science Programme (ESP), Department of Biochemistry, Microbiology and Bioinformatics, Rhodes University, Makhanda 6140, South Africa; groblercoleen@gmail.com (C.E.G.); bmabate@gmail.com or blessing.mabate@mandela.ac.za (B.M.)
[2] Department of Biochemistry and Microbiology, Nelson Mandela University, Port Elizabeth 6031, South Africa; s224702815@mandela.ac.za (B.M.); carminita.frost@mandela.ac.za (C.L.F.)
* Correspondence: b.pletschke@ru.ac.za; Tel.: +27-46-603-8081

Abstract

Blood coagulation is a vital physiological process; however, its dysregulation may result in excessive clotting and adverse health outcomes. Current anticoagulant therapies, such as heparin, are commonly employed to prevent and treat thrombosis, but these agents have notable limitations, including poor oral bioavailability and the risk of haemorrhage. Therefore, there is growing interest in discovering alternative natural compounds with anticoagulant properties. This study investigated the in vitro anticoagulant potential of previously characterised fucoidan-rich extracts from three South African brown seaweed species, namely *Ecklonia maxima*, *Ecklonia radiata*, and *Sargassum incisifolium*. Anticoagulant activity was evaluated using prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin-induced clotting using FIB-C reagent; D-dimer was assessed separately as an exploratory endpoint. Mean PT and aPTT increased across the tested extract concentration ranges. At 350 μg·mL^{-1}, *E. maxima* and *E. radiata* produced PT values 4.13 and 3.95 times the control value, respectively; both exceeded *S. incisifolium* but did not differ significantly from one another. Among the extracts, *E. maxima* produced the highest mean aPTT response, reaching 2.61 times the control value at 50 μg·mL^{-1}. Thrombin-induced clotting times measured with the FIB-C reagent were prolonged at the highest tested concentration for each preparation, with responses varying among preparations and concentrations. Together, the coagulation assays support the in vitro anticoagulant potential of these fucoidan-rich extracts, while their specific mechanisms of action remain to be determined.

Keywords: prothrombin time; activated partial thromboplastin time; sulphated polysaccharides; haemostasis; marine bioproducts

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1. Introduction

Oceans are rich reservoirs of chemically and biologically diverse compounds [1]. Macroalgae (seaweeds) are diverse marine eukaryotes distributed throughout coastal and oceanic environments worldwide [2,3] and are important renewable resources for both humans and marine organisms [4].

Macroalgae are abundant sources of nutrients, including dietary fibres, proteins, vitamins, minerals, and a wide range of polysaccharides [5]. Due to their extensive

biological diversity and chemical complexity, macroalgae have attracted a growing interest in biomedical research and in the development of functional foods and nutraceuticals [5,6]. Polysaccharides derived from brown algae, such as fucoidan, alginate, and laminarin, are of particular interest for these applications [3,4].

Fucoidans are a structurally diverse group of sulphated, fucose-rich polysaccharides [7,8]. Their composition and size vary with algal species, extraction technique, purification method, geographic origin, harvesting season, and seaweed growth stage [1,5,7,9]. These differences provide a rationale for evaluating the biological activity of individual preparations. Fucoidans are gaining increased attention for their potential health benefits [7], with reported activities including anti-obesity, antioxidant, antidiabetic, and anticoagulant effects [10]. Oral tolerability has been reported for specific fucoidan preparations under defined study conditions; for example, no treatment-related adverse events were reported during a 12-week placebo-controlled study of *Cladosiphon okamuranus* fucoidan in healthy adults [11]. However, these findings do not establish the safety of all fucoidan-rich extracts, including the preparations examined here.

Cardiovascular diseases (CVDs) are disorders of the heart and blood vessels, and thrombosis contributes to serious cardiovascular complications [12]. Thrombosis involves the formation of a blood clot within a blood vessel, which can obstruct blood flow and contribute to myocardial infarction, ischaemic stroke, pulmonary embolism, or deep vein thrombosis [13]. Although blood coagulation is an essential physiological process that prevents excessive bleeding following vascular injury, its dysregulation can have severe consequences [14].

Current therapeutic strategies for thrombosis include antiplatelet agents, thrombolytic therapies, and anticoagulants such as heparin, which are selected according to the clinical setting [15]. Heparin and low-molecular-weight heparins (LMWHs) are widely used anticoagulants, but their poor oral bioavailability motivates investigation of alternative approaches [16]. Fucoidans have been investigated for anticoagulant and antithrombotic activity in experimental systems [17]. For example, Sun et al. [16] reported differences in absorption and coagulation responses following oral administration of fucoidan and its lower-molecular-weight derivatives in animal models. Using structurally defined synthetic fucoidan oligosaccharides, Chen et al. [18] demonstrated that molecular size and sulphation influence anticoagulant activity. These findings support considering molecular-weight distributions and structural features alongside clotting responses, rather than assuming that bulk sulphate content alone determines activity. Furthermore, changes in plasma clotting times provide screening evidence of anticoagulant activity but do not, by themselves, establish specific molecular targets or clinical efficacy.

Given the health burden posed by thrombosis and the limitations of available anticoagulants, a further investigation of natural compounds with anticoagulant activity is warranted. South African brown macroalgae, including *Ecklonia maxima*, *Ecklonia radiata*, and *Sargassum incisifolium*, provide fucoidan-rich extracts with distinct physicochemical characteristics [19]. Earlier studies investigated inhibition of diabetes-related enzymes by fucoidans from *E. maxima* and *E. radiata* [20,21]. These findings establish a metabolic-bioactivity context, but do not establish the coagulation effects of the preparations examined here. However, these characteristics alone do not establish how the preparations affect plasma coagulation. The present study evaluates the in vitro coagulation effects of the same fucoidan-rich extract preparations previously characterised by Grobler et al. [19]. Specifically, it compares their responses across concentrations using prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin-induced clotting with FIB-C reagent, with heparin and commercial *Macrocystis pyrifera* fucoidan as reference preparations. D-dimer measurements are considered separately as an exploratory endpoint. Previously published compositional,

molecular-weight, and spectroscopic data provide context for the new coagulation findings, allowing the examination of preparation- and assay-dependent responses without attributing activity to a single structural feature or excluding contributions from co-extracted constituents.

