

Extraction of Fucoidan from *Turbinaria decurrens* and the Synthesis of Fucoidan-Coated AgNPs for Anticoagulant Application

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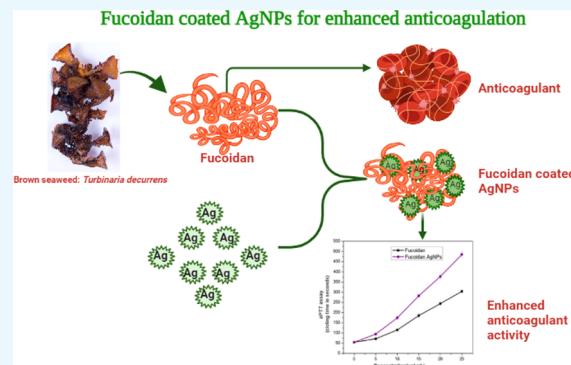
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ABSTRACT: Brown seaweeds usually contain alginate as a major polymer. The second major sulfated polymer in brown seaweeds is fucoidan, which has huge potential in medicinal applications. In this study, the photosynthetic pigments from *Turbinaria decurrens* were first extracted using chloroform/methanol in the ratio of 1:1 (v/v), followed by fucoidan extraction with yields of 5.58% (crude) and 1.28% (purified fucoidan) from the dry weight of seaweed, whereas alginate was extracted with a yield of 14.7% DW of seaweed. The isolated fucoidan possessing anticoagulation property was identified and characterized as (1–3)- α -L-fucopyranosyl residues with sulfate groups primarily at the C₄ position and to a lesser extent at the C₂ position, whereas in the case of galactose, at the C₃ and C₆ positions. The AgNPs synthesized using isolated fucoidan exhibit strong anticoagulant activity and possess a good antibacterial property against Gram-negative clinical bacteria. Functional groups such as O–H, C–H, and S=O associated with sugar residues in sulfated fucoidan are involved in the synthesis of the nanoparticles with a spherical shape, size ranging from 10 to 60 nm, and showing polydispersity. From this study, we conclude that fucoidan-coated anionic AgNPs synthesized from *T. decurrens* have tremendous potential in drug development.



1. INTRODUCTION

Seaweeds (marine macroalgae) that occur in the intertidal and subtidal regions of coastal waters are categorized based on their pigmentation as red (*Rhodophyceae*), green (*Chlorophyceae*), and brown (*Phaeophyceae*). The cell wall of seaweeds contains commercially important polysaccharides (agar and carrageenan in red; cellulose and ulvan in green; and alginate, laminarin, and fucoidan in brown seaweeds) that exhibit various biological activities.^{1,2} Even though sulfated fucans are found in some marine organisms such as sea cucumber,³ sea urchin,⁴ and bacteria, brown algae are the major source of fucoidan.⁵ Isolation of fucoidan polymers from brown seaweeds reveals structural diversities in length, linear or branched, branching pattern by glycosidic linkage, the proportion of monomers (galactose, mannose, fucose, xylose, uronic acid, etc.), sulfate level, and its position in monosugar residues besides fucose (the principle sugar).^{6–8} These structurally varied fucoidans, which depend on species, part and age of the thallus, seasons and geography, and the extraction methods followed, are responsible for different bioactive activities.^{8–10} Further, being of algal origin with a wide range of bioactive properties, such as antioxidant, antiviral, antithrombic, anticancer, etc., particularly anticoagulation,¹¹ the use of fucoidan in place of heparin as an anticoagulant certainly helps in avoiding the risk of virus contamination and its associated infections caused by animal-

origin heparin.⁶ Anticoagulant usage in medical applications is tremendous. Anticoagulants are nothing but blood thinners or antiplatelet drugs. They prevent thromboembolic diseases,¹² atrial fibrillation in stroke prevention, etc.¹³ However, fucoidan with promising anticoagulation property on par with heparin is yet to be isolated from brown seaweeds and applied as an anticoagulant.

Having a high surface area and a high fraction of surface atoms, silver nanoparticles (AgNPs) exhibit unique physicochemical and biological characteristics.¹⁴ Due to their outstanding antimicrobial properties, AgNPs are being employed for developing various biomedical products such as nanosilver-coated wound dressing and contraceptive devices, surgical instruments, implants, etc.^{15,16} Even though AgNPs are synthesized by various means through chemical and photochemical reactions such as reverse micelle thermal decomposition,¹⁷ electro- and ultrasound-facilitated reactions,^{18,19} and microwave-assisted processes,²⁰ green synthesis

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by biological means using microbes and plant extracts^{21,22} is considered to be cost-effective, easy to handle, readily available, and eco-friendly.^{23,24} But nanoparticles (NPs) synthesized using selective biomolecules as functionalizing ligands are effective for specific applications and requirements.^{25,26} Although polysaccharides have long been used for preparing scaffolds, fibers, and hydrogels because of their abundance, biocompatibility, hydrophilicity, and ability to modify into different forms, employing them for synthesizing NPs of a specific size with targeted bioactive properties is considered promising for desired use and applications.^{27,28} A few studies have characterized the NPs synthesized using seaweed extracts, but studies using specific polysaccharides (agar, carrageenan, and alginate) and compounds are limited. The NPs synthesized using sulfated fucoidan polymers have a broad scope for biomedical applications in the field of drug delivery²⁸ because as an anionic polymer they can readily ligand with any cationic drug. Based on the view of Jang et al.,²⁹ we can obtain effective fucoidan and its NPs for drug delivery based on the structure and bioactivity isolated from a specific algae source. In this paper, AgNPs synthesized using fucoidan extracted from *T. decurrents* and showing strong antibacterial activity against two Gram-negative pathogens and also exhibiting an anticoagulant property are presented.

2. EXPERIMENTAL SECTION

2.1. Seaweed Collection. Brown alga (*Turbinaria decurrents* Bory de Saint-Vincent) weighing about 1 kg was collected at the subtidal region of the Mandapam coast (Lat. 09°17' N; Long 79°07' E, Gulf of Mannar, India). The collected *Turbinaria decurrents* was washed with distilled water to remove salt, sand, and epiphytes and then shade-dried in a room under air for 5 days. The shade-dried specimen was chopped and powdered using a mixer grinder.

