Wound healing Potential of Fucoidan Extracted Microwavically from *Sargassum wightii* Possibly Mediated by Collagen-1 Expression in Vero Cell Line

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ABSTRACT

Background: Fucoidan, a natural macromolecule extracted from *Sargassum wightii*, has shown promise in various therapeutic areas, including anti-tumor, antioxidant, antithrombotic, and wound healing applications. This study explores the wound healing potential of fucoidan derived from *Sargassum wightii* collected from the Gulf of Mannar, Tamil Nadu, India.

Aims and Objectives: This research aims to assess the effectiveness of fucoidan extracted via microwave-assisted extraction (MAE) in promoting wound healing in Vero cells, a line of African green monkey kidney cells. The study also investigates the impact of fucoidan on collagen-1 expression, a critical protein involved in the wound healing process.

Materials and Methods: Fucoidan was extracted using MAE, and its cytotoxicity was evaluated using the Sulforhodamine B (SRB) Assay. The wound healing efficacy was tested through a scratch assay, measuring the closure of wounds over 24 and 48 hours.

Results: The SRB Assay demonstrated that fucoidan did not exhibit cytotoxicity to Vero cells, with an IC50 value of 61.30 µM. The scratch assay revealed wound closure of 46.15% at 24 hours and 76.9% at 48 hours, compared to 50% and 81.25% in the control group. Fucoidan treatment significantly increased collagen-1 expression, with 77.92% of cells showing elevated levels of this crucial protein.

Conclusions: This study confirms the in-vitro wound healing capabilities of fucoidan extracted from *Sargassum wightii*. These findings support the potential of fucoidan as a natural agent for wound healing and restoration.

Keywords: *Sargassum wightii*, Fucoidan, macromolecule, Wound healing, SRB assay, Vero cells, collagen-1 expression.

1. BACKGROUND

The world's oceans are teeming with a diverse array of multicellular marine plants known as seaweeds, or macroalgae. These marine plants are categorized into three primary groups based on color: red, green, and brown algae [1]. Seaweed has been a dietary staple in Asia for millennia, highlighting its importance as a food source [2]. Among these, the brown algae genus Sargassum thrives in warm, subtropical oceans, forming vast floating islands known as Sargasso Seas. These islands, consisting of dense mats of Sargassum, can travel extensive distances via ocean currents. Sargassum (figure1) has been utilized in various applications, including food, traditional medicine, and fertilizers [3]. However, further research is essential to fully understand its biological functions and optimize its extraction and utilization methods [4].

Brown seaweeds, such as species from the genera

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Fucus, Undaria, and Laminaria, contain a complex polymer called fucoidan. This sulfated polysaccharide consists of repeating units of glucuronic acid, fucose, and sulfate residues [5]. Fucoidans are believed to interact with various growth factors, such as basic fibroblast growth factor (bFGF) and remodeling growth factor, potentially through heparin-binding interactions, to exert beneficial effects. The chemical structure of fucoidan (figure 2) is highly variable and complex, depending on the source seaweed, extraction method, and environmental conditions. Typically, it comprises a backbone of α-(1→3)- and α-(1→4)-linked L-fucose residues, with branches of sulfate groups and occasional acetyl groups. This structural diversity contributes to its broad spectrum of biological activities [6]. Reducing the molecular weight of fucoidans is thought to enhance their bioactivity, as high molecular weight fucoidans (HMF) are less absorbable and exhibit low tissue absorption [7]. The chemical composition and quantity of fucoidan vary depending on the source seaweed, making it a promising candidate for developing novel therapeutics for various diseases. Yet, comprehensive studies are necessary to elucidate its mechanisms of action and therapeutic potential [8].

Extraction techniques, such as solvent extraction, isolate target compounds from complex mixtures or botanical sources based on their physical or chemical properties [9]. Microwave-assisted extraction (MAE) has successfully extracted numerous biologically active substances from natural sources. This technique uses microwave energy to induce molecular friction through the dipolar rotation of polar molecules, resulting in a volumetrically distributed heat source. MAE often achieves faster, more selective extraction with higher yields and significantly reduced energy and solvent consumption, making it environmentally friendly [10].

The purification process involves removing contaminants to produce a high-quality product. Ion exchange chromatography is a chromatographic method used to separate and purify crude chemicals. Resins like DEAE Sephadex A-25 are commonly used in biochemistry and molecular biology for protein purification and separation [11]. Fucoidan's complex and variable nature makes it challenging to describe its chemical structure using a single method. Techniques such as mass spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and nuclear magnetic resonance (NMR) are employed to gain a deeper understanding of its composition [12].

The Sulforhodamine B (SRB) assay is a colorimetric method used to measure cell death and growth, frequently applied in drug development, pharmacology, and cancer research to assess drug effects on cell viability. Cytotoxicity refers to the capacity of various substances, including chemicals, drugs, or environmental pollutants, to harm or kill cells. It is typically assessed in vitro by evaluating changes in cell viability or metabolic activity following exposure to the substance [13].

Developed in the 1960s, the Vero cell line originates from the kidney tissue of the African green monkey. These adherent cells grow as a monolayer on culture tube surfaces, exhibiting fibroblast-like morphology. Vero cells are known for their rapid growth and ability to form a dense cell layer. Cryopreserved at -80°C in glycerol or dimethyl sulfoxide, Vero cells are utilized for drug screening and toxicity testing to understand the cytotoxic effects of potential medicinal compounds [14].

A wound, encompassing injuries such as cuts, scrapes, punctures, burns, or ulcers, disrupts the normal structure and function of skin or tissue, leading to pain, inflammation, infection, and delayed healing [15]. The scratch test, also known as the wound repair assay, is a laboratory method used to observe cell migration. A scratch is made on a cell monolayer, and the closure of this scratch over time is monitored to study cell migration and wound healing [16].

Recent studies have explored the therapeutic potential of marine-derived compounds, particularly in wound healing applications. Fucoidan from various seaweed

species has demonstrated anti-inflammatory, antioxidant, and antimicrobial properties, making it a promising candidate for wound healing treatments [17]. Moreover, fucoidan's ability to enhance collagen production and promote fibroblast migration has been documented, highlighting its potential in tissue regeneration and repair [18].

This study investigates the wound-healing properties of fucoidan derived from Sargassum wightii by evaluating fibroblast migration in an in vitro scratch assay and measuring collagen-1 levels, an essential extracellular matrix component. The findings could pave the way for the development of new, fucoidan-based therapies for improved wound management and skin regeneration [19, 20].

To advance the application of fucoidan in biomedical fields, it is crucial to establish efficient extraction, purification, and characterization protocols. Integrating advanced techniques such as microwave-assisted extraction and ion exchange chromatography can optimize the yield and purity of fucoidan, ensuring its efficacy in therapeutic applications. Additionally, comprehensive in vitro and in vivo studies are necessary to fully understand fucoidan's bioactivities and mechanisms of action, providing a robust foundation for its clinical use [21, 22].

