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Development of Bioactive Cellulose Films from *Drynaria quercifolia* Rhizome: A Sustainable Approach to Advanced Natural Materials

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Abstract

Investigating underutilized botanical resources for developing cellulose-based polymers has gained momentum due to the need for sustainable biomaterials. With the added potential of phytochemical infusion, *Drynaria quercifolia,* a traditionally prized medicinal fern, provides an unexplored source for cellulose extraction. This study explores the green extraction of cellulose from the rhizome part of *D. quercifolia* using the alkaline peroxide method, which

produced 27.41 ± 4.65% (%w/w) cellulose. The resultant biopolymer was fabricated into glycerol plasticized films with 70% ethanolic extract. After functional property evaluation and physicochemical characterization, the extracted cellulose proved suitable for hydration-based applications by exhibiting high water retention capacity and settling volume compared to commercial cellulose. While TGA and DSC showed promising thermal stability, XRD verified the crystalline cellulose I structure. A porous fibrous surface that promotes film formation was depicted via SEM imaging. Cytocompatibility was verified by *in vitro* tests using RAW 264.7 macrophages, which proved >80% cell viability and no morphological anomalies. Qualitative scratch assay results confirmed cell migration, although the quantitative wound closure could not be obtained due to the optical interference from the film. Alkaloids, flavonoids, glycosides, and carbohydrates were detected through phytochemical analysis and UV-Vis spectroscopy, which verified the phytochemical release into PBS at pH 7.4 over 24 h. The value of *D. quercifolia* rhizome, which has long been utilized in medicine but is rarely investigated in biomaterial science, as a dual-purpose source of cellulose and bioactive compounds, makes this study novel.

Keywords: *Drynaria quercifolia*; Cellulose extraction; Biopolymer film; Phytochemical infusion; Characterization; *In vitro* analysis

1. Introduction

Cellulose-based biopolymers have become increasingly prominent in biomedical applications owing to their biodegradability, mechanical properties, and adaptations for functionalization. (Rose and Palkovits 2011)Combined with medicinal plant extracts, these polymers can achieve sustained bioactivity, making them ideal for advanced wound dressing applications. (Maver et al. 2020)However, incorporating native phytochemical-rich fern species into cellulose structures remains largely unexplored, especially for species like *Drynaria quercifolia*, which has a deep-rooted history in traditional medicine but is often overlooked in modern biomedical contexts. (Mani et al. 2023) Known as '*Mudavattukal kizhangu*' in Tamil, *Drynaria quercifolia* is widely used in Asia to treat inflammation, bone injuries, and skin ailments. (Devanesan et al. 2025)

Various studies confirmed that *D. quercifolia* possesses membrane-stabilizing and thrombolytic action *in vitro* and showed anti-rheumatoid effects. (Jegan et al. 2025) Broader phytochemical profiling indicates the rhizome contains alkaloids, tannins, terpenes, phenolics, flavonoids, glycosides, proteins, carbohydrates, and other metabolites. Coupled with evidence that the fern fronds show potent antioxidant and tyrosinase inhibitory activities. The rhizome possesses a rich spectrum of bioactive compounds (Thulasi et al. 2023). Despite these bioactive compounds, the plant has not yet been applied to develop bioactive cellulose-based films. Therefore, the study aims to extract cellulose from *Drynaria quercifolia* using the alkaline-peroxide method, fabricate glycerol-plasticized cellulose films infused with ethanolic rhizome extract, and characterize functional properties that are relative to commercial cellulose. Evaluate *in vitro* cytocompatibility, morphology, and cell migration using RAW 264.7 macrophages. Profile phytochemical release at physiological pH and assess the class of compounds released. By integrating traditional phytomedicine and modern biomaterials

science, this work seeks to develop *D. quercifolia* as novel, bioactive, and biodegradable materials.

2. Materials and Methods

2.1. Sample procurement and preparation

The *Drynaria quercifolia* rhizomes were procured from *Ueir Organic Foods* based in Erode district, Tamil Nadu, India. The samples are initially authenticated based on the macroscopic and morphological features by Dr. Fr. Jobi Xavier, Associate Professor and Head, Department of Life Sciences, CHRIST (Deemed to be University). The identity was confirmed via Sanger sequencing of the sample via Barcode biotechnology and matched with reference sequences of *Drynaria quercifolia* in the NCBI blast database with the accession number LC496676.1. The rhizome scales were processed manually, followed by rinsing with distilled water to remove excess debris. The cleaned rhizome sample was then dried at 60°C for three days and ground to fine powder. The dried sample was then kept in a clear Ziploc cover with Silica gel packets to avoid moisture trapping and was stored for further use

2.2. Extraction of Cellulose

The cellulose extraction was performed using alkali peroxide method. 10 g of the dried powdered rhizome was added to 150 mL of 3% (%w/v) Sodium Hydroxide for alkali treatment and the sample was mixed on a magnetic stirrer at 500 rpm for 90 min at 60°C. The mixture was filtered and immediately neutralized with warm distilled water. The biomass was again

treated with 150 mL of 4% (%w/v) sodium hydroxide as secondary treatment. The biomass was finally bleached with 150 mL of 4% (%v/v) hydrogen peroxide and 4% (w/v) sodium hydroxide and was stirred on a magnetic stirrer at 500 rpm for one and a half hours at 60°C. the extracted cellulose from *D. quercifolia* was dried overnight at 60°C and stored for further studies. (Chacko et al. 2025)

2.3. Characterization of the extracted cellulose

The extracted cellulose from *Drynaria quercifolia* was characterized to investigate and understand its physicochemical properties like crystallinity and thermal stability. Various analytical techniques were employed for its characterization, including X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

2.3.1. Fourier Transform Infrared Spectroscopy

FTIR is one of the most frequently used methods of identification of various functional groups present in a compound. (Hospodarova et al. 2018) The FTIR analysis was performed by scanning the sample in a range between 4000 and 400 cm^{-1} infrared frequency using the JASCO FTIR 4100 FTIR spectrophotometer at CLRI-CATERS, Chennai. The scanning was performed at a resolution of 4 cm^{-1}.

2.3.2. X-ray diffraction

XRD analyses the angular diffraction pattern (2θ) of the biopolymer within the range of 5° and 90°. This technique allows to observe crystal and amorphous phase and structure of

the cellulose.(Swain and Badamali 2020) The Malvern PANalytical Powder X-Ray Diffractometer Empyrean Series III instrument at CLRI-CATERS, Chennai, was used for XRD analysis of the extracted cellulose.

2.3.3. Differential Scanning Calorimetry

DSC provides information about the thermal properties, phase transitions like melting, crystallisation, glass transitions, and the stability of various compounds. (Gill et al. 2010) The sample was heated at a rate of 10°C per minute to the temperatures between 20 and 200°C using the DSC2A-00837 thermal analyser from the T A Instruments at CLRI-CATERS, Chennai.

2.3.4. Thermogravimetric analysis

TGA describes the stability and degradation of the isolated biopolymer at varying temperatures. (Cabrales and Abidi 2019). The sample was heated at a steady 20°C per minute reaching temperatures between 40°C and 800°C using Perklin Elmer (STA 6000), at the Central Instrumentation Facility, St. Joseph's University, Lalbagh Road, Bengaluru.