2.2. Successive Extraction of Fucoidan and Alginate. Fucoidan and alginate were extracted by adopting a successive extraction method³⁰ with slight modifications. The powdered specimen of 100 g was soaked in 500 mL of chloroform/methanol (1:1 v/v) solvent overnight to remove the pigments. Then, the residue was sequentially extracted in 2% CaCl₂ (2 × 100 mL for 3 h at 70 °C), followed by 0.01 N HCl (2 × 100 mL at 70 °C for 3 h) and 3% Na₂CO₃ (1 × 100 mL at 70 °C for 3 h). Extracts obtained in 2% CaCl₂ and 0.01 N HCl were combined and concentrated using a freeze dryer as crude fucoidan. The final residue extracted in aqueous 3% Na₂CO₃ was precipitated in acetone as sodium alginate. The total sugar³¹ and sulfate content³² in the crude fucoidan were recorded. Fucose, galactose, and mannose were estimated from the respective monomer standard graph prepared by a phenol-sulfuric acid method³¹ using the absorbance measured for total sugar estimation.

2.3. Qualitative Fucose Test. The presence of fucose in the crude fucoidan was confirmed by the cysteine–sulfuric acid–methyl pentose test.³³ The crude fucoidan (50 µg/mL) and standard L-fucose (100 µg/mL) were taken in separate test tubes, and 4.5 mL of H₂SO₄ (conc. H₂SO₄/H₂O 6:1 v/v) was added and mixed well. The mixture was cooled in an ice-water bath at 25 °C for 3–4 min and placed in a boiling water bath for 3 min subsequently. Then, the tubes were cooled under running tap water, and 0.1 mL of 5% (v/v) cysteine hydrochloride solution was added to each tube. The appearance of a yellowish-green color indicating the presence of L-fucose in the test solutions was observed.

2.4. Fucoidan Separation Using Ion-Exchange Chromatography. The crude fucoidan weighing 200 mg was separated through the DEAE-cellulose 52 column (42 × 3 cm) using increasing concentrations of 200 mL of NaCl (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 M). Eluents of 25 mL were collected until no more sugar was detected in the eluents by the phenol-sulfuric acid method against L-fucose standard.³¹ Based on the sugar content, the successive eluents such as 42nd, 43rd, 44th, 45th, and 46th fractions were pooled and confirmed the presence of the L-fucose content by a qualitative test.³³ Then, the combined eluents were dialyzed (cutoff 3.5 kDa) for 24 h in distilled water and lyophilized and weighed (46 mg). Using the phenol-sulfuric acid method,³¹ total sugar was calculated using glucose as the standard, and individual monomers such as fucose, galactose, and mannose were recorded in purified fucoidan using its standard graph. The sulfate content³² of the purified fucoidan was recorded.

2.5. In Vitro Anticoagulation Activity. *In vitro* anticoagulation activity was tested using the activated partial thromboplastin time (aPTT) assay (Randox kit-APTT2749).³⁴ The platelet-poor plasma was collected from blood samples of five voluntary healthy persons in a vial containing 3.8% sodium citrate (9:1 v/v). The collected blood samples were centrifuged at 4000 rpm for 20 min, and the plasma (100 µL) from the blood was mixed with purified fucoidan (5, 10, 15, 20, and 25 µg/mL) and incubated for 1 min at 37 °C. Then, 100 µL of the prewarmed aPTT reagent was added to the mixture and incubated for 5 min at 37 °C. Thereafter, clotting was initiated by adding 100 µL of 0.25 M CaCl₂, and the clotting time was recorded against the control sample plus reagents without fucoidan.

2.6. Characterization of Fucoidan. The FTIR spectrum was recorded in the range between 4000 and 400 cm⁻¹ using a Bruker Optik GmbH, Germany (model no. Tensor 27; Software, Opus version 6.5). The ¹H and ¹³C NMR spectra of fucoidan in 0.5 mL of D₂O were measured using a Bruker Biospin Avance 400 NMR spectrometer (¹H frequency 1/4 400.13 MHz, ¹³C frequency 1/4 100.62 MHz) at 298 K (using a 5 mm broad-band inverse probe head) equipped with a shielded z-gradient and XWIN-NMR software version 3.5 and using tetramethylsilane as an internal reference. One-dimensional ¹H and ¹³C spectra were obtained using a one-pulse sequence. One-dimensional ¹³C spectra using spin echo Fourier transform and quaternary carbon detection of 42 sequences were also obtained to aid the structural detection.^{35,36}

2.7. Synthesis of Fucoidan-Coated AgNPs. Fucoidan-AgNP synthesis³⁷ was carried out using 1 mg of fucoidan polymer dissolved in 3 mL of distilled water and 97 mL of AgNO₃ (1 × 10⁻¹ M) and stirred well at room temperature and left undisturbed. The appearance of a yellowish-brown color was observed after 1 h indicating the formation of AgNPs, which was confirmed using UV-visible spectroscopy between 200 and 800 nm. A control group was maintained without fucoidan. The synthesized mixture was then centrifuged at 5000 rpm for 20 min, and the pellet containing AgNPs was collected. To remove the uncapped fucoidan, the pellet was again dissolved in 10 mL of distilled water and centrifuged at 5000 rpm for 20 min. Centrifugation was repeated thrice to collect fucoidan-capped AgNPs. The obtained pellet was freeze-dried and stored at 4 °C until further use.

2.8. Anticoagulant and Antibacterial Assay of Fucoidan-Coated AgNPs. The *in vitro* test of the anticoagulant activity using 5, 10, 15, 20, and 25 $\mu\text{g}/\text{mL}$ fucoidan-coated AgNPs was carried out as described in Section 2.5. The antibacterial assay³⁸ of 10 μg of AgNPs was evaluated against four clinical pathogens (Gram-positive: *Streptococcus mutans* MTCC 896 and *Staphylococcus aureus* MTCC 96 and Gram-negative: *Pseudomonas aeruginosa* MTCC 2642 and *Escherichia coli* MTCC 40). The assay was carried out using the disc diffusion method on Mueller–Hinton (MH) agar plates. For this, a sterile 5 mm (dia) disc loaded with AgNPs (10 $\mu\text{g}/\text{mL}^{-1}$ sterile distilled water) was impregnated on MH agar Petri plates spread with the exponential test bacteria and was then incubated at 37 °C for 48 h. The antibacterial activity was recorded by measuring the clear zone around the disc as the diameter in millimeters. The discs loaded with sterile distilled H₂O and 10 μg of gentamycin were maintained as the negative and the positive controls, respectively. All of the experiments were carried out in triplicate, and the mean value was calculated with deviation.