2. Results

2.1. Previously Reported Extract Characteristics and Reducing Sugar Content

The coagulation experiments used the same fucoidan-rich extracts from *E. maxima*, *E. radiata* and *S. incisifolium* previously characterised by Grobler et al. [19]. Table 1 consolidates selected published extract characteristics and the reducing sugar results reported here; the previously published measurements are not presented as new experimental findings.

Extraction yields ranged from 3.73 to 8.76 g extract/100 g depigmented macroalgae, with *E. maxima* giving the highest yield (Table 1). Previously published compositional data [19] are summarised alongside the reducing sugar results in Table 1. Total carbohydrate contents were 38.5 ± 4.57, 90.23 ± 3.69 and 96.27 ± 4.79 g glucose equivalents/100 g dried extract for *S. incisifolium*, *E. radiata* and *E. maxima*, respectively. The two *Ecklonia* extracts had similar carbohydrate contents, both more than twice that of *S. incisifolium*. *S. incisifolium* had the highest total phenolic, reducing sugar and sulphate contents, whereas *E. radiata* had the highest L-fucose content.

Total carbohydrate and L-fucose values are reported as glucose equivalents and L-fucose equivalents, respectively, per 100 g dried extract. These estimates derive from separate colourimetric assays using different calibration standards and are not directly interchangeable gravimetric measurements. Monosaccharide-dependent colour responses and co-extracted constituents may influence the estimates [19]. The assay-derived composition values should therefore not be summed as an additive mass balance.

Table 1. Extraction yield, chemical characteristics and molecular weights of the dried fucoidan-rich extracts. Published values are reproduced from Grobler et al. [19], with reducing sugar results added from the present study.

Parameter	*Sargassum incisifolium*	*Ecklonia radiata*	*Ecklonia maxima*
Extraction yield (g extract/100 g depigmented macroalgae)	3.73 ± 0.54 ^{b}	3.92 ± 0.49 ^{b}	8.76 ± 0.81 ^{a}
Total carbohydrate (g GE/100 g dried extract)	38.50 ± 4.57 ^{b}	90.23 ± 3.69 ^{a}	96.27 ± 4.79 ^{a}
Total phenolic content (g GAE/100 g dried extract)	6.76 ± 0.85 ^{a}	4.02 ± 1.08 ^{b}	1.08 ± 0.53 ^{c}
Protein (g BSAE/100 g dried extract)	6.43 ± 0.43 ^{a}	6.75 ± 1.69 ^{a}	4.76 ± 1.74 ^{a}
Sulphate (g sulphate equivalents/100 g dried extract)	24.01 ± 3.36 ^{a}	15.27 ± 0.92 ^{b}	10.70 ± 1.04 ^{b}
L-fucose (g L-fucose equivalents/100 g dried extract)	36.74 ± 1.84 ^{b}	55.86 ± 3.77 ^{a}	49.55 ± 2.64 ^{a}
Total reducing sugar (g GE/100 g dried extract)	40.07 ± 3.4 ^{a}	27.70 ± 1.22 ^{b}	25.77 ± 1.41 ^{b}
Molecular weight (kDa)	226	~11	~19

Composition and yield values are means ± SD (n = 3). Extraction yield is expressed per 100 g depigmented macroalgae; composition values are expressed per 100 g dried extract. For the published composition and yield rows, n = 3 independent biological replicates, and different superscript lowercase letters within a row indicate significant differences (one-way ANOVA followed by Tukey's multiple-comparisons test, $p < 0.05$); values sharing a letter are not significantly different. GE, glucose equivalents; GAE, gallic acid equivalents; BSAE, bovine serum albumin equivalents. Molecular weights are estimates relative to pullulan standards. *S. incisifolium*: peak-area-weighted average of 226 kDa, with fractions at approximately 450 and 5 kDa; the *Ecklonia* values represent the single major peaks.

2.2. Anticoagulant Activity

The coagulation effects of fucoidan-rich extracts from *E. maxima*, *E. radiata* and *S. incisifolium* were investigated using PT, aPTT and thrombin-induced clotting with FIB-C reagent. Commercial *M. pyrifera* fucoidan was included as a reference preparation and heparin as a positive control. D-dimer measurements were evaluated separately as an exploratory endpoint.

2.2.1. Effects of Fucoidan-Rich Extracts on Prothrombin Time (PT)

PT was measured to assess changes in clot formation following tissue-factor activation (Figure 1). Heparin and commercial *M. pyrifera* fucoidan provided reference responses within their respective concentration ranges. All three fucoidan-rich extracts prolonged PT relative to untreated plasma control at the displayed concentrations of 150–350 μg·mL^{-1} (unadjusted $p < 0.01$). Mean PT increased with concentration, reaching 50.47 ± 1.61, 48.30 ± 2.01 and 20.63 ± 0.85 s for *E. maxima*, *E. radiata* and *S. incisifolium*, respectively, at 350 μg·mL^{-1}, compared with 12.23 ± 0.23 s for the plasma control. These values corresponded to 4.13-, 3.95-, and 1.69-fold increases over the control value. At 150 and 250 μg·mL^{-1}, Holm-adjusted comparisons distinguished all three extracts, with the highest PT for *E. maxima*, followed by *E. radiata* and *S. incisifolium* (adjusted $p < 0.05$). At 350 μg·mL^{-1}, both *Ecklonia* extracts produced higher PT values than *S. incisifolium* but did not differ significantly from one another (adjusted $p = 0.222$).