2.3.5. Scanning Electron Microscopy

Microscopy gives a detailed account of the surface morphology of the cellulose.(Radoor et al. 2020) The images were obtained using TESCAN CLARA SEM, at CLRI-CATERS, Chennai.

2.4. Functional Property Analysis of the Extracted Cellulose

Cellulose's functional characteristics are essential for identifying its possible applications in various fields. Bulk density, hydrated density, packed density, water retention capacity, and settling volume are some of the important functional parameters applied to the extracted cellulose in this investigation. (Okeke et al.) The compactness and flow properties of the biopolymer to absorb water is determined by the hydrated density and water retention capacity, which are crucial for structural and biomedical applications.(Prakongpan et al. 2002)Settling volume supports the use of the material in the formulation of sols, emulsion, foams and aerosols, by indicating the propensity to form colloidal dispersions. (Santhosh and Umesh 2024) **Table 1** summarizes the experimental procedure used to ascertain these functional characteristics.

Table 1. Methodology for the calculation of functional properties (Santhosh and Umesh 2024)

FUNCTIONAL PROPERTY	METHODOLOGY	EQUATION
Bulk Density (BD)	0.1g of extracted cellulose was taken in a 10mL graduated cylinder. The volume occupied by the sample was measured.	***BD(g/ml) = wt. of sample(g)/vol. Occupied by the sample***
Packed Density (PD)	0.1g of extracted cellulose was taken in a 5mL graduated syringe, and pressure was applied to completely minimize the volume occupied by the sample. The final volume was measured.	***PD (g/mL) = wt. of the sample (g)/least vol. occupied by the sample (mL)***
Hydrated Density (HD)	0.1g of the extracted cellulose was added to a 10mL graduated cylinder containing 5mL of distilled water. The volume dispersed was noted down.	***HD (g/mL) = wt. of the sample (g)/vol. Of water displaced (mL)***
Water Retention Capacity (WRC)	2 g of extracted cellulose was mixed with 20 mL of distilled water and incubated for 10 min, followed by centrifugation at 2000 rpm	***WRC (g/g) = wt. of water in the sample (g)/wt. Of dry sample (g)***

	for 15 min. The supernatant was discarded, and the weight of water retained in the sample was calculated	
Settling Volume (SV)	1 g of extracted cellulose was mixed with 70 mL of distilled water, followed by sonication for 30 min to disperse gas between the fibers. Further, the mixture was degassed in a vacuum for 30 min and was stored at 4 °C for 24 h. After degassing, the volume was 100 mL using distilled water. This final mixture was kept undisturbed for 24 h in a graduated cylinder. The volume occupied by the sample was recorded.	***SV (mL/g) = (Vol. of the settled sample residue (mL)/ wt. of sample taken (g))***

2.5. Extraction of *Drynaria quercifolia* using 70% ethanol

The ethanolic extraction was performed using 70% ethanol, which is selected for its optimal polarity in solubilizing polar and moderately polar phytochemicals such as flavonoids, polyphenols, and glycosides typically present in the medicinal plant rhizomes. A total of 20 g of powdered rhizome was added into a 500 mL conical flask containing 250 mL of 70% (%v/v) ethanol. The mixture was then subjected to maceration on an orbital shaker at 120 rpm for 72 hours at ambient temperature to facilitate the efficient compound diffusion. Post extraction, the solution was filtered using Whatman No. 1 filter paper, and the filtrate was dried in hot air oven at around 50°C until the volume reduced to approximately 150 mL. The concentration of the extract was determined gravimetrically by evaporating a known aliquot and weighing the residual mass, expressed in mg/mL. (Prasanna and Chitra; Nithin et al. 2020)

2.6. Synthesis of plant extract-infused cellulose sheet

To prepare the plant extract-infused film, 2.5 g of alkali-extracted cellulose was dispersed in 200 mL of distilled water, along with 0.75 mL of glycerol, which was used as a plasticizer to enhance the flexibility of the film. The mixture was continuously mixed at room temperature for 6 h to ensure uniform dispersion in the solution. The hydrated cellulose matrix was homogenized using a blender until a smooth consistency was observed. The resulting slurry was incubated overnight at 4°C to allow structural stabilization. On the following day, the mixture was briefly homogenized again, after which 7 mL of the 70% ethanolic *Drynaria quercifolia* extract was incorporated and uniformly mixed via gentle swirling. The final solution was cast into a leveled casting tray and allowed to dry in the hot air oven at 60-70°C overnight or until the sheet becomes dry. After drying, the film was carefully removed from the tray and stored in a desiccator for further characterization (Reshmy R. et al. 2021) A control film was also prepared using the same protocol, excluding the plant extract, to serve as a reference for subsequent comparative analysis.

2.7.Characterization of the Cellulose Sheets

2.7.1. FTIR

The FTIR analysis was performed using a Shimadzu IR Spirit-L FTIR spectrophotometer at the Common Instrumentation Lab, Department of Life Sciences, CHRIST (Deemed to be University). The samples were scanned in the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. The method was used for spectral analysis of the control and the extract-infused films.(Hospodarova et al. 2018)

2.7.2. SEM

The intricate microscopic structure of the cellulose films was obtained via SEM imaging. Thermo Scientific Apreo 2 SEM was employed for obtaining the images at the Central Instrumentation Facilities, CHRIST (Deemed to be University), Bengaluru.(Radoor et al. 2020)

2.8.Release Kinetics Study

The release profile of the components infused in the *Drynaria quercifolia* extract was measured with a Thermo Scientific BIOMATE 160 UV-Visible Spectrophotometer at a scanning range of 200 to 1000nm. Briefly, the sample was weighed at ~0.003 g and was incubated in 3 mL phosphate buffer saline solution (PBS) at various pH ranges of 4.5, 6, 7.4, and 8.5 and incubated at room temperature. At specific time intervals, the scanning absorbance range was measured. The absorbance of the plain extract was taken as a reference. (Motelica et al. 2024)

2.9.Phytochemical Analysis

Qualitative phytochemical analysis was performed in the released media of the infused Cellulose sheet in phosphate buffer saline solution to check for the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, carbohydrates, proteins, saponins, terpenoids, steroids, quinones, and phenolics. The procedure used is provided in the Online resources Table 1. (Banu and Cathrine; Raj; Balamurugan 2019; R. Bhattacharyya * 2019)

2.10. *In vitro* analysis

RAW 264.7 cells, a murine macrophage cell line, were employed in the *in vitro* assays due to their well-established role in modelling cellular responses related to inflammation, cytotoxicity, and cell migration studies. As macrophages are central mediators of immune response and tissue regeneration, this cell line provides relevant ideas to assess the biocompatibility and bioactivity of the extract-infused cellulose films intended for external biomedical applications.(Jamalzadeh et al. 2016)

2.10.1. Morphological Analysis

For morphology analysis, the RAW264.7 cells were seeded in a 24-well plate and exposed to the cellulose films ranging from 2 mg/mL to 10 mg/mL. Following 24 h of incubation at 37°C in a humidified CO_2 incubator, the cellular morphology was observed under an inverted microscope. Changes in the cell shape, adherence, and membrane integrity were documented to assess cytocompatibility and potential extract-mediated morphological alterations. (Li et al. 2022)