2.9. Characterization of Fucoidan-Coated AgNPs. The FTIR spectrum was recorded in the range between 4000 and 400 cm^{-1} using a Bruker Optik GmbH; model no., TENSOR 27; Software, OPUS version 6.5.²³ The X-ray diffraction (XRD) pattern was measured using a powder X-ray diffractometer (PXRD-6000 Shimadzu) at angle 2 θ and a scan axis of 2:1 sym. The formation of AgNPs was confirmed from the PXRD peak positions using Bragg's law.³⁹ The morphology of the AgNPs was examined using field emission scanning electron microscopy (Hitachi SU6600) on a carbon-coated copper grid equipped with an EDAX attachment. The size and the morphology of the AgNPs were examined using atomic force microscopy (AFM, diCP II Veecs) and transmission electron microscopy (TEM). For measuring AgNPs using TEM, the freeze-dried AgNPs were placed on the carbon-coated copper grids and kept overnight in a vacuum desiccator before loading them onto a specimen holder. TEM micrographs of the AgNPs were obtained using a JEOL (JSM 100 cx) TEM instrument operated at an accelerating voltage of 200 kV.

3. RESULTS AND DISCUSSION

3.1. Quantification of Fucoidan and Alginate. The results of the yield of crude and purified fucoidans, crude alginate, and their constituents obtained from the residue of *T. decurrens* after removing the pigments by chloroform/methanol (1:1 v/v) are presented in Table 1. The cell wall

Table 1. Yield of Fucoidan and Alginate and Their Constituents Extracted from *T. decurrens* on a Dry Weight Basis^a

constituents	sulfated polysaccharides		
	crude fucoidan	purified fucoidan	crude alginate
yield (%)	5.58	1.28	14.70
total sugar ($\mu\text{g}/\text{mg}$)	34.00 ± 4.54	62.50 ± 4.21	78.32 ± 3.44
fucose ($\mu\text{g}/\text{mg}$)	12.39 ± 1.38	19.00 ± 3.72	05.61 ± 4.21
mannose ($\mu\text{g}/\text{mg}$)	06.63 ± 2.29	05.73 ± 6.81	19.51 ± 4.22
galactose ($\mu\text{g}/\text{mg}$)	11.82 ± 2.71	18.51 ± 6.38	20.78 ± 7.61
sulfate ($\mu\text{g}/\text{mg}$)	05.90 ± 0.93	09.25 ± 1.71	02.22 ± 0.46

^aAll constituents in the table are calculated using crude polysaccharides extracted from *T. decurrens*.

of the brown seaweeds mainly constitutes alginate and some extend fucoidan as matrix substances. The yield, structure, and constituents of fucoidan vary depending on brown algae species,^{9,10} and further, the yield was influenced by the choice of the solvents and/or methods adopted for extraction.^{40–42} Further, a difference in the yield was recorded when the sample of species was subjected only to fucoidan extraction compared to successive extraction for fucoidan and then alginate. The fucoidan yield obtained by direct aqueous extraction in *Laminaria japonica* was 2.04%⁴³ and 2.74% by direct mild acid (0.1 N HCl) in *Sargassum polycystum*.⁴⁴ Variation in the fucoidan yield among the 11 brown algae species was recorded depending on the methods followed.⁴¹ By successive extraction in *Fucus serratus*, *Fucus vesiculosus*, and *Ascophyllum nodosum*, the fucoidan yield was 6.0, 9.8, and 8.0%, respectively,¹⁰ and 4.24% in *Saccharina latissima*.⁴⁵ Previous studies demonstrated that fucoidan was extracted using 2% CaCl₂, followed by 0.01 N HCl, and alginate was extracted using 3% Na₂CO₃ successively after removing pigments using 85% ethanol from *Saccharina longicurvis*, *A. nodosum*, and *F. vesiculosus*.³⁰ In another study, 3.62% yield of fucoidan (using 2% CaCl₂ and 0.01 N HCl) and 11.2% yield of alginate (using 3% Na₂CO₃) were obtained by successive extraction from the residues of *Sargassum wightii* after removing the pigments using chloroform/methanol (1:1 v/v), followed by 85% ethanol.⁴⁰ Whereas, in the present study, after removing the pigments by treating samples in chloroform/methanol (1:1 v/v) and considering the thallus hardness of *Turbinaria decurrens*, successive extraction using 200 mL of 2% CaCl₂, 200 mL of 0.01 N HCl, and 100 mL of 3% Na₂CO₃ was performed that resulted in a high fucoidan yield of 5.58% (w/w) and a 14.7% (w/w) alginate yield.

3.2. Fucoidan Constituents. Sulfated polymers dominated by fucose monomer (fucoidan) have been isolated from various brown algae species.^{11,46} On the other hand, recently, fucoidans with equimolar fucose and galactose, so-called galactofucoidan, have also been isolated in some brown seaweed species.¹⁰ These two major types of fucoidan isolated from the brown seaweed species are implicated mainly by extraction techniques, season, harvest location, age of the thallus, and species.⁴⁷ The monomer residues and the sulfate content of fucoidan extracted using the direct method⁴⁸ are varied compared to those of fucoidan extracted using the successive method⁴⁹ from *Undaria pinnatifida*. The sulfated galactofucans are reported in various brown seaweeds such as *Adenocystis utricularis*,⁵⁰ *Undaria pinnatifida*,⁵¹ *Spatoglossum schröederi*,⁶ *Lobophora variegata*,⁷ *Sargassum wightii*,⁸ and *Turbinaria decurrens*.⁴⁰ In this study, fucoidan extracted from brown seaweed (*T. decurrens*) through the successive extraction method³⁰ contains a high amount of fucose followed by galactose,^{51,52} and the sulfate content was more in purified fucoidan than the crude (Table 1), which implies increased bioactivity,⁵³ and also an increased sulfate content shows increasing anticoagulation property.⁵⁴

3.3. Fucoidan Isolation and Purification. In this study, crude fucoidan extracted by the successive method from *Turbinaria decurrens* was separated through cationic DEAE column elution by increasing NaCl gradients. Fucoidan containing sulfate as an anionic functional group and thereby giving the polymer a negative charge⁵⁰ was introduced into the positively charged ion-exchange DEAE column. The strong ionic solvent (NaCl) promoted the release of sulfate groups from the gel column. As the concentration of NaCl increased