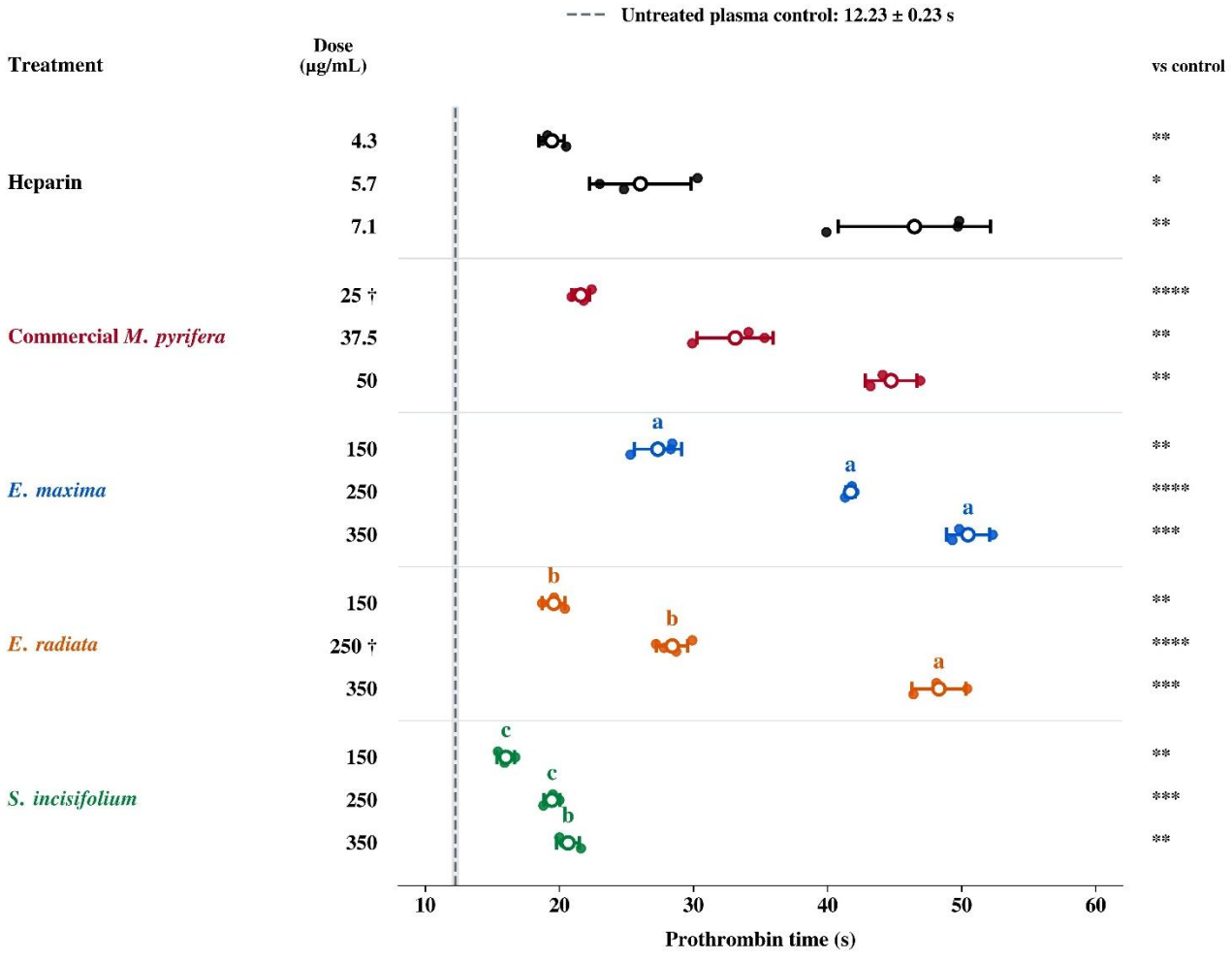


Figure 1. Effects of heparin and fucoidan preparations on prothrombin time (PT). Open circles and whiskers show means ± SD; filled points show individual observations ($n = 3$; † $n = 4$). The dashed line and shaded band represent the untreated plasma control mean ± SD. Stars indicate unadjusted

two-sided Welch's *t*-tests versus control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. Letters compare *E. maxima*, *E. radiata* and *S. incisifolium* at matching concentrations using Welch's *t*-tests with Holm correction across nine comparisons. Within each concentration, groups sharing a letter do not differ significantly (adjusted $p \geq 0.05$).

Commercial *M. pyrifera* fucoidan and heparin also prolonged PT within their lower tested concentration ranges. At 50 μg·mL^{-1}, *M. pyrifera* produced a PT of 44.73 ± 1.93 s (3.66 times control; unadjusted $p = 0.001$), whereas heparin at 7.1 μg·mL^{-1} produced 46.47 ± 5.69 s (3.8 times control; unadjusted $p = 0.009$). These observations demonstrate activity at different tested concentrations.

2.2.2. Effects of Fucoidan-Rich Extracts on Activated Partial Thromboplastin Time (aPTT)

aPTT was measured to assess clotting following contact activation (Figure 2). The fucoidan-rich extracts were tested at 25, 37.5 and 50 μg·mL^{-1}, commercial *M. pyrifera* fucoidan at 5, 7.5 and 10 μg·mL^{-1}, and heparin at 0.357 and 0.714 μg·mL^{-1}. Responses were interpreted within these tested ranges.