Figure 1: Image of *Sargassum wightii*

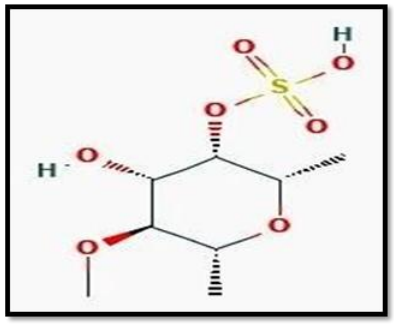

Figure 2: ChemicalStructure of Fucoidan

2. MATERIALS AND METHODS

Materials

The Vero cells (kidney fibroblasts from African green monkeys) were procured from the National Centre for Cell Science (NCCS) in Pune. All chemicals and products used in the study were sourced from Research Lab Fine Chem, Inc., including antibiotics, FBS (from PAA Labs, catalog number A11-043), and DMEM (Dulbecco's Modified Eagle's Medium) serum.

The study utilized various instruments: an FTIR Hyperion 3000 from Bruker (Germany), an NMR ECZR series 600MHz from Jeol (Japan), a mass spectrometer

Trift V nano TOF from Physical Electronics (USA), DEAE Sephadex A-25 from GE Healthcare (USA), a 96-well tissue culture plate from Corning (catalog number 3599), an inverted microscope from TMS (Nikon), and a microwave MSD-2000 from CEM Corporation (Matthews, NC).

Both the SRB Assay and the Scratch Assay were conducted at Aakaar Biotechnologies Pvt Ltd in Lucknow.

Methods

Source of Fucoidan

The freshly harvested Sargassum wightii came from Mandapam on the Indian coast, in the Gulf of Mannar. To get rid of any physical contaminants, like mud and particle matter stuck to the plant, it was completely washed. It was dried under shadow at room temperature for 10 to 15 days.

Authentication

The Regional Facility for DNA Fingerprinting, Thiruvananthapuram, Kerala, India's PG Department of Botany and Research Centre of Rajiv Gandhi Centre for Biotechnology authenticated and correctly identified the *Sargassum wightii* member of the *Sargassaceae* family by referring to a herbarium.

Extraction of fucoidan by Microwave Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) has been a well-established method for processing plant materials [23, 24]. The procedure involves placing reaction tanks, connected by tubes, into a rotating carousel within the sample holder. One of the tubes is equipped with a pressure monitor to track and maintain the set pressure. The extracted fucoidan from *Sargassum wightii* has demonstrated potential in promoting wound healing, as depicted by the Figure 1.

An equation was used to figure out the fucoidan extraction yield (% F) after the extraction was helped by microwaves. WMOHis the dry mass weight that was found after ethanol precipitation. WA is the weight of the algae that was used in each trial.

%F = WMOH/WA × 100

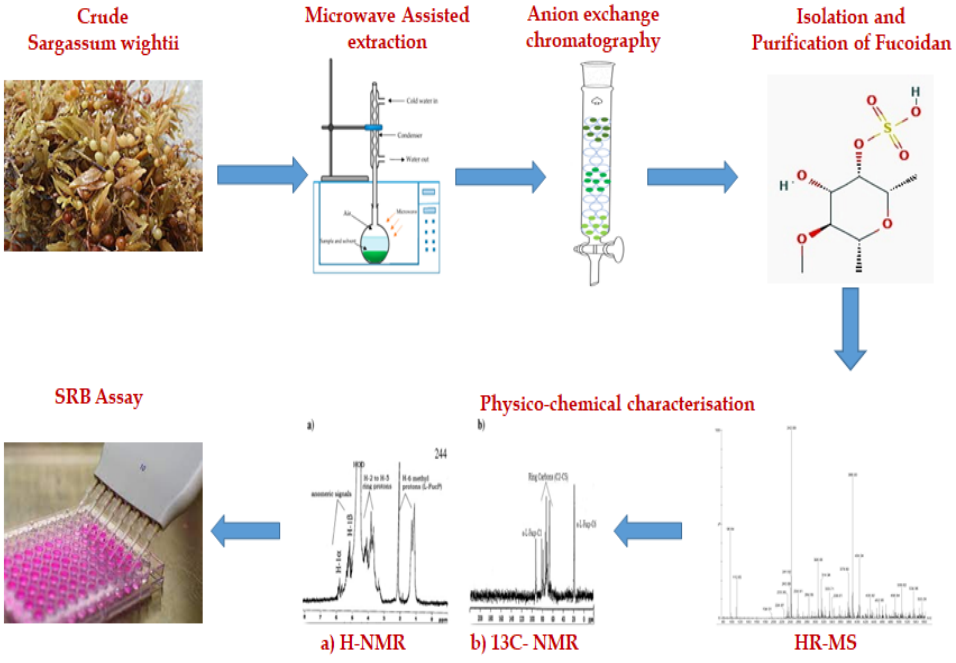

Figure 3: Illustrative depiction of the extraction and characterization process of fucoidan obtained from *Sargassum wightii*

Fucoidan Purification Using Anion Exchange Chromatography

The fucoidan extracted and precipitated with ethanol underwent further purification through anion-exchange chromatography (AEC) [25, 26]. To eliminate proteins and uronic acids, calcium chloride was added, followed by isoelectric point precipitation. Figure 4 presents a flow chart detailing the purification process of fucoidan from *Sargassum wightii*.