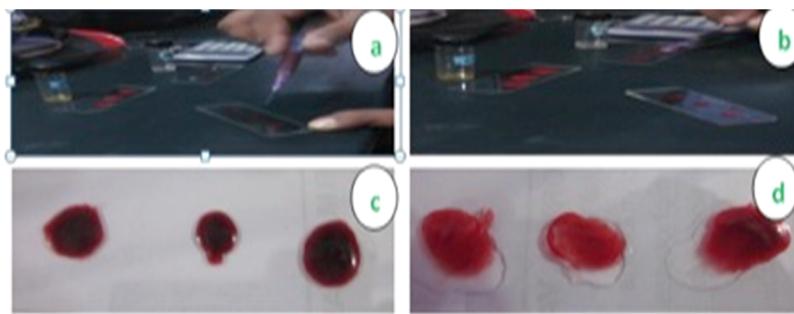


Figure 1a. *In vitro* anticoagulation by the activated partial thromboplastin time (aPTT) assay of fucoidan-coated AgNPs (a, b: preparation of the assay; c: blood sample showing coagulation at 54.23 ± 4.8 s by $0 \mu\text{g}/\text{mL}$; and d: blood mixed with fucoidan-coated AgNPs showing delayed coagulation at 387.8 ± 10.3 s by $20 \mu\text{g}/\text{mL}$).

in the mobile phase, more sulfate groups were released.^{8,9} Hence, the sulfate constituents of eluents were increased proportionally on increasing the NaCl gradient (Figure S1). In this study, the fucoidan polymer eluted using 3 M NaCl contains a high sulfate content suggesting the possibility of increased biological activities.⁴⁰

3.4. Anticoagulation Activity of Isolated Fucoidan.

The aPTT assay results depend on the clot detection method and assay kits used. The normal blood clotting time ranges from 25 to 43 s.⁵⁵ The blood maintains fluidity as long as it is inside the vasculature and clots quickly at the sites of vascular injury as soon as it is exposed to subendothelial surfaces. In normal conditions, thrombosis and hemorrhage are prevented by a balance action between coagulation and fibrinolysis. Any imbalance that occurs favors coagulation that causes thrombosis leading to platelet aggregation, fibrin formation, and trapped red blood cells in arteries or veins. There are different types of antithrombotic drugs available in the market to treat thrombosis. Antiplatelet drugs inhibit platelet activation or aggregation, whereas anticoagulants attenuate fibrin formation and the fibrinolytic agents degrade fibrin formation.⁵⁶ The compounds possessing anticoagulant properties have been evaluated using assays such as activated partial thromboplastin time (aPTT), prothrombin time, and thrombin time.⁵⁷ Like heparin, fucoidan also inhibits thrombin formation, but its anticoagulant efficacy is not the same as that of the latter. The anticoagulant and antithrombotic effects of fucoidan have been mediated by catalyzing thrombin inhibition by mainly inhibiting heparin cofactor II.⁵⁸ Fucoidan, like heparin, inhibits thrombin formation by forming complexes with the inhibitor. Specifically, heparin possesses antithrombin property, and in the case of fucoidan, it exhibits heparin cofactor inhibition.⁵⁹ Fucoidan inhibits thrombin activity or fibrin polymerization.⁵⁷

In this study, the anticoagulation activity of the isolated fucoidan from *T. decurrents* was assayed by *in vitro* aPTT activity using platelet-poor plasma.³⁴ Using the aPTT assay, heparin ($0.4 \text{ IU}/\text{mL}$) exhibits a clotting time of $68\text{--}75$ s.⁵⁵ Fucoidan of *Undaria pinnatifida* exhibits a five times higher anticoagulation property than the control.⁶⁰ The present study emphasizes that purified fucoidan of *T. decurrents* shows dose-dependent anticoagulation evidenced from the aPTT assay (Figure 1a) and increased anticoagulation activity on increasing the concentration up to $20 \mu\text{g}/\text{mL}$ fucoidan in 387.8 ± 10.3 s (Figure 1b). This result shows fucoidan of brown seaweed *T. decurrents* as a promising anticoagulant.

3.5. Fucoidan Characterization. 3.5.1. FTIR Analysis.

The FTIR spectra (Figure 2) of fucoidan isolated from *T.*

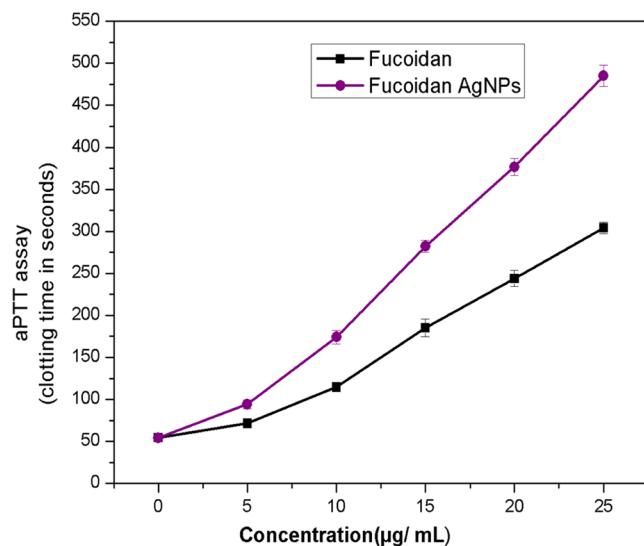


Figure 1b. Anticoagulation activity of *T. decurrents* fucoidan and its AgNPs.

decurrents were assigned to the spectral similarities of fucoidan isolated from brown seaweeds such as *Laminaria saccharina*, *L. digitata*, *Fucus evanescens*, *F. serratus*, *Fucus distichus*, *Fucus spiralis*, and *Ascophyllum nodosum*.^{61,62} A broad band at 3431 cm^{-1} assigned to the OH group of monosugar and the presence of aliphatic C–H at 2827 cm^{-1} are recorded. The C=O stretching vibration for acetate groups is noted by the peak at 1788 cm^{-1} , and the presence of O–C–O stretching is shown by the sharp peak at 1621 cm^{-1} . The C–O ether bond (1079 cm^{-1}) and the C–S–O band (872 cm^{-1}), characteristics of the fucose principle monomer of fucoidan polymer, are observed in the FTIR spectra. Besides, sharp peaks observed at 872 cm^{-1} ($\text{S}=\text{O}$) and 775 cm^{-1} ($\text{C}=\text{S}-\text{O}$) confirm the sulfate attachment at the C4 position in the fucose monomers. As reported by Singthong,⁶³ the band at around $\sim 465 \text{ cm}^{-1}$ is related to the presence of the sulfate group at the C6 position of the galactose unit. The signal close to $\sim 1466 \text{ cm}^{-1}$ is attributed to the asymmetric and symmetric stretching vibrations of COO of uronic acid. Further, signals at 1466 and 1388 cm^{-1} could be an indication of the presence of uronic acid residue in the fucoidan polymer isolated from *T. decurrents*.