Mean aPTT increased across the tested concentration range for each fucoidan-rich extract. At 50 μg·mL^{-1}, values were 93.37 ± 9.37, 64.13 ± 2.80 and 56.27 ± 3.53 s for *E. maxima*, *E. radiata* and *S. incisifolium*, respectively, compared with 35.73 ± 1.45 s for control. These represented 2.61, 1.79, and 1.57-fold increases over the control value and were significant in unadjusted comparisons ($p < 0.05$). Although *E. maxima* consistently produced the highest mean aPTT among the extracts, between-extract differences did not remain significant after Holm correction across nine comparisons (adjusted $p \geq 0.110$). Commercial *M. pyrifera* fucoidan produced 75.63 ± 8.36 s at 10 μg·mL^{-1} (2.12 times control; unadjusted $p = 0.0124$), whereas heparin produced 99.07 ± 9.30 s at 0.714 μg·mL^{-1} (2.77 times control; unadjusted $p = 0.0061$). *M. pyrifera* at 5 μg·mL^{-1} and heparin at 0.357 μg·mL^{-1} did not differ significantly from the control (unadjusted $p = 0.322$ and 0.888, respectively).

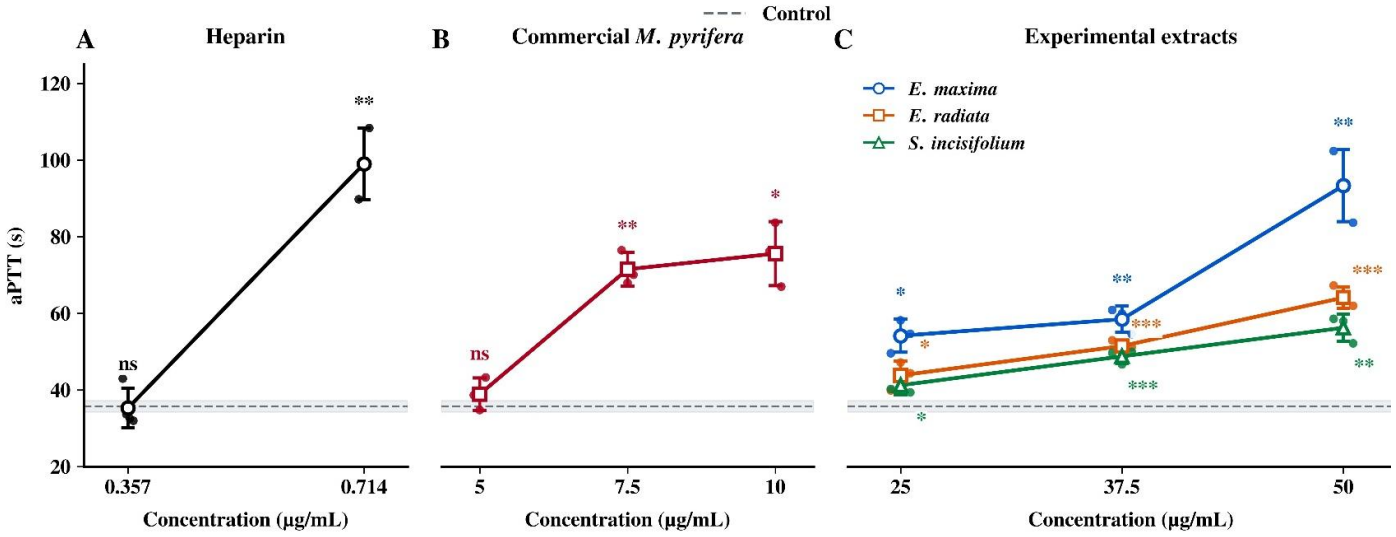


Figure 2. Effects of heparin and fucoidan preparations on activated partial thromboplastin time (aPTT). (**A**) Heparin; (**B**) commercial *Macrocystis pyrifera* fucoidan; and (**C**) fucoidan-rich extracts from *Ecklonia maxima*, *Ecklonia radiata* and *Sargassum incisifolium*. Open symbols and error bars represent SD; smaller filled points indicate individual independent observations. The dashed horizontal line and shaded band indicate the control mean ± SD (35.73 ± 1.45 s; $n = 3$). Each treatment–concentration combination was compared with the control using a two-sided Welch's *t*-test without correction for multiple comparisons. Asterisks indicate unadjusted *p*-values: $p < 0.05$

(*), $p < 0.01$ (**), and $p < 0.001$ (***); ns, $p \geq 0.05$. Symbols refer only to comparisons with control; comparisons between fucoidan extracts at matching concentrations were not significant after Holm correction across nine comparisons.

2.2.3. Effects of Fucoidan-Rich Extracts on Thrombin-Induced Clotting Time Using FIB-C Reagent

The FIB-C assay recorded clot formation following addition of a thrombin-containing reagent to diluted plasma (Figure 3). This endpoint is described as thrombin-induced clotting time; its equivalence to a conventional thrombin time assay has not been established. At the highest concentration tested for each preparation, FIB-C clotting time was longer than control in unadjusted comparisons ($p < 0.01$). Commercial *M. pyrifera* fucoidan was active at lower concentrations than heparin but showed no monotonic increase in mean clotting time: values were 28.47, 30.63, and 29.23 s at 5, 10, and 12.5 μg·mL^{-1}, respectively. Its response at 10 μg·mL^{-1} did not differ significantly from the control (unadjusted $p = 0.0755$). In contrast, mean clotting time increased across the tested heparin concentrations. At 12.5 μg·mL^{-1}, *M. pyrifera* produced 29.23 ± 0.83 s (1.4 times the control), whereas heparin at 20 μg·mL^{-1} produced 30.97 ± 2.41 s (1.48 times the control).