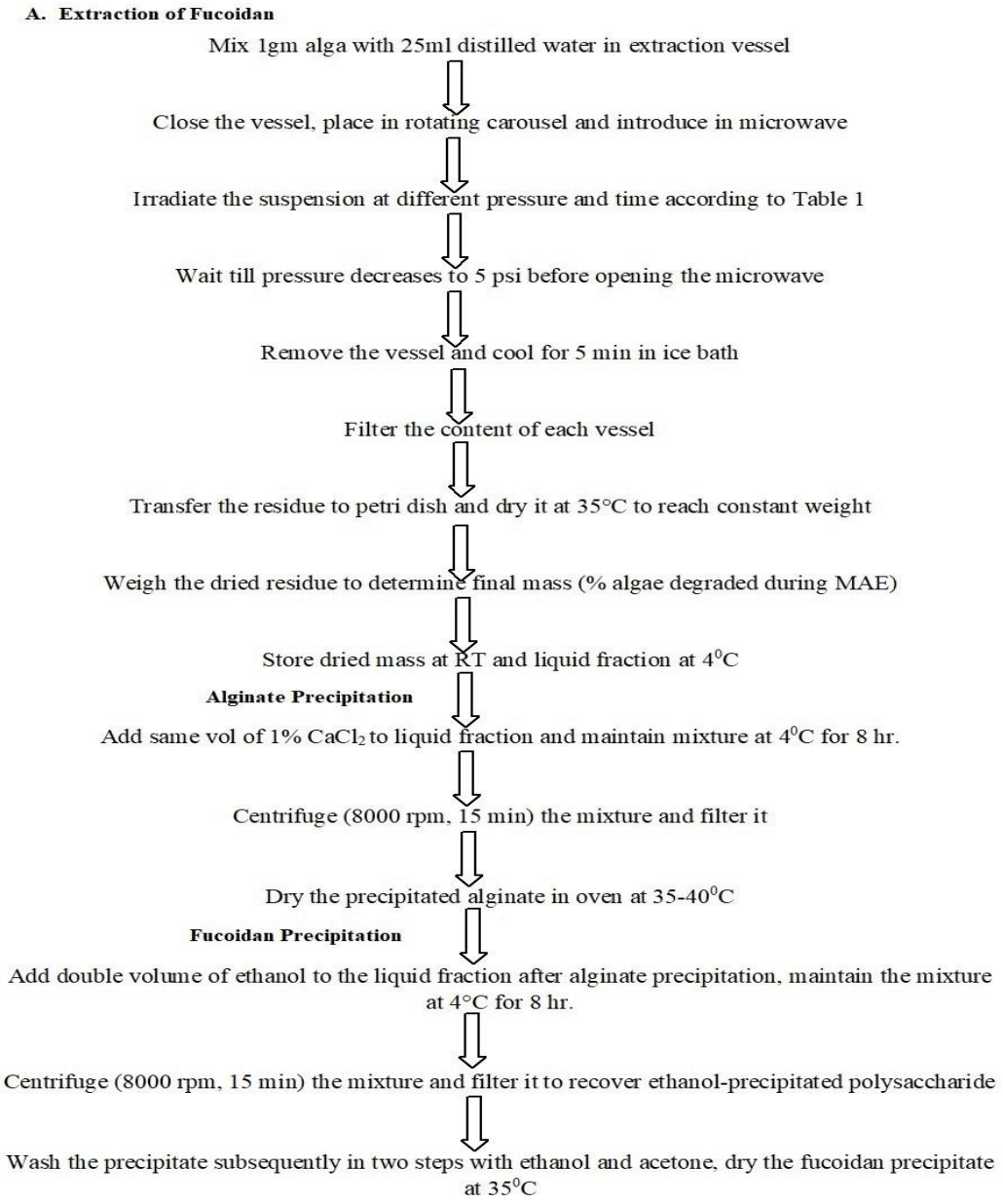

Fig 4: Flow chart presenting A) Extraction of fucoidan from *Sargassum wightii*

B. Purification of fucoidan

Protein Precipitation

Isoelectric point precipitation: Add HCL acid from pH 6-3 to fucoidan until isoelectric point is reached. Incubated the solution of pH 3 at RT for 4 hrs.

⇩

Salting out: Add ammonium sulphate to final concentration of 80% and incubate at 4^0C for 12 hrs

⇩

Trichloroacetic acid (TCA) denaturation: Add dilute TCA solution to final concentration of 10%, incubate at RT for 4 hrs and centrifuge the resulting solution at 6000rpm for 3 min to precipitate proteins

⇩

Uronic Acid precipitation

To the supernatant add different concentrations (1,2,3 and 4%) of calcium chloride, incubate at 4^0C for 4 hrs and centrifuge at 6000rpm for 3 min to precipitate uronic acid

⇩

The supernatant was then filtered, and lyophilized for further purification through anion-exchange chromatography

⇩

After protein and uronic acid precipitation 300mg of fucoidan was dissolved in distilled water at a concentration of 10 mg/mL and applied to DEAE Sephadex A-25 column

⇩

After loading with fucoidan, elution was performed using a NaCl gradient with four different NaCl concentrations (1, 2, 3, and 4 M) at a flow rate of 1 mL/min.

⇩

. Fractions containing fucoidan were collected, filtered, and lyophilized to obtain purified fucoidan.

⇩

The total carbohydrate content of each tube was measured at 490 nm by the phenol–sulfuric acid colorimetric method

Fig 4: Flow chart presenting B) Purification of fucoidan

Characterization of fucoidan

Fourier Transform Infra-Red Spectroscopy (FTIR)

To analyze the infrared spectra of fucoidan samples, we utilized Fourier transform infrared spectroscopy (FTIR) on a Bruker Hyperion 3000 with Vertex 80, carried out at SAIF IIT Bombay, Powai, Mumbai. For this analysis, we created pellets by blending about 1 mg of the fucoidan sample with 100 mg of KBr salt in a 1:100 ratio and pressing them into flakes using a hydraulic pellet press. This method enabled us to capture the sample spectra across the range of 400 to 4000 cm^{-1} with a precision of 4 cm^{-1}. We then meticulously examined the spectra to identify possible linkage types and functional groups [27, 28].

Nuclear Magnetic Resonance Spectroscopy (NMR)

The fucoidan fraction was dissolved in D2O at 10 mg/mL for 2D NMR analysis [27, 28]. This was performed at 300 K using a 500 MHz Bruker Avance DRX spectrometer with a TXI 5 mm probe. The HSQC pulse sequence from Bruker's database was used, and spectra were recorded at 360/120 ppm for 1H and 1024/9 ppm for 13C [27, 28].

Q-TOF Mass spectroscopy (MS)

Mass spectrometry was employed to analyze the Fucoidan substance. The Xevo G2-XS Q-TOF, manufactured by Waters Corporation USA [31–33], was replicated. The mass spectrometer may generate negative-ion mode results within the m/z range of 100 to 3000. The capillary was supplied with a power of 3 kilovolts, while the cone received a power of 45 volts. The gas desolvation rate was adjusted to 800 liters per hour, while the cone gas flow rate was set to 50 liters per hour. The samples were introduced at a flow rate of 10 µl/min after being mixed with methanol and filtered.

SRB Assay

The samples were evaluated for their cytotoxicity on the Vero cell line (kidney fibroblasts from African green monkeys) [34–36]. Vero cells were cultured in a 96-well plate at 37°C with 5% CO2 in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin antibiotic for 24 hours. The following day, cells were treated with varying concentrations of fucoidan (1–1000 µM).

For the cytotoxicity assessment, the Sulforhodamine B (SRB) assay was used. After 24 hours of fucoidan treatment, 100 µL of 10% Trichloroacetic Acid (TCA) was added to each well to fix the cells. The plate was washed with deionized water and air-dried. Subsequently, 0.04% SRB solution was added to each well and incubated for one hour to stain the proteins. Excess dye was removed by washing with 1% acetic acid, and the plate was dried at room temperature. The bound dye was solubilized with a tris base solution (pH 10.5) and mixed on an orbital shaker for 10 minutes. The absorbance was measured at 510 nm using an Elisa plate reader (iMark, Biorad, USA).

Scratch Assay

The wound healing capabilities of the fucoidan extract were assessed through in vitro cell migration studies on Vero cells. Initially, 10,000 Vero cells per well were cultured in a 96-well plate for 24 hours in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% CO2. On the following day, a scratch was made in the cell monolayer using a 200 µL pipette tip. The cells were then treated with 51.6 µg/mL of fucoidan extract and 5 µg/mL of povidone-iodine (positive control) and incubated for 24 hours.

Cell migration and morphological changes were captured using an inverted microscope equipped with a digital camera. The width of the scratch and wound closure at 0, 24, and 48 hours were analyzed using ImageJ software (NCBI) and presented graphically. The wound area percentage, A(t), was tracked to quantify cell migration indirectly:

$$A(t) = A(t)/A(0) \times 100\%$$

where A(t) is the wound area at time t and A(0) is the initial wound area.This method allows for the indirect evaluation of the migration rate by measuring the percentage of wound area at specific time points.