3.5.2. NMR Analysis. Monomer residues with sulfates in the polymer chain are formed by different linkages that produce less stable signals in the NMR spectra.^{14,64} The ^1H NMR data (Figure 3) support the identification of the position of

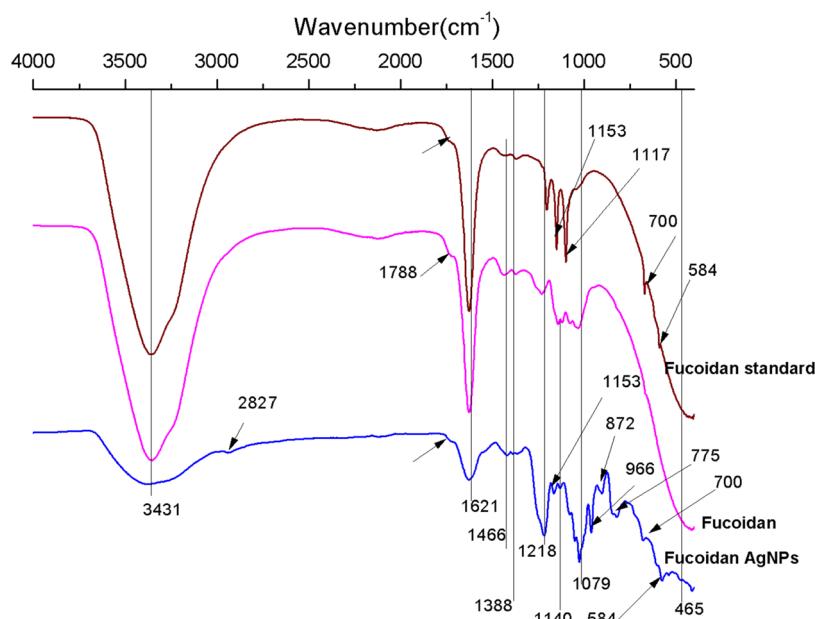


Figure 2. FTIR spectra of fucoidan standard, fucoidan of *T. decurrents*, and its AgNPs.

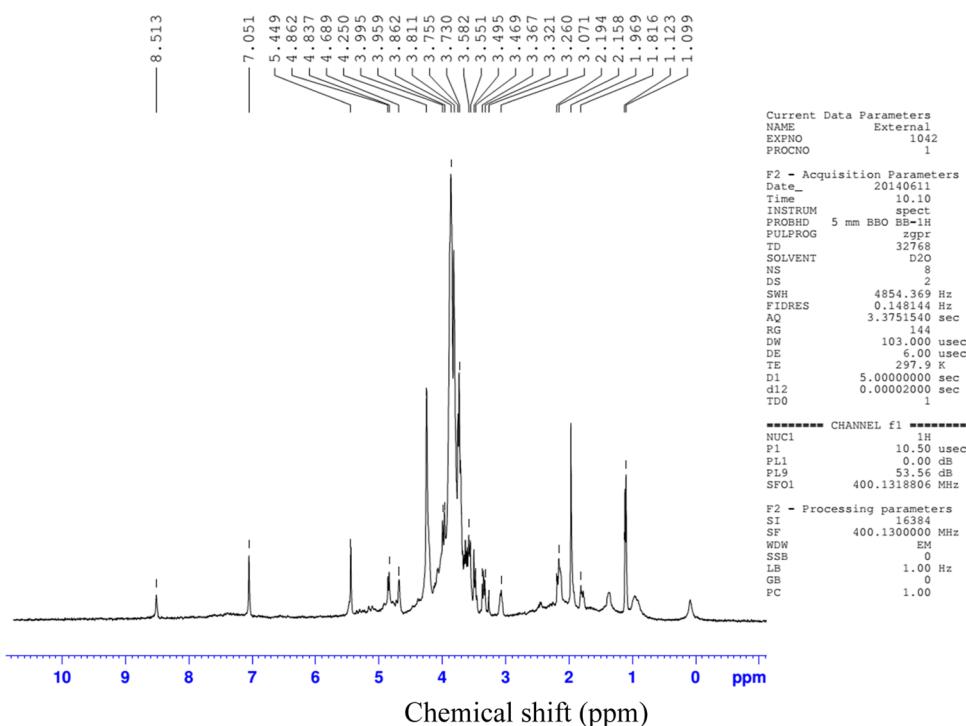


Figure 3. ^1H NMR spectrum of fucoidan isolated from *T. decurrents*.

glycosidic linkages and sulfate in the monosugar residues in the polymer. The spin systems attributable to the anomeric protons of α -L-fucopyranose residues and β -D-galactose residues were found in the ^1H NMR spectrum. Signals observed from the ring protons (H_2 – H_5) between 3.071 and 4.25 ppm are resonance characteristics of α -L-fucopyranose. Intense signals from the methyl protons H_6 , a minor signal at around 1.099 ppm, and a major signal at 1.23 ppm were observed. Methyl signals appeared at around 1.816–1.969 ppm representing α -L-fucopyranose.^{64,65} Similar to fucoidan characterized from various brown seaweeds, the ^{13}C NMR spectrum of isolated polymer (Figure 4) has several intense

signals in the anomeric (64.77–69.88 and 75.53–87.73 ppm) and high-field (16.62 ppm) regions that are typical of the α -L-fucopyranosyl unit. The resonance of the methyl group (C6) appeared as a strong signal at 16.62 ppm. The signal at 57.27 ppm indicates six nonlinked galactose units, and the signal at around 69.80 ppm indicates six linked galactose units. The absence of signals at around 83.0–86.0 ppm suggests a lack of C2-, C3-, or C4-linked galactose units, and the galactose residues are C1- and C6-linked. The NMR data suggest that fucoidan extracted from *T. decurrents* consists of (1–3)- α -L-fucopyranose residues with sulfate groups primarily at the C4 position and to a lesser extent at the C2 position and C3 and