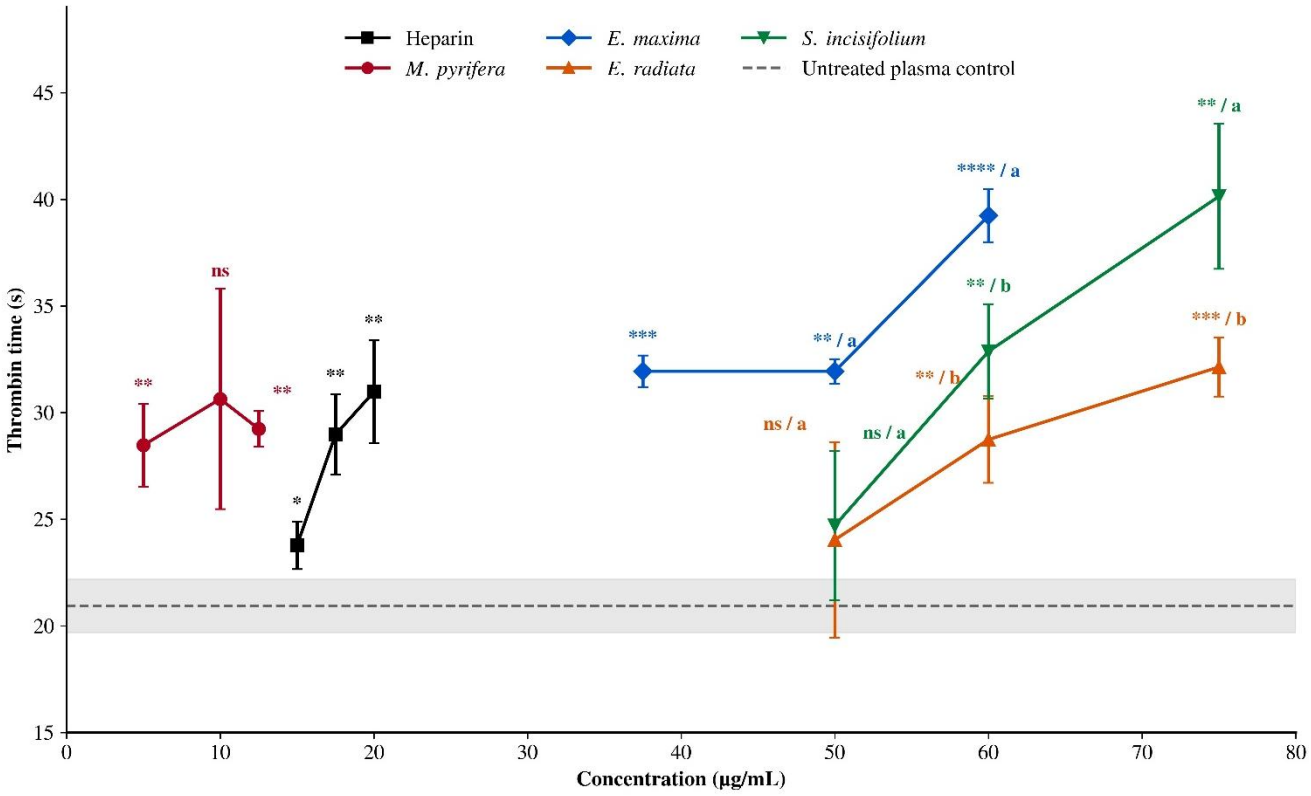


Figure 3. Effects of heparin and fucoidan preparations on thrombin-induced clotting time measured using FIB-C reagent. Points and error bars represent means ± SD ($n = 3$). The dashed line and shaded band indicate the untreated plasma control mean ± SD. Comparisons used two-sided Welch's *t*-tests without multiple-comparison correction. Stars indicate comparisons against control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$; ns, $p \geq 0.05$. Within each concentration, different letters indicate significant differences between extracts ($p < 0.05$); shared letters indicate no significant difference.

At 60 μg·mL^{-1}, *E. maxima* produced a FIB-C clotting time of 39.23 ± 1.24 s, compared with 28.73 ± 2.03 s for *E. radiata* and 32.87 ± 2.21 s for *S. incisifolium*. The *E. maxima* response exceeded those of both extracts (unadjusted $p < 0.05$). At 75 μg·mL^{-1}, *S. incisifolium* produced a longer clotting time than *E. radiata* (40.13 ± 3.40 versus 32.13 ± 1.39 s;

unadjusted $p < 0.05$); *E. maxima* was not tested at this concentration. The *S. incisifolium* value was 1.92 times the control (20.93 ± 1.25 s). No between-extract difference was detected at 50 μg·mL^{-1} (unadjusted $p \geq 0.0663$).

2.2.4. Effects of Fucoidan-Rich Extracts on D-Dimer Test

D-dimer measurements provided an exploratory comparison of plasma–extract mixtures (Table 2). The carbohydrate-rich *Ecklonia* extracts yielded higher readings (*E. maxima*, 429.67; *E. radiata*, 490 ng·mL^{-1}) than *S. incisifolium* (240.33 ng·mL^{-1}), suggesting that preparation-specific composition may influence the measured response. Commercial *M. pyrifera* yielded 475.67 ng·mL^{-1}. The control-material ranges were analytical quality-control limits, not biological reference intervals. These observations provide a hypothesis for subsequent interference-controlled studies; without defined clot formation and lysis, they do not establish effects on fibrinolysis.

Table 2. The D-dimer test was completed for the fucoidan extracts from various South African brown seaweeds, compared to a commercial fucoidan sample and two controls (high and low D-dimer control).

Sample	[D-Dimer] (ng·mL^{-1})	Role
Blank	271.00	Assay blank
PBS	290.33	Vehicle
Control 1 (low D-dimer)	423.00	Low quality-control material
Control 2 (high D-dimer)	669.67	High quality-control material
E. maxima Fucoidan	429.67	Test preparation
E. radiata Fucoidan	490.00	Test preparation
S. incisifolium Fucoidan	240.33	Test preparation
M. pyrifera Fucoidan	475.67	Reference preparation

3. Discussion

3.1. Extract Characteristics in Relation to Anticoagulant Activity

The physicochemical and structural characteristics of these same fucoidan-rich extracts were previously reported by Grobler et al. [19]. SEC-HPLC-RID (Size-Exclusion Chromatography – High-Performance Liquid Chromatography – Refractive Index Detection) indicated single major peaks corresponding to molecular weights of approximately 19 and 11 kDa for *E. maxima* and *E. radiata*, respectively, whereas *S. incisifolium* exhibited a heterogeneous distribution, with fractions near 450 and 5 kDa and a peak-area-weighted average of 226 kDa (Table 1; [19], Figure 1). FTIR and ^{1}H NMR provided supporting evidence for sulphated, fucose-containing polysaccharides ([19], Figures 2 and 3). Considered alongside the present coagulation results, the relatively high mean PT and aPTT responses of *E. maxima* despite its lower sulphate content suggest that bulk sulphate content alone does not explain the differences among the extracts. These comparisons highlight the importance of considering molecular-weight distribution, composition and sulphation together when interpreting anticoagulant activity, rather than relying on a single bulk compositional measurement.