2.10.2. MTT Assay

The cytotoxic potential of the control and extract-infused films was evaluated using the EZcount^{TM} MTT Cell Assay Kit (HIMEDIA Labs). RAW cells were seeded into the sterile 24-well plates and incubated for 24 h at 37°C in a 5% CO_2 humidified incubator to allow cell attachment. The cellulose films (control and extract loaded) were introduced into the wells at

concentrations ranging from 1- 4 mg/mL. Following 24 h of exposure, 100 µL of MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium) reagent was added directly to the wells and incubated in the dark for 4 h. Subsequently, the formazan crystals formed were dissolved using the solubilization buffer provided in the kit, and the solution was incubated for 30 min with gentle shaking. The absorbance values were recorded at 570 nm. The cell viability was then calculated relative to untreated control wells and expressed as percentage viability. (Prasanna et al. 2019)

2.10.3. Cell Migration Assay

The cell migration assay was performed using the scratch method to evaluate the effect of cellulose films on RAW264.7 cell motility. Cells were seeded in a 24-well plate and cultured until ~90% confluence. A sterile 200 µL pipette tip was used to create a uniform linear scratch through the monolayer of each well. The wells were gently washed with PBS to remove the detached cells. The UV-sterilized discs of the control and extract-infused films were placed directly over the scratch area at concentrations ranging from 1 - 3 mg/mL. The plates were incubated at 37°C in a 5% CO_2 atmosphere, and the wound area was imaged at 24 h using an inverted microscope at 100x magnification. (Orlando et al. 2020)

3. Results and discussion

3.1. Extraction of Cellulose from *Drynaria quercifolia* rhizome

Cellulose is a complex carbohydrate that forms the cell wall of plant cells, along with various other non-cellulosic elements such as starch, hemicellulose, pectin, and lignin. Therefore, treating the selected substrate with chemicals that solubilize and remove these components is essential, leaving the cellulose fibers behind. The extraction was performed through alkaline treatment and bleaching. The alkaline treatment with 3% and 4% Sodium Hydroxide solubilizes starch, hemicellulose impurities, and removes the tannins in the substrate. The bleaching process through the alkaline peroxide method (4% NaOH and H_2O_2) proves efficiency in removing the residual components. (Figure 1) Peroxide radical degradation is the key chemical reaction that takes place in this process. The cellulose yield was calculated as 27.20 ± 4.65 % (%w/w) based on the initial dry biomass weight of 10 g. Although no prior reports exist for this species, the fiber content in *D. quercifolia* has been reported at ~37%, suggesting that cellulose constitutes the majority of fibrous components. (Raj) In a related study by Ma et al used the Nitric acid and ethanol method to extract cellulose from chili stems. According to the study, the yield ranged from 15 to 34.5% (%w/w) (Ma et al. 2023). Additionally, in a study on the cellulose from grape stalks by Araújo et al. obtained a yield of 21.98% (%w/w). (Araújo et al. 2023)

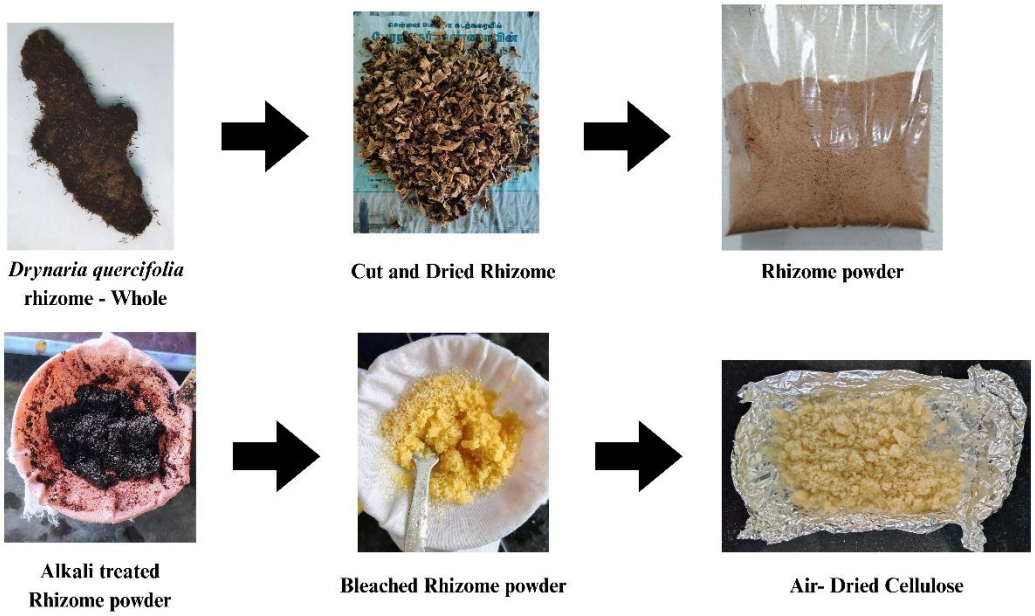


Figure 1 *Overall procedure of sample processing and extraction of cellulose from Drynaria quercifolia rhizome*

3.2. Characterization of Extracted Cellulose

3.2.1. FTIR

FTIR provides the structural and functional group details of the compound. **Figure 2**(A) illustrates the FTIR spectrum, and **Table 2** offers information on the cellulose extracted from the *Drynaria quercifolia* rhizome. The spectrum exhibits a broad peak at 3332.43 cm^{-1}, which indicates the stretching vibrations of the -OH groups involved in the inter- and intra-molecular hydrogen bonding. This peak is characteristic of the cellulose and confirms its hydrophilic nature and hydrogen-bonded network (Apaydın Varol and Mutlu 2023). A moderate peak at 2898.15 cm^{-1} can be attributed to the C-H stretching vibrations of the aliphatic -CH and -CH_2 groups in the cellulose backbone (Kim et al. 2018). The peak at 1604.88 corresponds to O-H bending of adsorbed water or residual carboxylic groups (Kassab et al. 2020). The absorption

at 1426.12 cm^{-1} is associated with the CH_2 scissoring vibrations, which are the typical fingerprint of crystalline cellulose. (Vârban et al. 2021) A distinctive peak at 1315.49 cm^{-1} reflects C-C and C-O skeletal vibrations (Yoganandam et al. 2020) and a band at 1156.84 cm^{-1} represents C-O-C asymmetric stretching, which is an indication of the glycosidic linkages present in the cellulose molecule (Oh et al. 2005a). The strong peak at 1025.78 cm^{-1} corresponds to the C-O stretching of the primary alcohol groups, and the signal at 1025.78 cm^{-1} is assigned to the pyranose ring vibration, confirming the cellulose backbone(Apaydın Varol and Mutlu 2023). Notably, the absence of the peaks near 1730-1750 cm^{-1} (assigned to C=O stretching of hemicellulose) and near 1510-1530 cm^{-1} (aromatic skeletal vibration of lignin) indicates the effective removal of the non-cellulosic components such as hemicellulose and lignin during the alkaline peroxide extraction(Santhosh and Umesh 2024). The minor peaks at 663.79 cm^{-1}, 555.99 cm^{-1}, and 436.54 cm^{-1} can be associated with bending vibrations and out-of-plane skeletal modes seen in highly purified cellulosic material. The spectral profile confirms that the extracted cellulose retains its structural integrity of Cellulose I with minimal contamination from residual plant matrix components.