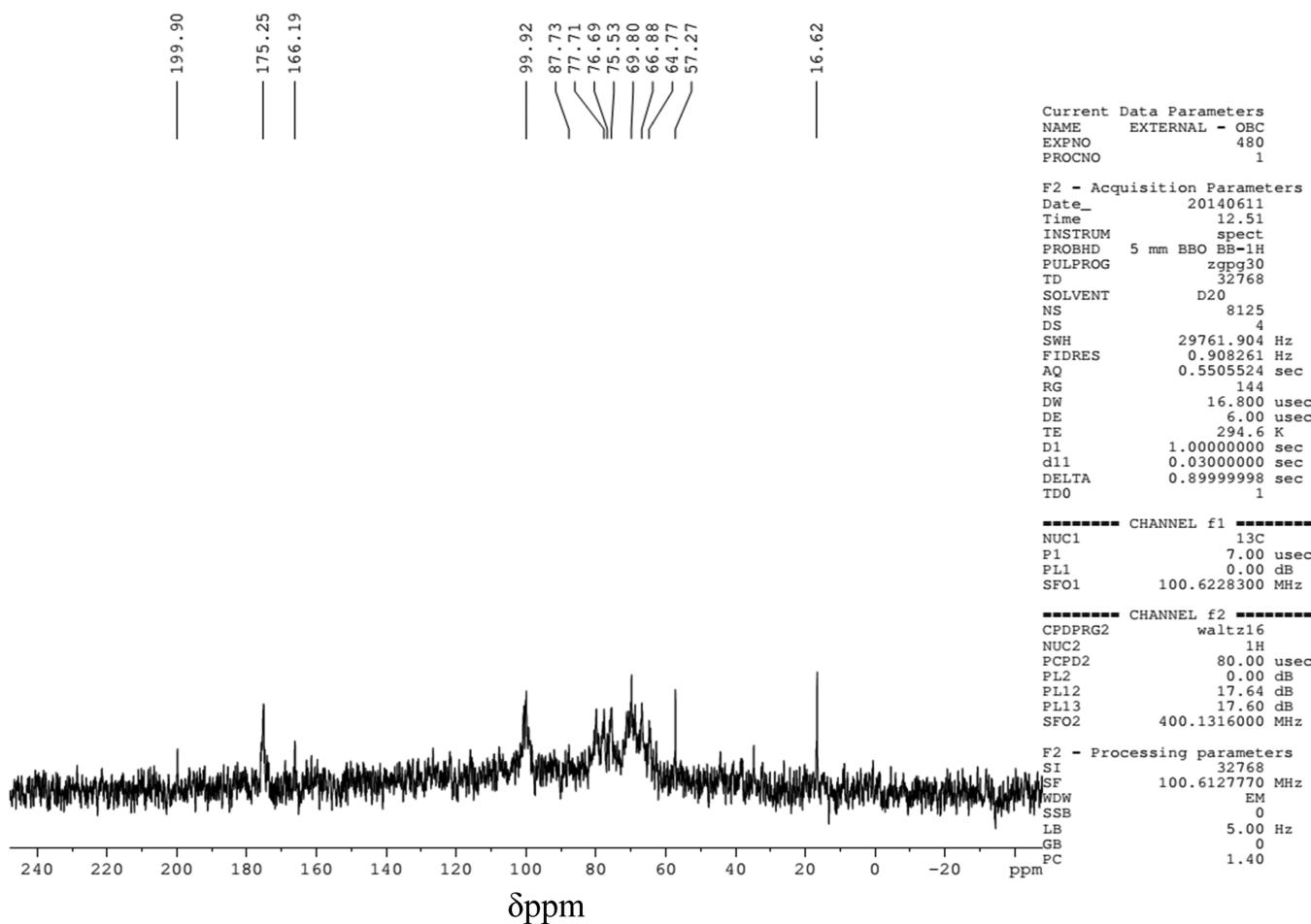


Figure 4. ^{13}C NMR spectrum of fucoidan isolated from *T. decurrents*.

C6 of galactose residues with numerous branches in the form of α -L-fucose and β -D-galactose residues linked by (1–3)-O-glycosidic linkages as reported by Wang et al.⁶⁶ in *Sargassum japonica* and⁶⁷ *U. pinnatifida* (Figure 4).

3.6. Spectral Analysis of Fucoidan-Coated AgNPs. NPs synthesized by selective biomolecules are considered to be most desired for target-specific biomedical applications rather than those synthesized from crude compounds.^{68,69} There are studies that have reported AgNPs synthesized using crude extracts of marine brown algae such as *Sargassum wightii*,⁷⁰ *Turbinaria conoides*,⁷¹ and other seaweeds.^{23,72,73} In this study, the synthesis of AgNPs by sulfated fucoidan isolated from the marine brown alga *T. decurrents* was confirmed based on the colorless solution of AgNO_3 turning brownish-yellow, indicating the formation of NPs, and monitored by the UV-visible spectral band at 420 nm (Figures 5a and S2). This band was identified as a “surface plasmon resonance band” attributed to the excitation of free electrons in the NPs. The shape of the band recorded was symmetrical, which indicates that the synthesized NPs are spherical and can disperse uniformly.⁷⁴