The compositional analyses provide complementary information about the fucoidan-rich extracts. Total carbohydrate and L-fucose contents were quantified using their respective calibration standards and are therefore reported as glucose and L-fucose equivalents, respectively [19]. Measurements within the standard-curve ranges supported estimation of sample concentrations. These results provide a basis for comparing the assay-derived compositional profiles of the extracts, while recognising that differences in

monosaccharide colour responses and potential matrix effects preclude treating the values as directly interchangeable mass fractions.

The previously reported SEC, FTIR, and ^{1}H NMR data provide a physicochemical context for interpreting the coagulation responses of these preparations [19]. Together, these analyses support the presence of sulphated, fucose-containing polysaccharides and reveal differences in the apparent molecular weight distribution. When considered alongside the present findings, they indicate that anticoagulant responses warrant interpretation in terms of several compositional and structural features. Extraction-dependent variation has also been reported for *S. elegans* fucoidans evaluated for α-glucosidase inhibition [22], reinforcing the importance of preparation-specific characterisation, although the biological endpoint differs. The present study thus connects existing characterisation with new coagulation findings and provides a basis for targeted structure–activity studies. Comprehensive monosaccharide profiling and fractionation would help identify the features associated with activity and distinguish contributions from fucoidan and co-extracted constituents.

3.2. Coagulation Studies

The three extracts produced distinct coagulation-response profiles rather than a uniform activity ranking across all assays. PT responses clearly separated the extracts at 150 and 250 μg·mL^{-1}, whereas the two *Ecklonia* extracts did not differ significantly at 350 μg·mL^{-1} after Holm correction. *E. maxima* also produced the highest mean aPTT values, but between-extract differences did not remain significant after adjustment. Thus, numerical differences in clotting time should be distinguished from statistically supported differences. These experiments extend the previous characterisation of the same preparations by establishing their plasma clotting responses; they do not identify the active molecular species.

Pronounced aPTT prolongation was observed at lower tested extract concentrations than those used in the PT experiments. This pattern is consistent with observations for *F. vesiculosus* fucoidan, for which aPTT and TT responded at lower concentrations than PT [23]. However, direct comparisons of clotting times between studies cannot establish relative potency because plasma, reagent composition, dilution and assay conditions differ. In the present study, *M. pyrifera* at 10 μg·mL^{-1} produced an aPTT 2.12 times the control, *E. maxima* at 50 μg·mL^{-1} produced 2.61 times the control, and heparin at 0.714 μg·mL^{-1} produced 2.77 times the control. These responses demonstrate activity within different tested ranges, without establishing formal potency ratios.

Earlier studies also reported source-dependent coagulation effects of brown-seaweed fucoidans [24,25]. Lin et al. [26] similarly combined monosaccharide composition, molecular-weight and FTIR analyses with in vitro antithrombotic assessment of *S. henslowianum* fucoidan fractions. Such findings support investigating each preparation individually rather than extrapolating activity from species identity alone. The lower concentrations used in the aPTT experiments are compatible with assay-dependent sensitivity, but do not prove selective inhibition of the intrinsic pathway. PT and aPTT are screening endpoints influenced by multiple reactions, and differences between them require targeted mechanistic investigation.

Fucoidan preparations can interact with coagulation proteins through several mechanisms. Ustyuzhanina et al. [27] demonstrated direct thrombin interactions and antithrombin-dependent inhibition of thrombin and factor Xa for particular structurally characterised fucoidans. These findings provide plausible mechanisms for prolonged clotting, but neither those mechanisms nor inhibition of the intrinsic tenase complex were measured in the present study.

The FIB-C assay demonstrated that the test preparations prolonged thrombin-induced clotting in diluted plasma, indicating delayed clot formation under the experimental conditions. The reagent supplies exogenous thrombin and is conventionally used to determine functional fibrinogen by the Clauss method [28]; in this study, clotting time was recorded directly as the response endpoint. These findings complement the PT and aPTT results by demonstrating effects on clot formation following thrombin addition. The endpoint is therefore described as FIB-C thrombin-induced clotting time, rather than conventional TT or fibrinogen concentration. Further studies using purified coagulation factors and defined fibrin-formation systems would help identify the mechanisms underlying the observed prolongation. Bulk sulphation and molecular weight should be interpreted in the context of the structure [9,17]. Ustyuzhanina et al. [27] found that additional sulphation increased activity markedly in one fucoidan but slightly decreased it in another, indicating that sulphate abundance alone does not determine activity. In the present study, *S. incisifolium* had higher bulk sulphate content and a larger apparent average molecular weight than the *Ecklonia* extracts, yet produced less PT prolongation at matched concentrations. Conversely, its FIB-C response exceeded that of *E. radiata* at 75 $\mu g \cdot mL^{-1}$ in the unadjusted analysis, although this difference did not survive Holm correction. The assay-dependent responses are therefore not explained by a simple ordering of sulphate content or apparent molecular weight.

The published SEC, FTIR and ^{1}H NMR results for these same preparations [19] provide supporting context, as discussed in Section 3.1. However, differences in monosaccharide composition, sulphation patterns, chain conformations, and co-extracted constituents could contribute to the responses. Fractionation, comprehensive monosaccharide profiling and matched characterisation and activity measurements across additional independent preparations would be required.