Expression Studies by Flow Cytometry

Flow cytometry [39, 40] was used by researchers to investigate the impact of fucoidan on collagen 1, a molecule involved in wound healing. Inoculate a 6-well microtiter plate with a density of 2 x 106 Vero cells/mL and allow them to proliferate overnight. The cells were exposed to a concentration of 125 ng/mL of plant extract and 10 ng/mL of positive control hEGF for a duration of 48 hours. Rinsing with DPBS following trypsinization to disperse cells. The cells were preserved in 70% methanol at a temperature of -20°C for an extended period of time to analyze the expression of collagen 1. The cells were labeled with an anticollagen 1 antibody and rinsed with DPBS. Subsequently, they endured a 30-minute period of darkness under ambient conditions. Fluorescence-based cell sorting Protein expressions were evaluated using Calibur flow cytometry, and the findings were analyzed using CellQuest Pro software.

Statistical Analysis

The results are shown as the mean percentage inhibition with standard deviation (SD) (n = 3). Each experiment was done three times. A one-way analysis of variance was used to find statistical significance. A value of $p < 0.05$ was considered statistically significant. After this, the Bonferroni post hoc test for multiple comparisons was done. All statistical tests and IC50 value estimates were done with GraphPad Prism (version 3.1) software.

3. RESULTS

Extraction of fucoidan

Through the optimization of microwave-assisted extraction parameters, the yield of fucoidan from *Sargassum wightii* was significantly enhanced. The highest yield, 88.22%, was achieved under conditions of 120 psi pressure for 1 minute and an algal-to-solvent ratio of 1:25 gm/ml. Purification of Fucoidan

The study explored the impact of varying NaCl concentrations (1, 2, 3, and 4 M) on the elution of fucoidan. It was found that using 1 M NaCl resulted in the highest total sugar content, but the lowest sugar recovery [26]. When 2 M NaCl was employed, the total sugar content decreased slightly, but the recovery efficiency significantly improved, reaching nearly 76%. Although higher NaCl concentrations (3 M and above) made sugar recovery easier, they resulted in much lower total sugar content. These findings demonstrated that 2 M NaCl was the optimal concentration for fucoidan elution, as stronger NaCl solutions did not enhance purity (Table 1).

Consequently, 2 M NaCl was chosen as the eluent for anion exchange chromatography (AEC). The implementation of microwave-assisted extraction (MAE) markedly increased the purity of total sugar extracted from *Sargassum wightii*. The purity soared from 6.94% to 64.5%, with a fucoidan carbohydrate recovery rate of 50.3% ± 3.5%. Following these advanced processing steps, the total sugar content reached 70.5 ± 2.56%, and the sulfate group content was 19.1 ± 0.58%. These results indicate that the fucoidan achieved an impressive purity level close to 90%, showcasing the efficacy of this extraction and purification method (Figure 5).

Table 1: Purification of Fucoidan Using Anion Exchange Chromatography

Parameters	**Control**	**NaCl concentration (Molar)**			
		1 M	2 M	3 M	4 M
Total sugar content (% of dry weight)	53.75	54.6	69.87	70.19	64.24
Sugar recovery (%)	100	51.08	77.09	80.93	82.65
Total sugar content after dialysis cut off (% of dry weight)	**47.3**	**48.97**	**64.95**	**55.74**	**56.95**

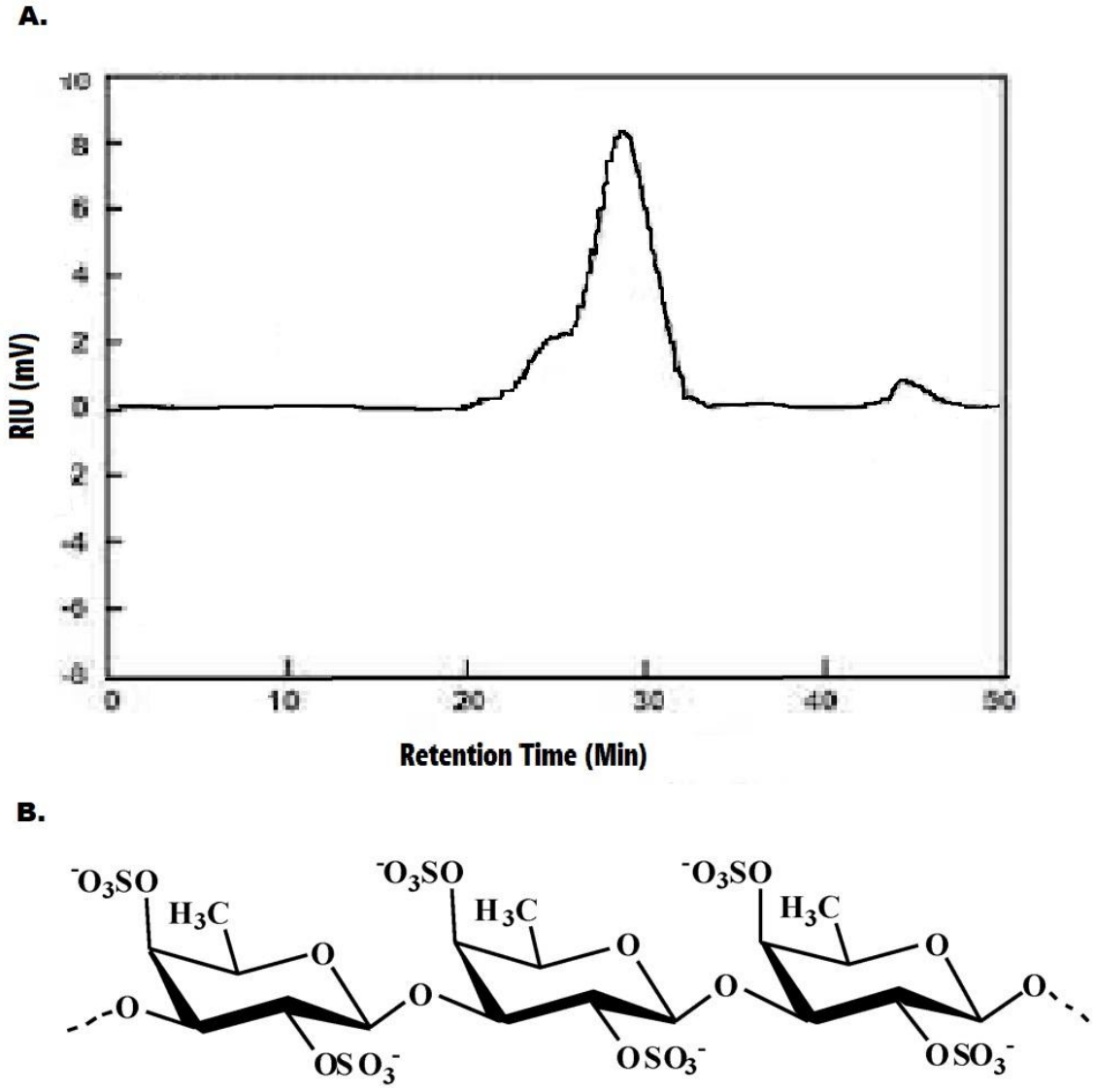

Fig 5: AEC Chromatogram of fucoidan separation using DEAE column

Characterisation of Fucoidan

FTIR analysis

The purpose of the FTIR investigation was to identify infrared absorption characteristics indicative of fucoidan [28]. The FTIR spectra (Figure 6) revealed distinctive fucoidan bands at 1256.6 and 1258.8 cm^{-1}. Absorption within this range indicates the presence of sulfates, corresponding to the asymmetric O=S=O stretching vibrations of sulfate esters. Additionally, an IR band at 839 cm^{-1} indicates the COS bending vibration of sulfate substituents at position C4. This suggests a strong sulfation pattern at the C4 position of the monosaccharide in the polysaccharide chain.