3.7. Enhanced Anticoagulant Activity of Fucoidan-Coated AgNPs and Their Antibacterial Activity. The metal NPs possessing various bioactive properties^{75,76} open an avenue for synthesizing the desired-size NPs using specific biomolecules for various applications to treat different diseases. Recently, the usage of biologically synthesized AgNPs using selective active molecules is found to be effective and requires

only a very less quantity when compared to active crude molecules.^{28,77} In this study, AgNPs synthesized using fucoidan exhibit strong anticoagulant activity compared to isolated fucoidan in a dose-dependent manner (Figure 1aa,b). Antibacterial activity against clinical pathogens was also strong, and the effect was pronounced against Gram-negative bacteria over -positive bacteria (Figure S3). This observation corroborated with an earlier study⁷⁸ where the bioactivity effect was superior and depended on the concentration of AgNPs and the type of pathogen tested. Because of the high surface-to-volume ratio, NPs can undergo a high level of interaction with the bacterial cell surface with an enhanced holding capacity, promoting bioavailability that ultimately exhibited high antibacterial activity against Gram-negative bacteria. A mechanism was proposed where close contact between AgNPs and the microorganisms enhances the transfer of Ag ions to the microbial cell, while the microorganisms’ metabolic activity dissociates fucoidan that promotes the release of silver ions.⁷⁹ From a previous study on fucoidan-based NPs, which were found to be effective against osteosarcoma,⁸⁰ fucoidan NPs prepared in this study were more effective than its polymer. This enhanced anticoagulation and antibacterial activities of fucoidan-coated AgNPs observed in this study, which were also ascertained to act as nanocarriers to promote the intracellular delivery of fucoidan²⁹ by controlled release with stable coated NPs that resulted in increased anticoagulant activity compared to the fucoidan polymer.⁸¹

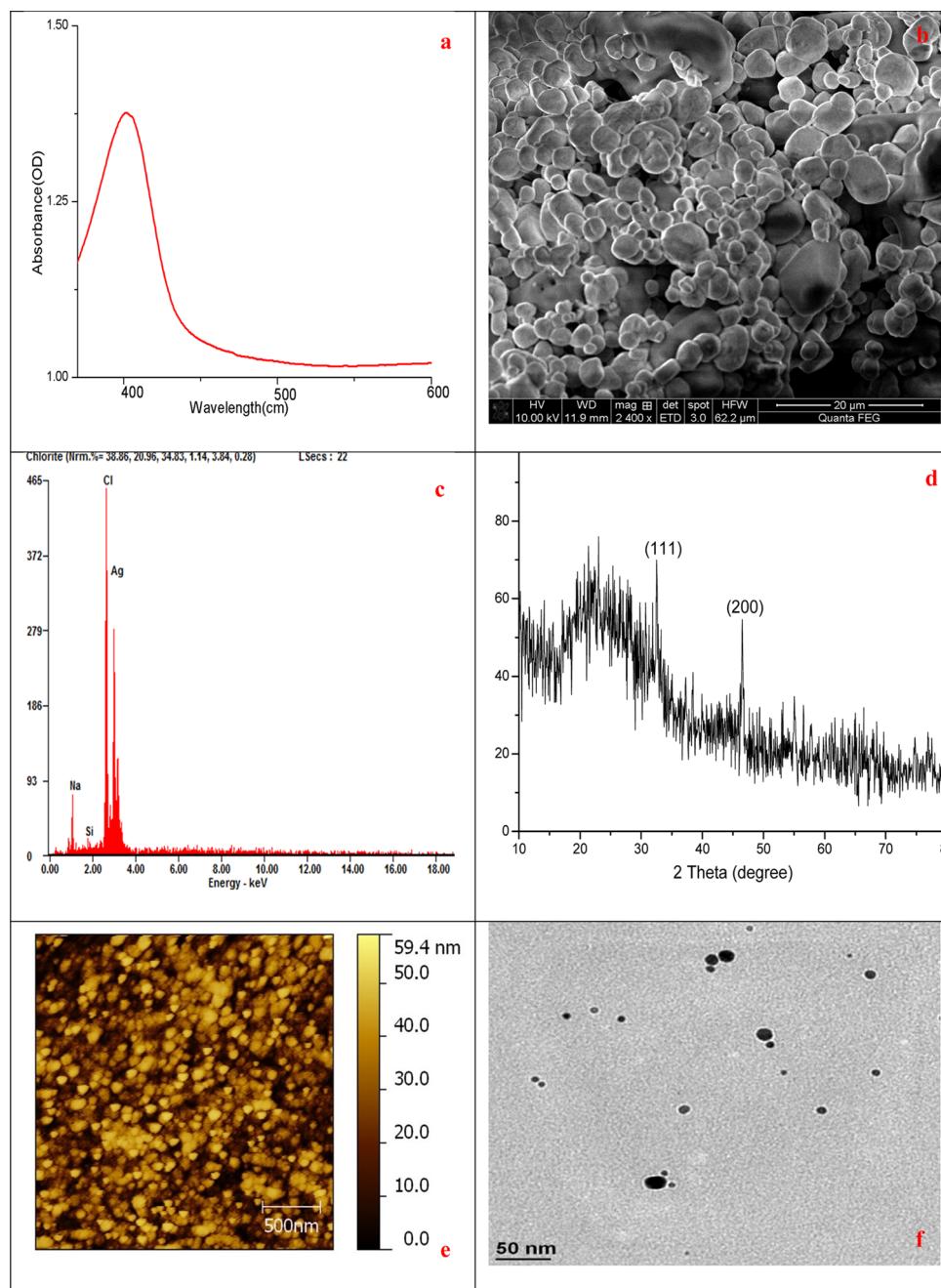


Figure 5. Fucoidan-coated AgNP characterization by the UV–visible spectrum (a), SEM (scale bar $20\ \mu\text{m}$) (b), EDAX (c), XRD (d), AFM (scale bar $500\ \text{nm}$) (e), and TEM (scale bar $50\ \text{nm}$) (f) analyses and their images.

3.8. Fucoidan-Coated AgNP Characterization.

3.8.1. FTIR Study. The absorption bands at $3431\ \text{cm}^{-1}$ of fucoidan standard, isolated fucoidan (Figure 2), and its AgNPs show the stretching vibrations of the OH groups of carbohydrates.^{82,83} Bands at $1466\ \text{cm}^{-1}$ of fucoidan and $1388\ \text{cm}^{-1}$ of AgNPs indicate the presence of uronic acid.⁸⁴ The above characteristics of FTIR spectra confirms the formation of fucoidan-coated AgNPs. The most important bands of fucoidan (Figure 2) are those found at 1388 and $700\ \text{cm}^{-1}$, corresponding to ester sulfate groups. The region at around $1466\ \text{cm}^{-1}$ is assigned to the scissoring vibration of CH_2 (galactose, xylose) and the asymmetric bending vibration of CH_3 (fucose, O-acetyls), and the absorption at around $872\ \text{cm}^{-1}$ indicates the presence of sulfate groups in the C4