Molecular weight may also influence biological response through exposure rather than intrinsic anticoagulant activity alone. Sun et al. [16] compared native fucoidan and lower-molecular-weight derivatives after oral administration in animal models and reported differences in absorption and coagulation responses. This provides a rationale for subsequent pharmacokinetic work but does not establish oral efficacy of the present extracts. The current experiments demonstrate only in vitro changes in clotting time; thrombus formation, bleeding risk, toxicity, and in vivo efficacy were not evaluated.

D-dimer measurements provided an exploratory assessment of the assay response in plasma mixtures containing the fucoidan preparations. D-dimer concentrations varied among the preparations, providing preliminary observations for subsequent investigation. Because the experiment measured an endpoint in calibration-plasma mixtures rather than D-dimer generation during controlled clot formation and lysis, these differences cannot yet be attributed to altered fibrinolysis. Future studies that combine a defined clot-lysis system with controls to address extract-related assay interference would clarify their biological significance.

4. Materials and Methods

4.1. Seaweed Harvesting and Preparation

The macroalgal material used to prepare the fucoidan-rich extracts was sourced and processed as described by Grobler et al. [19]. *Ecklonia maxima* was supplied by AfriKelp (Cape Town, South Africa), whereas *Ecklonia radiata* and *Sargassum incisifolium* were collected as beach-cast material along the South African Indian Ocean coastline (32°50′13.3″ S, 28°06′58.9″ E) and identified by Prof. J.J. Bolton (University of Cape Town). Material was transported to the laboratory on ice and rinsed with distilled water. After cutting into approximately 5–10 cm pieces, the seaweeds were dried in a Biobase incubator (Biobase, Jinan, China) at 40 °C for approximately 48 h without agitation. The dried biomass was

milled with a coffee grinder and kept in airtight containers at room temperature until extraction.

4.2. Fucoidan Extraction

The coagulation experiments used the same fucoidan-rich extract preparations previously characterised by Grobler et al. [19]. Briefly, ground macroalgae were depigmented with absolute ethanol (1:20, *w*/*v*) overnight at room temperature and extracted with distilled water (1:30, *w*/*v*) at 70 °C overnight with agitation. After centrifugation at 10,000× *g* for 10 min, the supernatant was treated with $CaCl_2$ at a final concentration of 2% (*w*/*v*) and incubated at 4 °C for 4 h. Precipitated calcium alginate was removed by centrifugation at 10,000× *g* for 10 min. Previously reported extraction yields are expressed as g extract per 100 g dried, depigmented macroalgae.

4.3. Chemical Characterisation and Previously Reported Physicochemical Analyses

The chemical composition, molecular weight profiles and spectroscopic characteristics of the fucoidan-rich extracts used in the present study were previously reported by Grobler et al. [19]. Briefly, total carbohydrate and L-fucose contents were quantified using the phenol–sulphuric acid assay [29] and the cysteine–sulphuric acid assay [30], respectively, with D-glucose and L-fucose as calibration standards. Sulphate was measured using a modified barium chloride–gelatine assay following treatment with 60% (*v*/*v*) formic acid, with sodium sulphate as the standard [31]. Protein and total phenolic contents were determined using the Bradford and modified Folin–Ciocalteu assays, calibrated with bovine serum albumin and gallic acid, respectively [32,33]. Total carbohydrate and L-fucose results were expressed as g glucose equivalents and g L-fucose equivalents per 100 g dried extract, respectively. All quantitative measurements were made within the linear ranges of the corresponding standard curves.

Molecular weights were estimated by SEC-HPLC with refractive index detection, as described by Grobler et al. [19]. Separation was performed using a Shodex OHpak SB-806M HQ column (8.0 × 300 mm) (Showa Denko K.K., Tokyo, Japan) maintained at 30 °C. The mobile phase was 0.1 M aqueous $NaNO_3$, filtered through a 0.22 μm nylon membrane and delivered at 0.5 mL min^{-1}. Samples were injected in a 20 μL volume, and molecular weights were estimated relative to a calibration curve prepared with pullulan standards.

Previously reported structural evidence for these same extracts included FTIR and ^{1}H NMR spectra ([19], Figures 2 and 3). The original analytical procedures are described in Sections 3.6 and 3.7 of that publication. These spectra are used here as supporting characterisation and were not newly acquired for the coagulation study. L-fucose was quantified colourimetrically; comprehensive chromatographic monosaccharide profiles were not available.

Reducing sugars were quantified after acid hydrolysis using the dinitrosalicylic acid (DNS) assay [34]. Extracts (10 $mg.mL^{-1}$) were hydrolysed with 2 M trifluoroacetic acid at 100 °C for 6 h, centrifuged at 13,000× *g* for 5 min, and evaporated at 40 °C. The hydrolysates were reconstituted in distilled water to 20 $mg.mL^{-1}$ and diluted to 1 $mg.mL^{-1}$ for analysis. Aliquots (150 μL) were mixed with 300 μL of DNS reagent, heated at 100 °C for 6 min, and cooled on ice for 6 min. The absorbance was measured at 540 nm, and reducing sugar content was calculated using a D-glucose calibration curve and expressed as glucose equivalents.

4.4. Anticoagulant Effect of Fucoidan

Coagulation studies were conducted using the fucoidan extracts to investigate their potential anticoagulant effects. These studies were carried out using purchased coagulation kits from ILEX (Pty) Ltd., Johannesburg, South Africa. The tests were conducted according to the protocol recommended in the kit's instructions. Commercial *Macrocystis pyrifera* fucoidan was included as a reference preparation; this comparison did not exclude contributions from other constituents of the fucoidan-rich extracts. Heparin, a known anticoagulant, was used as a positive control. The samples were dissolved in phosphate-buffered saline (10 mM Na_2HPO_4; 1.8 mM KH_2PO_4; 2.7 mM KCl; 140 mM NaCl, pH 7.4) and 0.1% (*v*/*v*) DMSO.