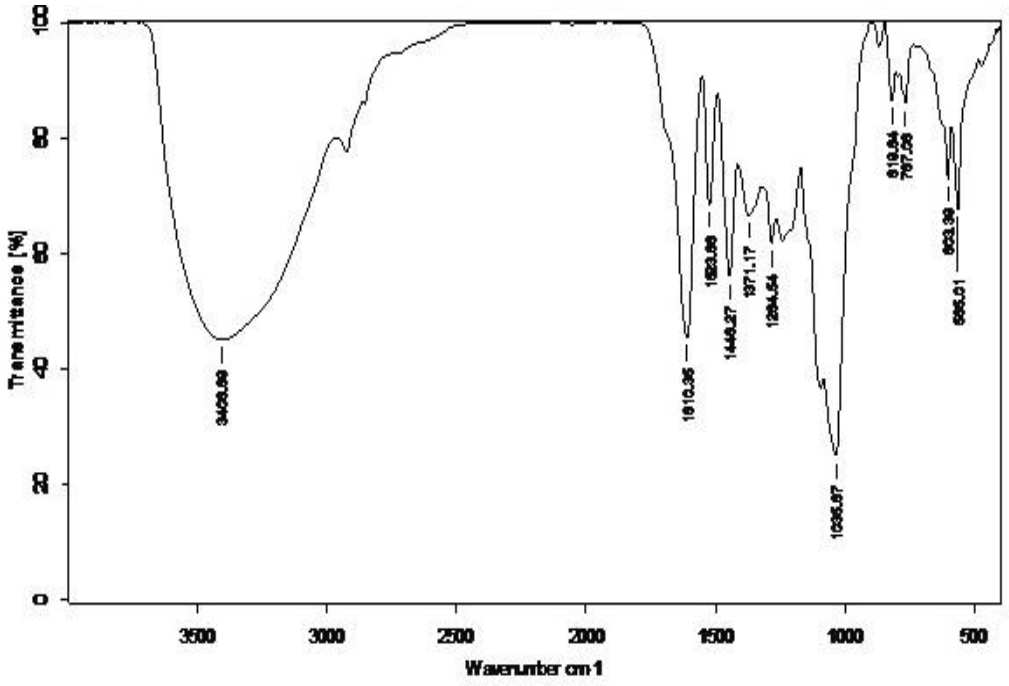

Fig 6: FTIR spectra of fucoidan from *Sargassum wightii*

NMR Analysis

To ensure the accuracy of these structures [30], we analyzed fucoidans using **H^1 and C^{13}** NMR spectra (Figure 7). The key for the labeled peaks can be found in Table 2.

Table 2: Assignment of chemical shift of H^1 and C^{13} NMR spectra of fucoidan extracted from *sargassum wightii*

Sr.No	Hydrogen and carbon of functional groups	Signal of H^1 proton	Signal of C^{13}
1.	H-1[α] = protons that are α-anomeric	5.0–5.1	90–105
2.	H-1[β] = protons that are β-anomeric	4.24 – 5.0	101 – 109
3.	H-(2/3/4) [O-Ac] = protons needed for O-acetylation at sites C2, C3, and C4	4.88, 4.97, 4.98, 5.09, 5.12	70.7, 71.0, 70.0, 70.2, 71.1
4.	[O-sulfate] (H-(2/3/4)) = protons connected to O-sulfation at places C2, C3, and C4	4.5– 5.0	75–85
5.	H-6[CH2] = protons of hexose -CH2OH	3.24, 3.90	67.39
6.	H-6[CH3] = fucose of O-Ac [CH3] = acetyl CH3 protons	5.25, 5.50	67.4, 69.1

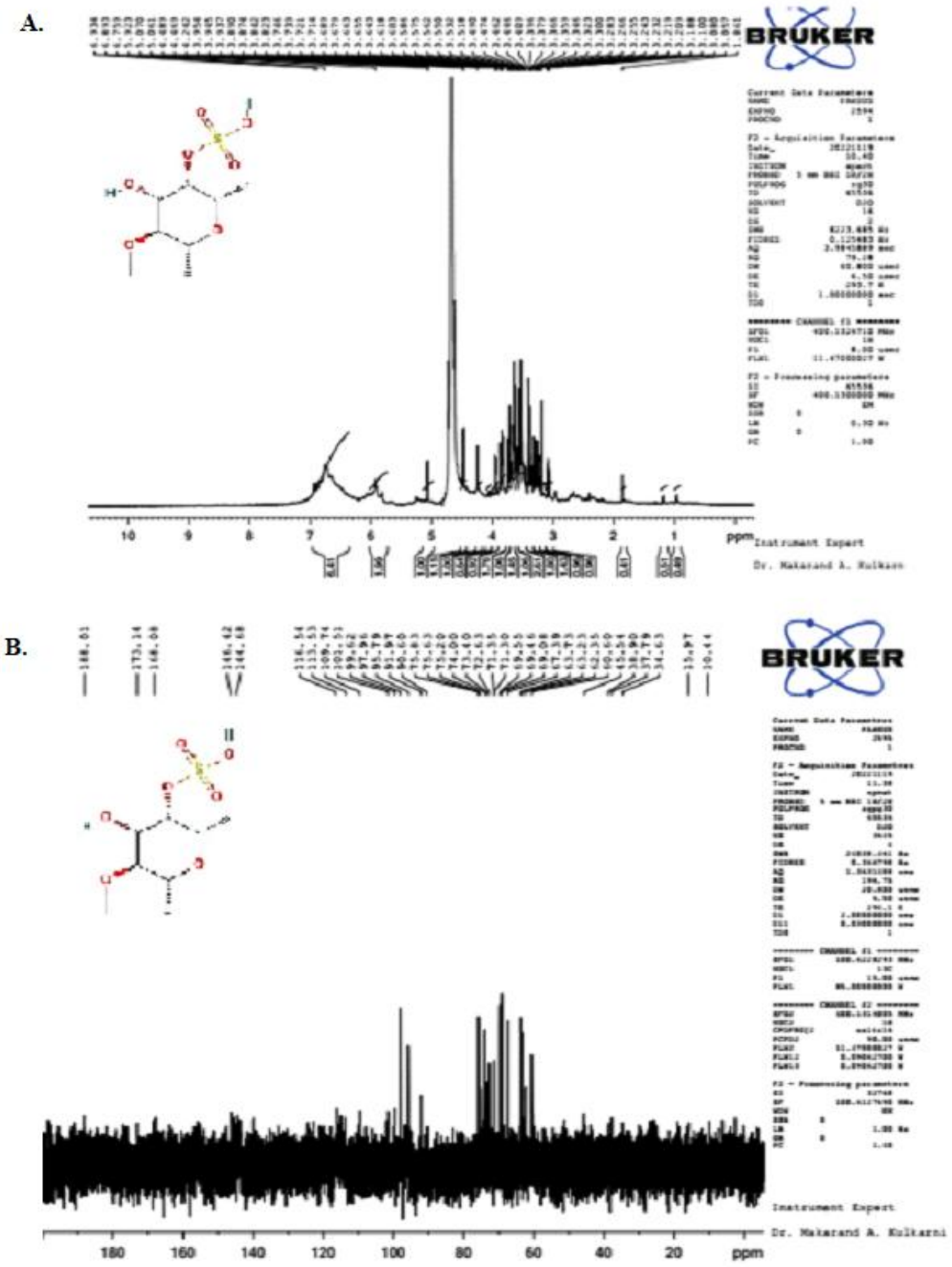

Fig 7: A) H^1 NMR and B) C^{13} NMR Spectrum of Fucoidan isolated from *Sargassum wightii*