position of galactose.⁶⁰ The peak at $1079\ \text{cm}^{-1}$ in fucoidan shows the CH stretching vibration of fucose and $\text{S}=\text{O}$ bound to the axial position of C4 (Figure 2),^{60,83} which disappeared after the synthesis of AgNPs (Figure 2). These observations on the sulfate and hydroxyl groups present in the galactose and CH and $\text{S}=\text{O}$ groups of fucose are probable functional groups involved in the reduction of AgNPs (Figure 2). In addition, a broad peak at $3431\ \text{cm}^{-1}$ (OH stretching) found in the AgNPs shows that the reduction of AgNO_3 decreases the intensity, which implies the involvement of the OH groups in the reduction process. This is in agreement with previous studies on sulfated polysaccharides of brown alga *Sargassum muticum* indicating a strong capacity to synthesize NPs from Fe_3O_4 ⁸⁵ and ZnO .⁸⁶ Similarly, Venkatpurwar reported that sulfated

polysaccharides isolated from the marine red alga *Porphyra vietnamensis* had a strong capacity to synthesize AgNPs.⁸⁷ A peak at 1140 cm⁻¹ was observed in all samples and in the standard, and the 1079 cm⁻¹ peak was assigned to the presence of the RO-SO₃⁻ bond of the sulfate groups. The additional peaks at 1153 and 1117 and 966, 872, and 775 cm⁻¹ in Figure 2 are related to AgNPs. It is observed that mostly redox-oxidation sites of reducing sugars of fucoidan are involved in silver ion reduction in the NP synthesis.

3.8.2. FESEM and EDAX Studies. Figure 5b shows the FESEM image that visualizes the size and shape of the AgNPs. A thin organic shell covering each individual particle is found to be responsible for reducing Ag⁺ ions supporting interparticle binding with NPs. The synthesized AgNPs appear spherical with the size ranging between 10 and 60 nm, which indicates the polydispersity nature. A few aggregations of AgNPs were also observed in some places in the FESEM images, indicating agglomeration after some time.⁸⁸ The result of EDAX (Figure 5c) gives clear information about the Ag present in the NPs, and a strong signal of the Ag atoms indicates the crystalline property. Energy-dispersive X-ray analysis is the typical technique for the analysis of metallic silver nanocrystallites.⁸⁹

3.8.3. XRD, AFM, and TEM Analyses. The XRD data (Figure 5d) shows AgNPs having Bragg reflection patterns at $2\theta = 32.4$ and 46.4 that clearly indicate the presence of 111 and 200 sets of lattice planes, and they can be indexed as the FCC structure of silver. AgNPs synthesized using fucoidan are crystalline in nature.⁹⁰ Similar to the FESEM observation (Figure 5b), the AFM image (Figure 5e) shows a three-dimensional view of the spherical shape with a 10–60 nm size supporting the polydispersity nature. The tendency of agglomeration indicates an attractive interaction among the NPs. The distribution of particle size ranging from 10 to 60 nm was calculated using Gwyddion 2.9 analysis software. The broad surface area of the NPs and the magnetic forces between them could cause considerable agglomeration between the AgNPs but not aggregation.⁹¹ The TEM analysis clearly shows that the largest size of the individual NPs is 50 nm and they are well dispersed. The shape of the NPs is almost spherical to cubical (Figure 5f) as observed.⁹²

3.9. Fucoidan Nanoparticles for Drug Delivery. Major challenges posed in drug delivery in disease treatment are poor reachability at the target site, *in vivo* instability, poor bioavailability, less solubility, and absorption, but the application of drugs through nanoparticle carriers overcomes these challenges.^{66,93} Drugs from natural compounds formulated as nanoparticles widely practiced in Indian and Chinese systems of medicine given as dose and delivery to the target site are considered to be quick and effective. Because of their miniature size of 1–100 nm, nanoparticles as effective carriers in nanoformulated drugs can also be capped with another active drug molecule of less reachability for enhancing delivery and activity. Formulating the desired-size nanoparticles as drug and/or carriers is very important in the present context of drug delivery in modern medicine and emphasizes the potential role of seaweed sulfated polysaccharides.^{28,94} In this study, anionic AgNPs in the range of 10–60 nm encapsulated by anticoagulant fucoidan of *T. decurrens* showing strong antibacterial activity against Gram-negative clinical pathogens suitable for loading cationic drugs were identified.

4. CONCLUSIONS AND PERSPECTIVES

This study concludes that the residue, after removing the pigments by chloroform/methanol (1:1 v/v) extraction, through successive extraction, crude fucoidan of 5.58% algal DW was extracted by 2% CaCl₂ and 0.01 N HCl, and alginate of 14.70% algal DW was extracted subsequently by 3% Na₂CO₃ from the biomass of hard brown alga thallus *Turbinaria decurrens*. The purified fucoidan polymer yield of 1.28% algal DW possesses an anticoagulation property and is characterized, consisting of (1–3)- α -L-fucopyranosyl monomers with sulfate groups primarily at the C₄ position and to a lesser extent at the C₂ position, and in galactose, at the C₃ and C₆ positions. The AgNPs synthesized using fucoidan exhibit strong anticoagulant activity and possess an antibacterial property against Gram-negative clinical bacteria. Functional groups such as OH, CH, and S=O associated with sugar residues in the sulfated fucoidan are involved in the synthesis of nanoparticles in the range of 10–60 nm with a spherical shape and showing polydispersity, which have a tendency to agglomerate. These anionic AgNPs encapsulated by anticoagulant fucoidan of *T. decurrens* showing strong antibacterial activity against Gram-negative clinical bacteria are suitable for loading cationic drugs.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c03776>.

Fucose content of fractions collected through the DEAE-cellulose column eluted by different molar concentrations of NaCl (Figure S1); aqueous white solution containing 97 mL of AgNO₃ (1×10^{-1} M) mixed with 3 mL of 1 mg of fucoidan polymer (a) turning brown as a result of fucoidan-AgNP synthesis (b) (Figure S2); and the antibacterial activity of fucoidan-coated AgNPs (Figure S3) (PDF)

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Nagarajan Shanthi: planning, execution, and writing; Arumugam Ponnan: experimental analysis; Murugan Marudhamuthu: experimental analysis; Muthiyal Prabakaran Sudhakar: writing and English language editing, formatting, and analysis of results; and Kulanthaiyesu Arunkumar: planning, writing, language correction, and project execution.

Notes

The authors declare no competing financial interest.

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