4.4.1. Prothrombin Time (PT) Assay

The prothrombin time (PT) test measures clotting time after tissue factor activation and assesses the extrinsic and common coagulation pathways. Human calibration plasma (100 µL) was purchased from ILEX (SA) and the samples (50 µL) were combined in a cuvette. Recombiplastin 2G (200 µL) was added to a separate cuvette. The calibration plasma and sample mixture, as well as the recombiplastin 2G were incubated in the incubation wells at 37 °C for 3 min. The cuvette containing recombiplastin 2G was then transferred to the reading cell, to which calibration plasma and sample mixture (150 µL) were added, initiating clot formation. The readings (time in seconds) were taken using a coagulometer (Beckman, Brea, CA, USA).

A final concentration of 150, 250, and 350 µg·mL^{-1} of *E. maxima* and *S. incisifolium* fucoidan was used. The same concentration range of *E. radiata* fucoidan was used, with an additional concentration of 500 µg·mL^{-1}. The concentrations of *M. pyrifera* fucoidan were 25, 37.5, and 50 µg·mL^{-1}, and those of heparin were 4.3, 5.7, and 7.1 µg·mL^{-1}.

4.4.2. Activated Partial Thromboplastin Time (aPTT) Assay

Activated partial thromboplastin time (aPTT) was measured to assess clotting following contact activation of the intrinsic and common coagulation pathways. Firstly, the $CaCl_2$ solution was incubated at 37 °C for 15 min before use. Synthasil phospholipid reagent (100 µL), calibration plasma (100 µL), and the samples (50 µL) were combined in a cuvette and incubated at 37 °C for 3 min. After incubation, the cuvette was transferred to the reading cell, and the $CaCl_2$ solution (100 µL) was added to initiate clot formation. The readings (time in seconds) were taken using a coagulometer (Beckman, CA, USA).

A final concentration of 25, 37.5, and 50 µg·mL^{-1} of *E. maxima*, *S. incisifolium*, and *E. radiata* fucoidan was used in this test. The concentration range of *M. pyrifera* fucoidan was 5, 7.5, and 10 µg·mL^{-1}, and 0.357 and 0.714 µg·mL^{-1} for heparin, respectively.

4.4.3. Thrombin-Induced Clotting Time Using FIB-C Reagent Assay

Thrombin-induced clotting time was recorded using FIB-C reagent in diluted plasma. Clotting times were not converted to fibrinogen concentrations, and equivalence to conventional thrombin time was not established. The calibration plasma was diluted with tissue diluent (a saline solution) in a 1:9 ratio (calibration plasma to tissue diluent). The diluted calibration plasma (200 µL) was mixed with the samples or controls (50 µL), then incubated at 37 °C for 3 min. After the incubation, the cuvette was transferred to the reading cell, and 100 µL of FIB-C reagent (bovine thrombin, 35 UNIH mL^{-1}) was added to initiate clot formation. The readings (time in seconds) were taken using a coagulometer (Beckman, CA, USA).

A final concentration of 50, 60, and 75 µg·mL^{-1} of *E. radiata* and *S. incisifolium* fucoidan was used, while a concentration range of 37.5, 50, and 60 µg·mL^{-1} of *E. maxima* fucoidan

was implemented. The concentration ranges for *M. pyrifera* fucoidan were 5, 10, and 12.5 μg·mL^{-1}, and for heparin, 15, 17.5, and 20 μg·mL^{-1}, respectively.

4.4.4. D-Dimer Test

D-dimer, a degradation product of cross-linked fibrin, was measured as an exploratory endpoint. The protocol did not include a controlled clot-formation and fibrinolysis system. Firstly, the D-dimer buffer was incubated at 37 °C for 15 min before use. The D-dimer buffer (150 μL) was combined with the samples or controls (50 μL) and calibration plasma (50 μL). Latex reagent (200 μL) was added to the mixture, and the cuvette was immediately transferred to the reading cell. The D-dimer content (ng·mL^{-1}) was determined using a coagulometer (Beckman, CA, USA).

Two HemosIL quality-control materials were used: control level 1 (low D-dimer) and control level 2 (high D-dimer). Their assigned ranges were used as analytical quality-control limits, not as physiological reference intervals for classifying the extract mixtures. The extracted fucoidan samples were tested at a final concentration of 0.5 mg.mL^{-1}, while commercial *M. pyrifera* fucoidan was tested at 0.15 mg.mL^{-1}.

4.5. Statistical Analysis

Results are expressed as means ± SD from three biological replicates (n = 3), unless otherwise stated. Coagulation data were analysed in Python 3.14 using SciPy 1.18.1 with two-sided Welch's t-tests. Between-extract comparisons were restricted to matching concentrations, with Holm correction applied separately to nine PT and nine aPTT comparisons. Figure 3 presents unadjusted comparisons; Holm-adjusted results were evaluated in sensitivity analyses. Statistical significance was set at $p < 0.05$, using adjusted p-values where applicable. The characterisation statistics in Table 1 follow those of Grobler et al. [19].

5. Conclusions

The fucoidan-rich extracts from *Ecklonia maxima*, *Ecklonia radiata* and *Sargassum incisifolium* prolonged plasma clotting times in vitro, with responses varying among preparations, concentrations and assays. *E. maxima* and *E. radiata* produced greater PT prolongation than *S. incisifolium* at matched concentrations, while *E. maxima* showed the highest mean aPTT response. These findings extend the previously reported characterisation of these extracts by demonstrating their anticoagulant potential and support further investigation of South African brown macroalgae as sources of bioactive preparations. Future work should combine fractionation and comprehensive monosaccharide and structural analyses with targeted coagulation assays to identify the constituents and mechanisms responsible for activity. Controlled clot-lysis experiments, followed by safety and in vivo efficacy studies, would clarify their effects on fibrinolysis and their potential for further development.

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