Mass analysis

The structure of fucoidan is better understood by using Q-TOF for mass studies [32, 33]. Hexoses (m/z 259), desoxyhexoses (m/z 243), and probably pentoses (m/z 229) all go through sulfation at C-2, which makes the cross-ring cleavage at m/z 138.9 send out a strong signal. Another sign that shows the sulfation at C-2 is at m/z [M-18]. It is possible to separate the C-4 and C-6 sulfations on hexoses to make a fragment ion with a mass of 198.9. The breaking apart of the ring-opening ion at m/z 168.9 points to sulfation at C-3. There is a strong signal at m/z 225, which means that the fucose residue on the non-reducing end is sulphated at position C-2. The sulphate is close to the glycosidic bond, which makes it easier to cut. Too much desulfation broke down the α-L-fucan, which was initially more strongly sulfated (3-linked and 2,4-disulfated). However, its main part (2,4-disulfate of α-L-Fuc p) and the 3-linked fuco-oligosaccharides of the main chain could still be seen clearly by Q-TOF-MS, as shown in figure 8.

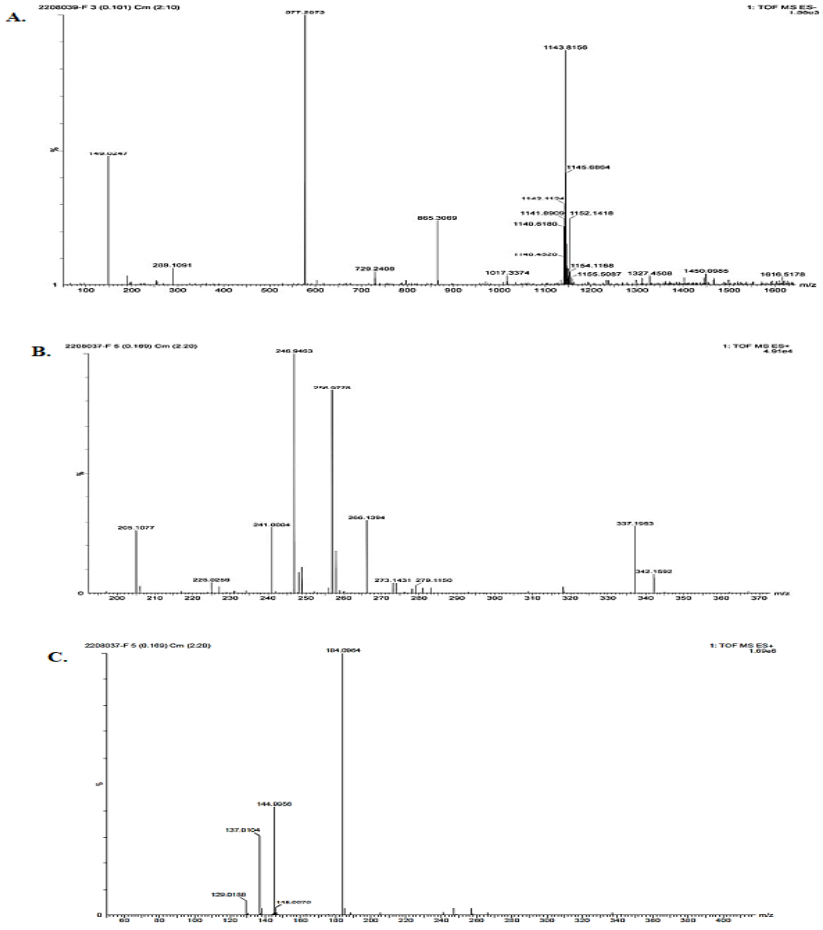

Fig 8: Q-TOF mass spectra of sulphated polysaccharide-Fucoidan from *Sargassum wightii*

SRB assay

To calculate the IC50 [34-36], we plotted the dose-response curve, showing cell viability at various fucoidan concentrations. We identified the point where cell viability was 50% of the maximum (125%). From this point on the y-axis, we drew a line intersecting the dose-response curve, then extended a vertical line down to the x-axis to find the corresponding concentration.

The IC50 value was determined to be 61.3 µM, as shown in Figure 9. This approach ensures the IC50 accurately represents the concentration required to achieve a 50% reduction in cell viability from the peak response.

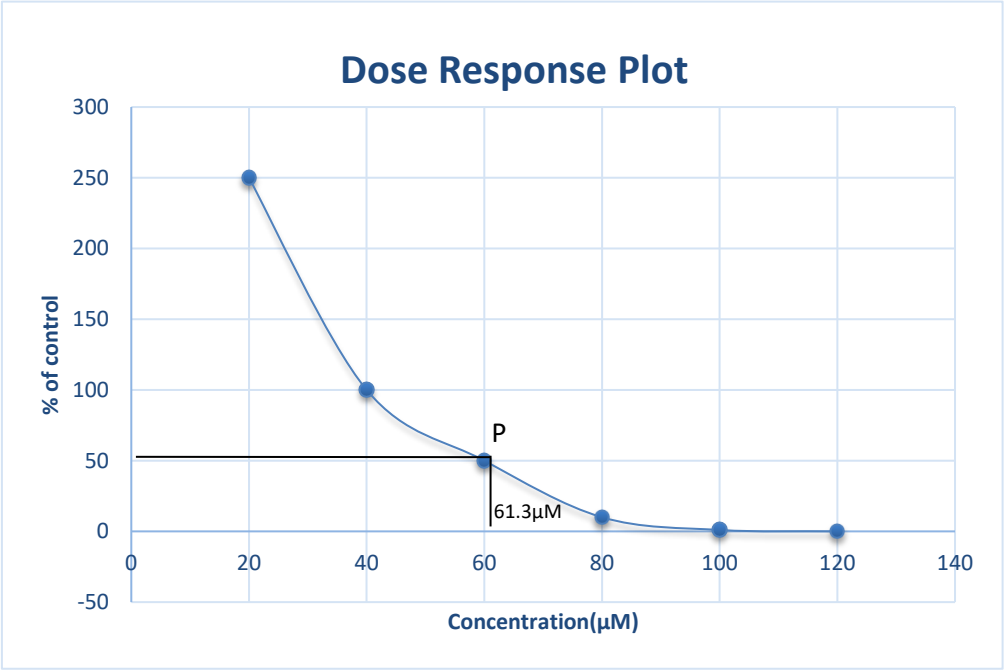

Fig 9: Graphic Determination of IC50 from dose response plots.