Table 2. FTIR peaks of the extracted cellulose from the rhizome

PEAK VALUE (cm^{-1})	**FUNCTIONAL GROUPS**	**REFERENCES**
3332.43	O-H Stretching Vibrations	(Araújo et al. 2023)
2898.15	C-H Stretching Vibrations	(Apaydın Varol and Mutlu 2023)
1604.88	Water O-H Bending	(Kim et al. 2018)
1426.12	CH_2 Scissoring Vibrations	(Kassab et al. 2020)
1315.49	C–C and C-O skeletal vibration	(Vârban et al. 2021)

1156.84	β-1→4 glycosidic linkages	(Yoganandam et al. 2020)
1103.85	C-O stretching and pyranose ring vibrations	(Araújo et al. 2023)
1025.78		

3.2.2. XRD

Figure 2(B) illustrates the XRD spectrum of the cellulose extracted from the *D. quercifolia* rhizome. The high intensity peak at 2θ=23.09°, which corresponds to the (200) crystallographic plane of cellulose I allomorph, confirms the extracted cellulose's semi-crystalline nature. Additionally, the lower intensity peaks were observed around 2θ=16.8° and 34.5°, which can be identified as the (110) and (004) planes, respectively, further proving the presence of cellulose I, the dominant crystalline form present in the native plant cellulose. The sharpness and the intensity of the central peak and the absence of significant amorphous peaks indicate the removal of non-cellulosic components during extraction. Although the crystallinity index was not calculated, the match with the standard ICDD reference pattern (JCPDS No. 00-050-2241) confirms the identity and structural integrity of the extracted cellulose. (French 2014; Santhosh and Umesh 2024)

3.2.3. DSC

The DSC thermogram of the extracted cellulose is seen in **Figure 2**(C). The obtained peak started at 87.34°C with a peak of 117.64°C with an enthalpy of 44.95 J/g. This can indicate the evaporation of bound water and the disruption of the intermolecular hydrogen bonds within the cellulose structure. The absence of multiple transitions suggests a homogenous, purified cellulose phase, with minimal non-cellulosic components. The observed thermal behaviour aligns with the previous findings for native cellulose I, where the endothermic peaks typically

appear between 100-130°C, indicating water loss and partial crystalline reorganization. These results underscore the successful removal of the amorphous non-cellulosic components and reinforce the suitability of the extracted cellulose for further modification. (Cichosz et al. 2019; Norizan et al. 2022)

3.2.4. TGA

The TGA profile of the extracted cellulose is shown in **Figure 2**(D). An initial weight loss was observed between 40°C and approximately 117°C, corresponding to the release of adsorbed and bound moisture and possible residual solvents. This data is consistent with the thermal profiles reported for purified cellulose and lignocellulosic compounds, where an initial 5-10% mass loss below 150°C is attributed to water loss and light volatiles(Oh et al. 2005a) . This event aligns with the endothermic peak observed in the DSC curve at 117.64°C, confirming that the loss corresponds to the physical transition rather than structural decomposition. Beyond this temperature, the TGA curve displayed a non-physical negative weight percentage. Since the instrument was calibrated and functioning correctly, these anomalies are likely due to the sample-specific factors, such as low sample stability during rapid volatilization, volatile compounds, and poor contact or uneven distribution in the crucible. Such behaviour has been reported in studies dealing with natural biomaterials, where sample volatility and uneven thermal conductivity lead to baseline instability(Ionita et al. 2023). Consequently, only the data up to 117°C was considered valid for interpretation. Despite these limitations, the initial thermal behaviour confirms that the cellulose extracted from the rhizome is stable up to ~117°C, supported by both DSC and structural data (FTIR, XRD). This aligns with similar observations in purified cellulosic samples. (Cabrales and Abidi 2019)

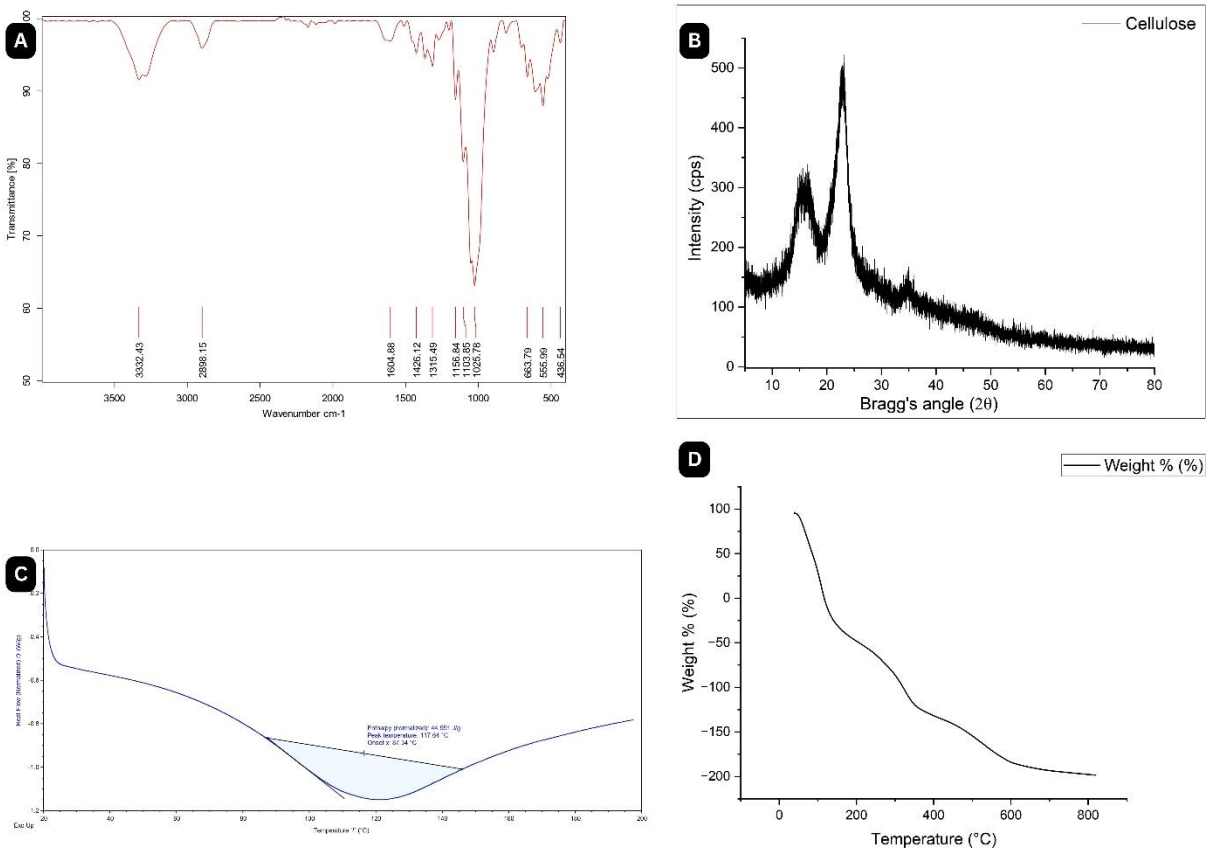
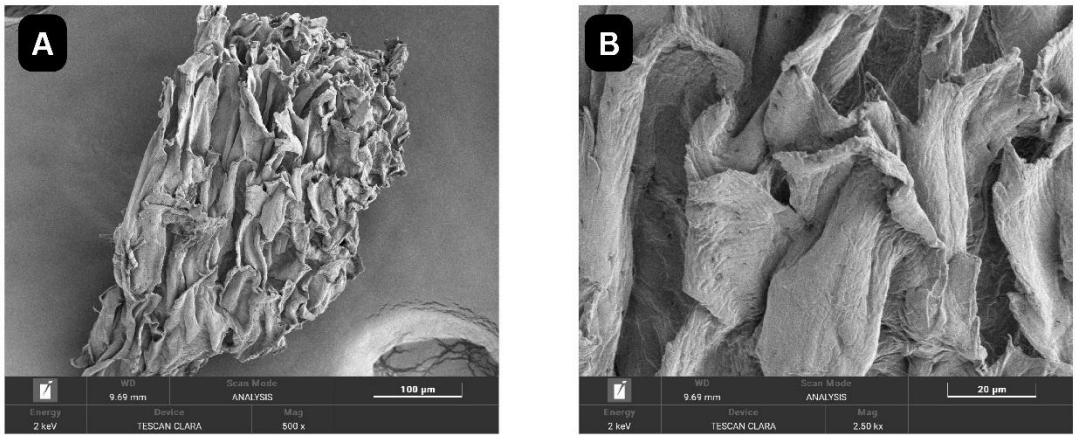