Scratch assay

The migration rate was directly evaluated as the percentage of wound area healed at specific time points [37-39]. For the control group treated with 1% povidone-iodine, the wound area healed was 43.75% at 24 hours and 81.25% at 48 hours. For the test group treated with fucoidan, the wound area healed was 46.15% at 24 hours and 76.92% at 48 hours. The statistical analysis yielded a p-value of 0.05, indicating that there is no statistically significant difference between the wound healing rates of the fucoidan-treated group and the control group. This suggests that fucoidan exhibits wound healing properties comparable to those of the control treatment.

The wound healing process was monitored using the ImageJ program to measure the scratch width at 0, 12, 24, and 48 hours. The experiments were performed in triplicate, and the results confirmed that fucoidan facilitated faster wound healing than the control group, as shown in Figure 10.

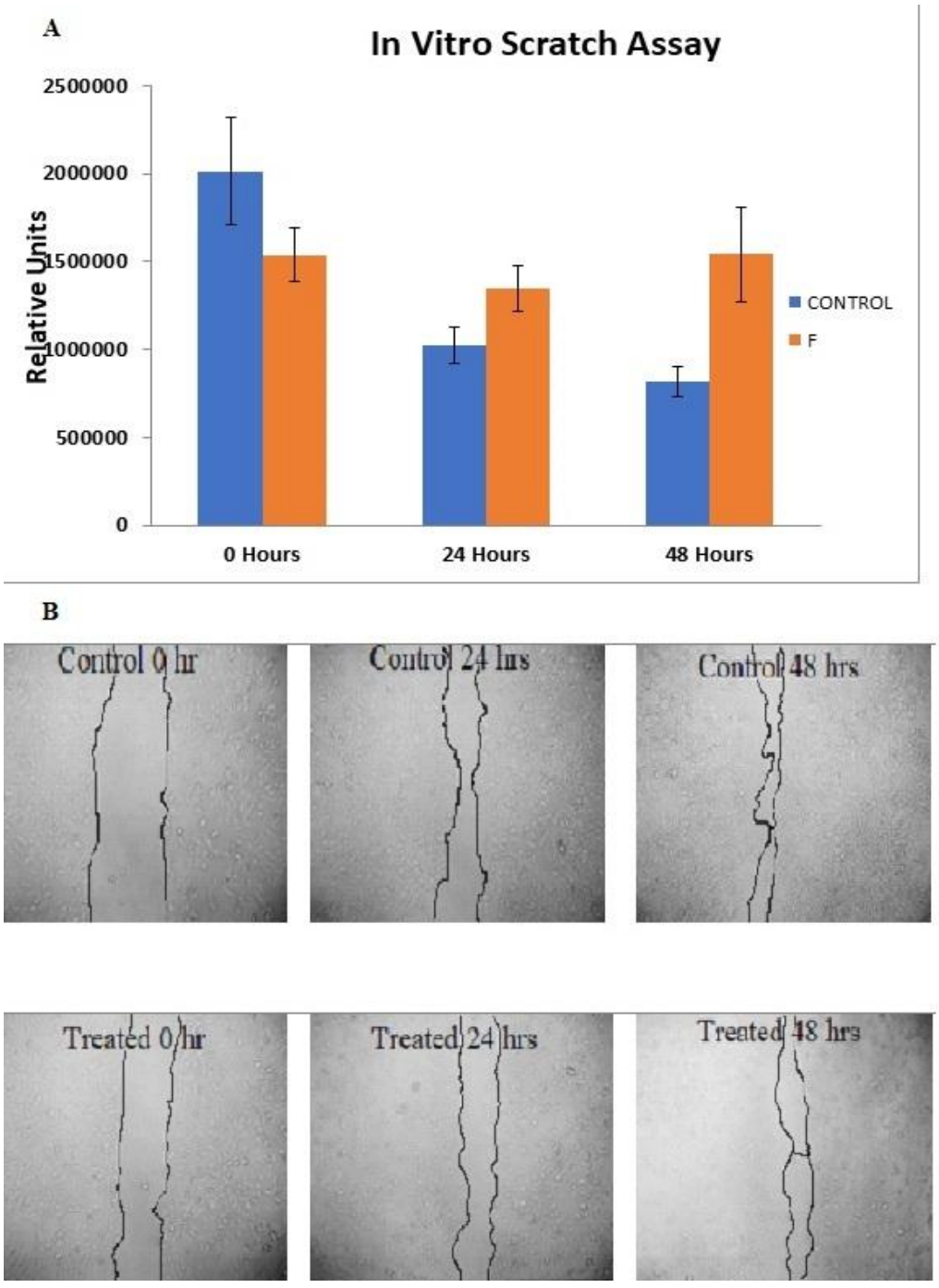

Fig 10: Time-lapse images of scratch assay

Collagen-1 expression

One of the main proteins in the extracellular matrix (ECM) is collagen-1. It helps make the ECM during the mending process and also encourages the growth, migration, differentiation, and production of other important proteins from skin cells [41, 42]. This study looked at how much Collagen type 1 was expressed in vero cells 48 hours after they were treated with either 125 µg/mL of fucoidan extract or 10 ng/mL of human Epidermal Growth Factor (hEGF). The experiments showed that cells treated with fucoidan and hEGF had higher levels of collagen type 1. In cells treated with extract, 77.92% of cells showed collagen type 1, and in cells treated with hEGF, 90.10% of cells did. Figure 11 shows the flow cytometric study of Collagen type 1 expression and the number of expressing cells in cells that have not been treated, cells that have been treated with extract, and cells that have been treated with hEGF. The results show that the extract clearly raises the expression of Collagen type 1 in cells treated with it compared to the control group that wasn't treated. This suggests that the extract raises the expression of Collagen type 1 in vero cells, which may help the wound heal.

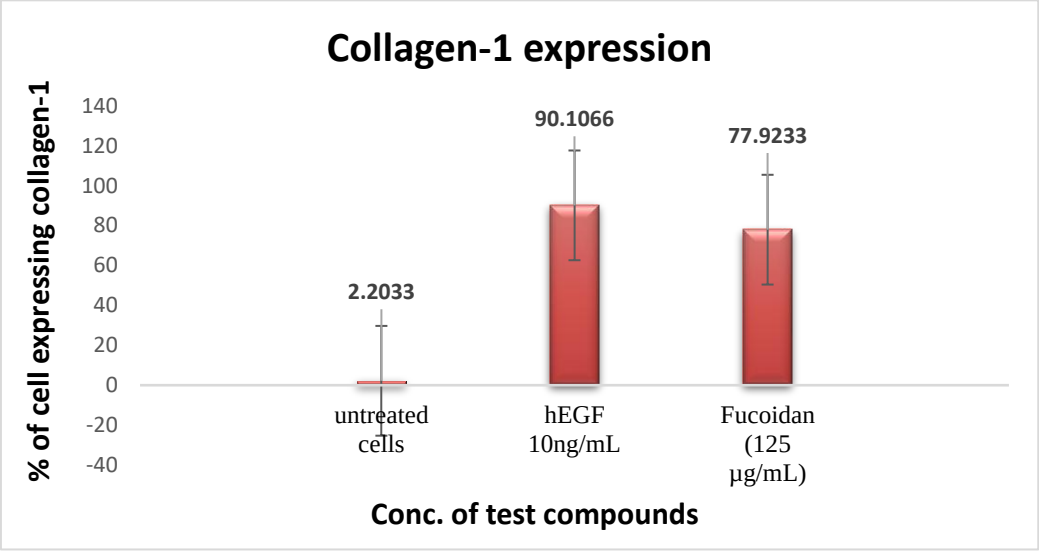

Fig 11: Bar graphs represent the % of Vero cells that expressed collagen-1 after being treated for 48 hours with 125 µg/ml of fucoidan and 10 ng/ml of hEGF

4. DISCUSSION

To thoroughly evaluate the wound healing efficacy of microwave-extracted fucoidan from **Sargassum wightii**, we performed scratch assays and analyzed collagen I expression levels. Our findings provide strong evidence that the extracted fucoidan significantly promotes fibroblast migration and proliferation—two critical processes essential for effective wound closure. These cellular activities are closely associated with the activation of key signaling molecules such as VEGF, PDGF-BB, and collagen, which play vital roles in epithelial regeneration, angiogenesis, and extracellular matrix (ECM) remodeling.

Collagen I, which constitutes approximately 70% of the body's collagen, is a major structural component of the ECM and plays a pivotal role in the wound healing process. The accelerated synthesis of connective tissue proteins, particularly collagen I, is indicative of active ECM formation and restructuring, which are essential for the

repair and regeneration of damaged tissue. The interaction between fibronectin and collagen is another crucial factor that enhances wound healing, as it supports the stabilization and integrity of the newly formed ECM [40].

The final phase of wound healing, tissue remodeling, is characterized by scar formation and the restructuring of the ECM, a process that further underscores the importance of collagen I and fibronectin. An increase in the levels of these proteins is a strong indicator of the wound healing potential of the materials tested in this study.

To further understand the role of fucoidan in wound healing, we analyzed collagen I expression in Vero cells treated with human epidermal growth factor (hEGF) as a reference, fucoidan extract, and a control. The results revealed a significant increase in collagen I expression in fucoidan-treated Vero cells, which was comparable to the hEGF-treated control. These findings suggest that fucoidan enhances wound healing by promoting fibroblast migration and proliferation, a process facilitated by the upregulation of collagen I. This aligns with previous studies that have demonstrated elevated fibronectin expression when plant polyphenols are utilized for wound healing [42-46].

The implications of these findings are significant, as they suggest that the fucoidan extracted from **Sargassum wightii** is not only effective in promoting wound healing but also non-toxic, making it a promising candidate for further research and potential therapeutic applications. This study is particularly noteworthy as it represents the first in vitro demonstration of the wound healing potential of fucoidan derived from **Sargassum wightii**. Further studies, including in vivo analyses, are warranted to explore the full therapeutic potential of this natural compound in wound care and tissue regeneration.

CONCLUSION

Fucoidan from Sargassum wightii can be recovered through MAE in the best response conditions. The fast extraction time and use of chemicals that don't damage the material kept costs down. It was suggested that MAE could be used to get fucoidan from brown kelp. The FTIR and 2D NMR results show that the fucoidan found in Sargassum wightii is made up of a fucose-galactose backbone that is linked by 1,3 glycosidic links and has sulfation at C2 and C4 sites. The fucoidan water mix helped the Vero cell line heal wounds faster. Furthermore, it was demonstrated that the extract did not have any damage to cells. Based on these findings, fucoidan may be a good source of natural chemicals that can help heal wounds.

In our study, we show that increased collagen 1 expression and fibroblast movement to the wound site affect fucoidan's ability to heal wounds. In addition, this extract does not harm cells and is safe for further study. According to these results, fucoidan may help wounds heal, and more research needs to be done to find the substances that make this happen.

Declarations

Ethics permission: The Ethics Committee has confirmed that there is no need for ethical permission.

Permission to publish: The writers have given permission to publish.

Data Access: All the data that was collected or analyzed for this study is in this published piece and the files that go with it.

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List of abbreviations:

MAE: Microwave-assisted extraction
bFGF: basic fibroblast growth factor
HMF: High molecular weight fucoidans
SRB: Sulforhodamine B
ECM: Extracellular matrix
DMEM: Dulbecco's Modified Eagles Medium
FBS: Fetal Bovine Serum
PBS: Phosphate Buffered Saline
FTIR: Fourier-transform infrared
NMR: Nuclear magnetic resonance
MS: Mass spectroscopy
AEC: Anion-exchange chromatography
HSQC: Heteronuclear single-quantum coherence

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إمكانية التئام الجروح باستخدام الفوكويدان المستخرج بالموجات الدقيقة من نبات سارجاسوم وايتي، ربما بوساطة التعبير عن الكولاجين-1 في سلالة خلايا فيرو

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ملخص

الخلفية: أظهر الفوكويدان، وهو جزيء طبيعي كبير مستخلص من نبات سارجاسوم وايتي، نتائج واعدة في مجالات علاجية متنوعة، بما في ذلك تطبيقات مضادة للأورام، ومضادة للأكسدة، ومضادة للتخثر، ومضادة للجروح. تستكشف هذه الدراسة إمكانات الفوكويدان المستخلص من نبات سارجاسوم وايتي، المُجمع من خليج مانار، تاميل نادو، الهند، في التئام الجروح.

الأهداف: يهدف هذا البحث إلى تقييم فعالية الفوكويدان المستخلص بتقنية الاستخلاص بمساعدة الميكروويف (MAE)في تعزيز التئام الجروح في خلايا فيرو، وهي سلالة من خلايا كلى القرد الأخضر الأفريقي. كما تبحث الدراسة في تأثير الفوكويدان على التعبير الجيني لبروتين الكولاجين-1، وهو بروتين أساسي يشارك في عملية التئام الجروح.

النتائج: أظهر اختبار SRB أن الفوكويدان لم يُظهر سمية خلوية لخلايا فيرو، حيث بلغت قيمة IC50 61.30 ميكرومولار. وكشف اختبار الخدش عن إغلاق جرح بنسبة 46.15% بعد 24 ساعة و76.9% بعد 48 ساعة، مقارنةً بـ 50% و81.25% في المجموعة الضابطة. وقد أدى علاج الفوكويدان إلى زيادة ملحوظة في التعبير عن الكولاجين-1، حيث أظهرت 77.92% من الخلايا مستويات مرتفعة من هذا البروتين الحيوي.

الخلاصة: تؤكد هذه الدراسة قدرة الفوكويدان المستخلص من نبات سارجاسوم وايتي على التئام الجروح في المختبر. وتدعم هذه النتائج إمكانات الفوكويدان كعامل طبيعي لالتئام الجروح وترميمها.

الكلمات الدالة: Sargassum wightii :، Fucoidan، جزيء كبير، التئام الجروح، اختبار SRB، خلايا Vero، التعبير عن الكولاجين-1.

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