Figure 2 *Combined FITR, XRD, DSC, and TGA characterization of cellulose extracted from Drynaria quercifolia rhizome, indicating its functional groups, crystallinity, and thermal stability*

3.2.5. SEM

The SEM image of the extracted cellulose at 500X and 2500X is shown in **Figure 3**(A) and **Figure 3**(B), respectively. The cellulose has a morphology that resembles an abrasive fiber. The results were similar to those of Balasubramani et al and Sankar et al. (Balasubramani et al. 2024; Santhosh and Umesh 2024). The texture indicates the successful removal of non-cellulosic components through chemical treatments. The structure also confirms the separation of fibril structures that bind the biomass.



370

371 ***Figure 3*** *SEM micrographs of the extracted cellulose at (a)500X and (b) 2500X magnification*

372

373 ## 3.3. Functional Properties of Extracted Cellulose

374

375 **Table 3.** Comparative analysis of functional properties between the extracted cellulose
376 from the rhizome and commercial cellulose powder[1] compares the key functional properties of
377 *D. quercifolia-derived* cellulose and the commercially available cellulose. The extracted
378 material exhibited significantly lower bulk density (0.0819 ± 0.0098 g/mL), showing a lighter,
379 more porous structure. Hydration density was also reduced (0.0387 ± 0.0098 g/mL vs. 0.4000
380 g/mL), highlighting enhanced water uptake capacity. Particle density was also consistent with
381 the lighter matrix (0.1940 ± 0.0485 g/mL vs. 0.5000 g/mL). Conversely, water retention
382 capacity was substantially higher than commercial cellulose (11.0677 ± 1.1099 g/g vs. 2.6710
383 ± 0.2729 g/g). The settling volume, an indicator of colloidal behaviour, was also elevated
384 (25.6667 ± 0.5774 mL/g vs. 4.8333 ± 0.4726 mL/g). These results indicate that *D. quercifolia*
385 cellulose proves to be a substrate that can be used for various applications, including in the

biomedical and food packaging industries. (Antony and thottiam Vasudevan 2018; Sankar Santhosh et al. 2024; Santhosh and Umesh 2024)

Table 3. Comparative analysis of functional properties between the extracted cellulose from the rhizome and commercial cellulose powder[1]

FUNCTIONAL PROPERTY	**COMMERCIAL CELLULOSE**	***DRYNARIA QUERCIFOLIA* CELLULOSE**
BULK DENSITY	0.1663±0.3350 g/mL	0.0819±0.0098 g/mL
PACKED DENSITY	0.5000 g/mL	0.1940±0.0485 g/mL
HYDRATION DENSITY	0.4000 g/mL	0.0387±0.0098 g/mL
WATER RETENTION CAPACITY	2.6710±0.2729 g/g	11.0677±1.1099 g/g
SETTLING VOLUME	4.8333 ± 0.4726 mL/g	25.6667 ± 0.5774 mL/g

[1]Observations are presented as mean ± SD values obtained in triplicate (n=3). Statistical significance was considered at $p < 0.05$

3.4. Extraction of *D. quercifolia* extract

The ethanolic extraction of *Drynaria quercifolia* rhizome yielded a dark orange colored solution, indicating the presence of phenolic and flavonoid-rich compounds typically soluble in 70% ethanol. The initial 250 mL of extract was reduced to 100 mL via evaporation to increase the phytochemical concentration for subsequent film formation. The final concentration was calculated as 17 mg/mL. The clarity of the filtrate through the Whatman No.1 paper indicated minimal residue retention and effective solubilization of bioactives. The color and solubility characteristics are consistent with literature reports of polyphenol-rich fern extracts, often orange to reddish, due to flavones, tannins, or quinones. The extract's

physicochemical profile supports its potential for integration into biopolymeric wound healing systems. (Nurhasnawati et al. 2019; Sankar Santhosh et al. 2024)

3.5. Fabrication of Infused Cellulose Sheet

Continuous stirring and homogenization made the fibrous cellulose from the rhizome into a colloidal solution. (**Figure 4** (A)) The fabricated sheet resembles those of Sankar et al. and Umesh et al. (Umesh et al. 2022)in texture and color. The sheet resembled paper, maintaining its integrity and shape after bending. This characteristic feature makes it valuable for various biomedical applications. (Seddiqi et al. 2021)

3.6. Characterization of the Infused Cellulose Sheet

3.6.1. FTIR

The FTIR analysis of the control and the extract-infused film shows subtle modifications in the graph (**Figure 4** (B)). A more pronounced O-H stretching band at ~3315 cm^{-1} suggests enhanced hydrogen bonding, likely between the cellulose and polyphenolic groups. A new peak at 2043 cm^{-1} indicates altered molecular vibrations. Differences in the fingerprint regions were also observed. C-O stretching peak at 1002 cm^{-1} becomes more curved in the extract-infused film, while a new peak at 885 cm^{-1} and the disappearance of the 905 cm^{-1} peak show extract incorporation. Minor narrowing at 1333 cm^{-1} shows small changes in the cellulose. These spectral shifts show non-covalent interactions between the cellulose and the bioactive molecules. (Liu et al. 2005; Oh et al. 2005b)

3.6.2. SEM

The surface morphology of the control and the extract-infused cellulose films was examined using Scanning Electron Microscopy at 500X and 2500X magnifications. All the samples were prepared using the same casting methodologies. (Reshmy R. et al. 2021) At 500X magnification, the control film exhibited a loosely layered and irregular fibrillar structure, a characteristic feature of the dried cellulose sheet. A sheet appeared with brittle, flake-like domains with visible voids appearing interstitially. At 2500X (**Figure 4** (C)), the surface showed an uneven drying process with relatively porous structures. In contrast, the extract-infused film showed more compactness at 500X (**Figure 4** (D)) with fewer voids and better sheet fusion. The sheets maintained a similar architecture, but less fracturing and tighter packing indicate improved film cohesion, due to the secondary interactions with the extract compounds. At 2500X, the surface showed fewer collapsed zones and less granular structures, which might indicate the localized embedding of the adsorption of the compounds, although no clear particulate aggregates are visible. Both films showed similar architecture, and the extract addition shows enhanced cohesion and reduced surface defects due to better plasticization effects of the compounds present in the extract. The corresponding SEM image of the control film is provided in online reference Figure 2 for reference (El Fawal et al. 2019; Alven et al. 2021)

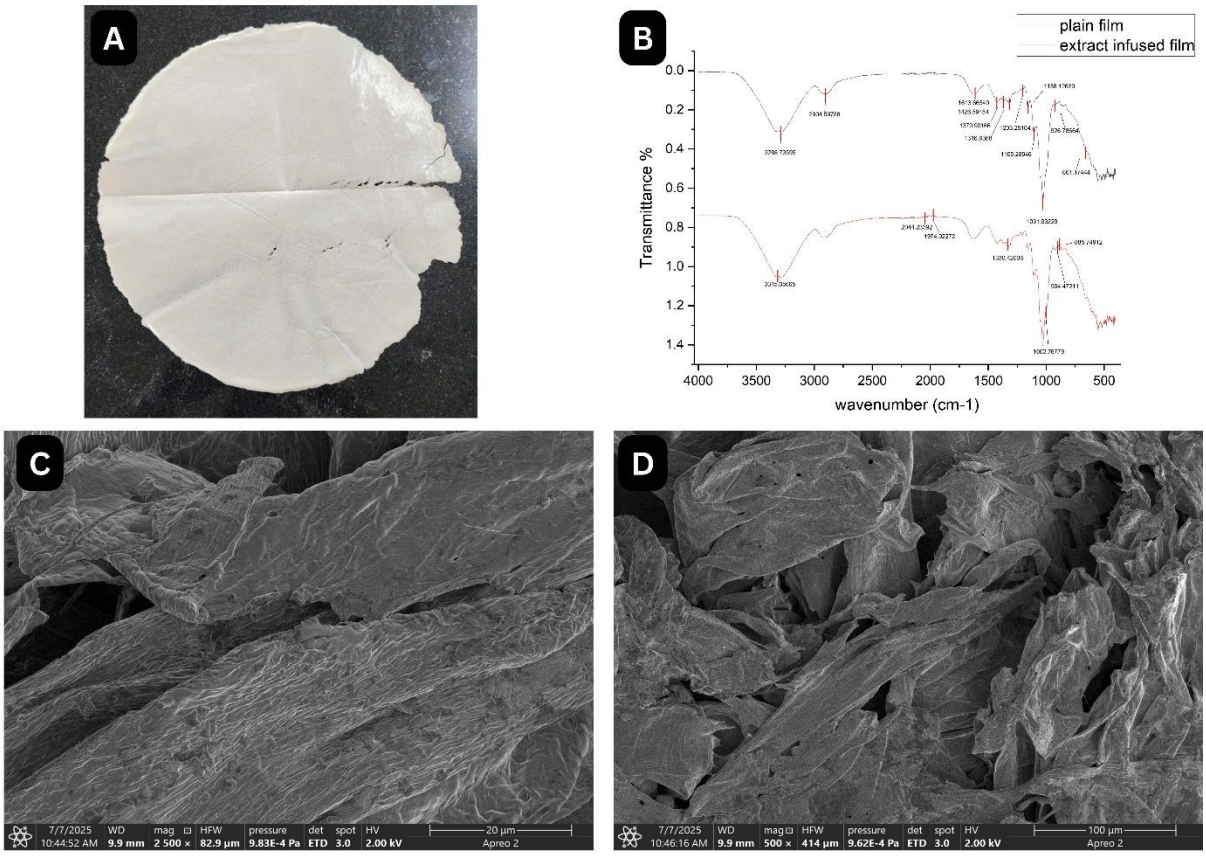
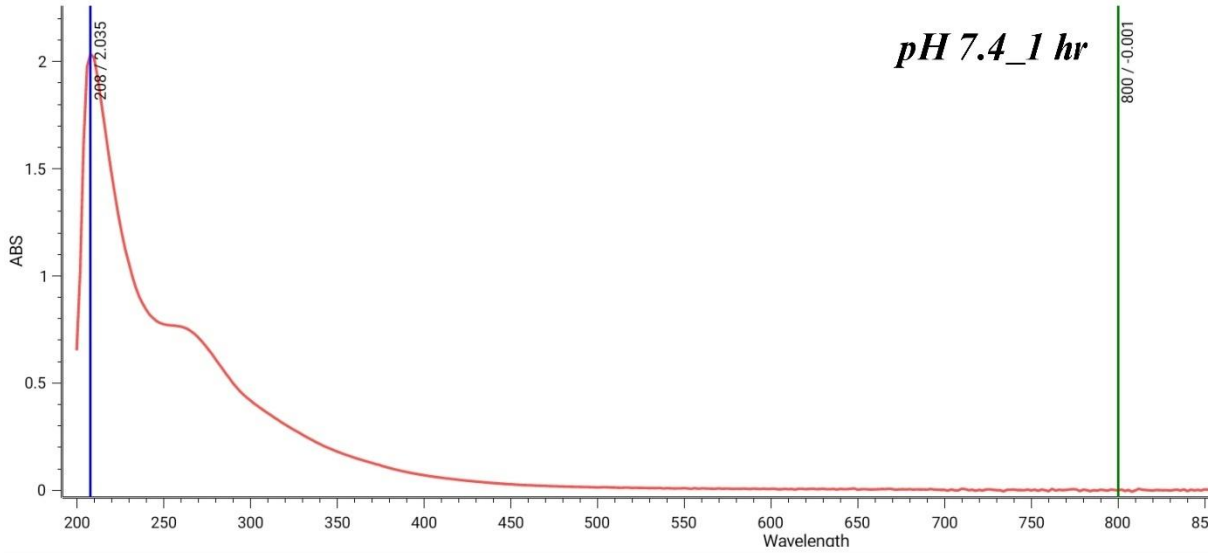


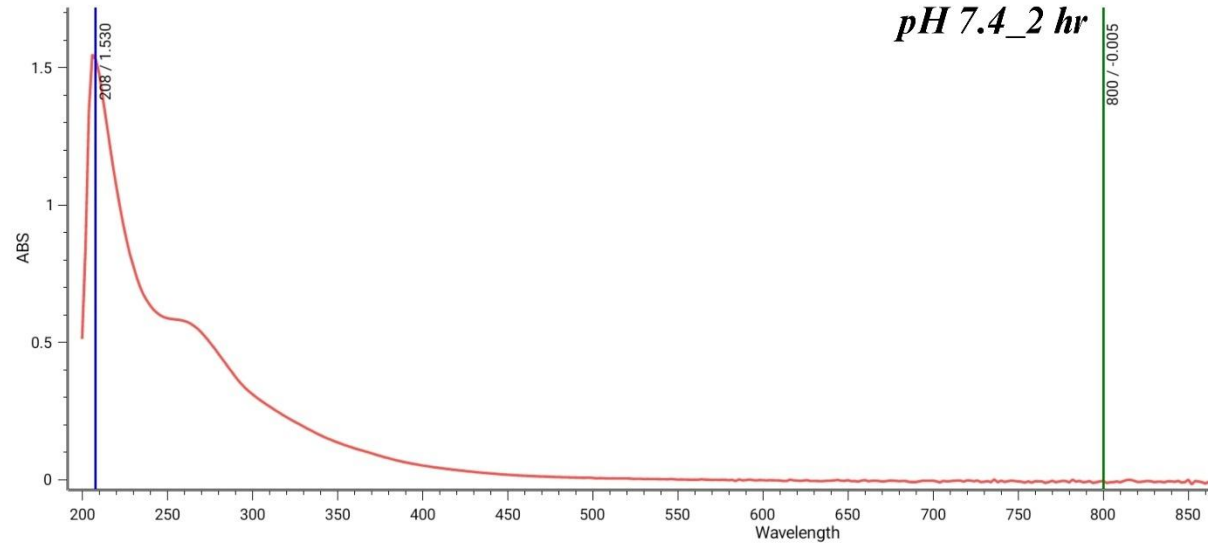
Figure 4 *Characterization of the extract-infused cellulose film: (A) Fabricated film; (B) FTIR spectra of the control and the extract-infused films; SEM images of the extract-infused film at (C) 2500X and (D) 500X magnification*

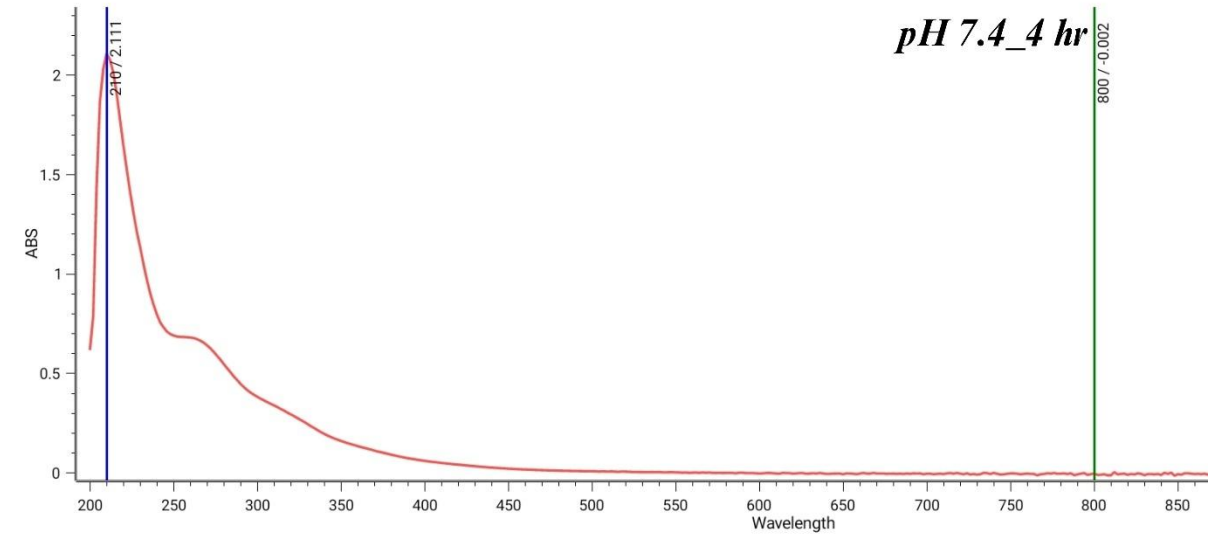
3.7. Release Kinetics Study

To evaluate the release of phytochemicals from the infused film, the samples were incubated for 48 h in PBS (pH 7.4) and analyzed using UV-Visible Spectroscopy in a range from 200-1000 nm. The absorbance was measured at different time intervals (1 h, 2 h, 4 h, 24 h, and 48 h). The release profile at pH 7.4 showed that after 1 h, the absorbance was 2.03, which dropped to 1.53 at 2 h. Then, at 4 h and 24 h, the absorbance increased to 2.11 and 2.18, respectively. Later at 48 h, the absorbance declined to 1.61, which exhibits a plateau-like trend, which is a characteristic feature for controlled and gradual diffusion of phytochemicals, beneficial in applications like localized therapeutic delivery.

The clear supernatants and persistent UV absorbance peaks matched those of the original ethanolic extract, confirming molecular-level diffusion rather than film breakdown. Comparable pH-dependent release patterns have been observed in flavonoid-loaded biopolymer matrices, where optimum release occurs near physiological pH due to favorable matrix swelling and compound stability. Quantitatively, ~2.17 absorbance at physiological PBS suggests sustained release, aligning with benchmarks of flavonoid-loaded cellulose films designed for wound healing. The reduced release under acidic conditions destabilizes certain phytochemicals important for designing pH-responsive dressings. Overall, the extract-loaded film demonstrates stable and meaningful release, reinforcing the potential of a long-acting bioactive wound dressing (Sharma et al. 2022; Pecorini et al. 2023; Ponjavic et al. 2023; Chalitangkoon et al. 2025) **Figure 5**(a,b)


pH 7.4_1 hr
208 / 2.035
800 / -0.001
ABS
Wavelength


pH 7.4_2 hr
208 / 1.530
800 / -0.005
ABS
Wavelength


pH 7.4_4 hr
210 / 2.111
800 / -0.002
ABS
Wavelength

476

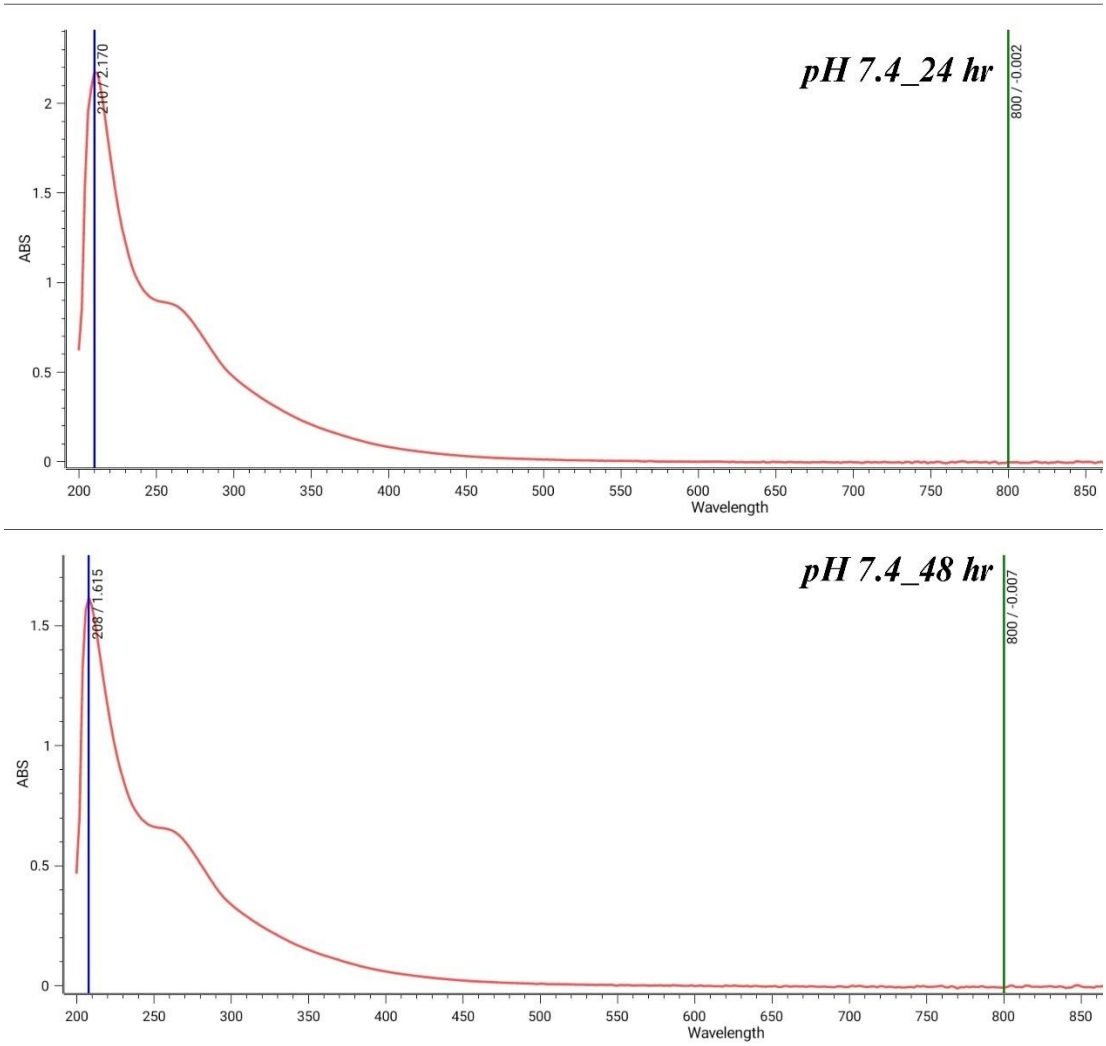


Figure 5(a,b) *UV-Vis Graph for the release profile of the infused cellulose film in PBS at pH 7.4 at various time intervals*

3.8. Phytochemical Analysis

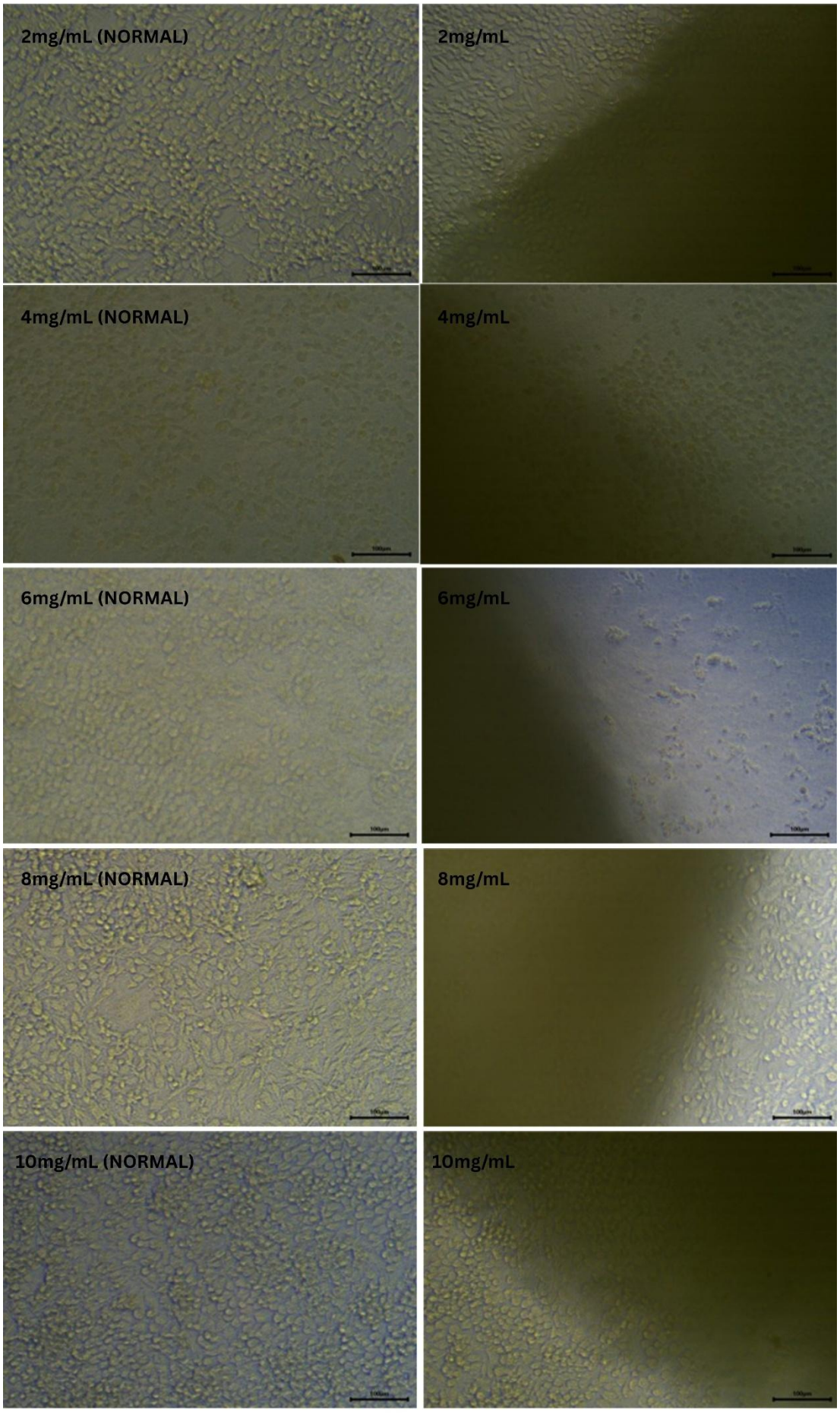
The qualitative analysis of the 24 h at pH 7.4 PBS release medium showed positive detection for alkaloids (Mayer's test), flavonoids (lead acetate test), glycosides (Keller-Killiani), and carbohydrates (Molisch test). In contrast, the tests for proteins, saponins, terpenoids, steroids, quinones, and phenols were negative. The presence of alkaloids and flavonoids aligns with existing profiles of *D. quercifolia*, where methanolic extracts consistently showed alkaloid, glycoside, flavonoid, and carbohydrate content among other

constituents. The flavonoids were leached into the release medium, consistent with prior findings with leaf and rhizome extracts of *D. quercifolia*, exhibiting high flavonoid levels and abundant phenolics. The absence of phenolics, steroids, and terpenoids suggests selective diffusion or degradation in water-based release media. The specific release of alkaloids, flavonoids, glycosides, and sugar compounds supports the hypothesis that the cellulose matrix favors the diffusion of moderately polar, low molecular weight phytochemicals, which supports it as a desirable trait for controlled biologically active applications.(Runa et al. 2013; Udayabhanu et al. 2014; Ahmed et al. 2015)

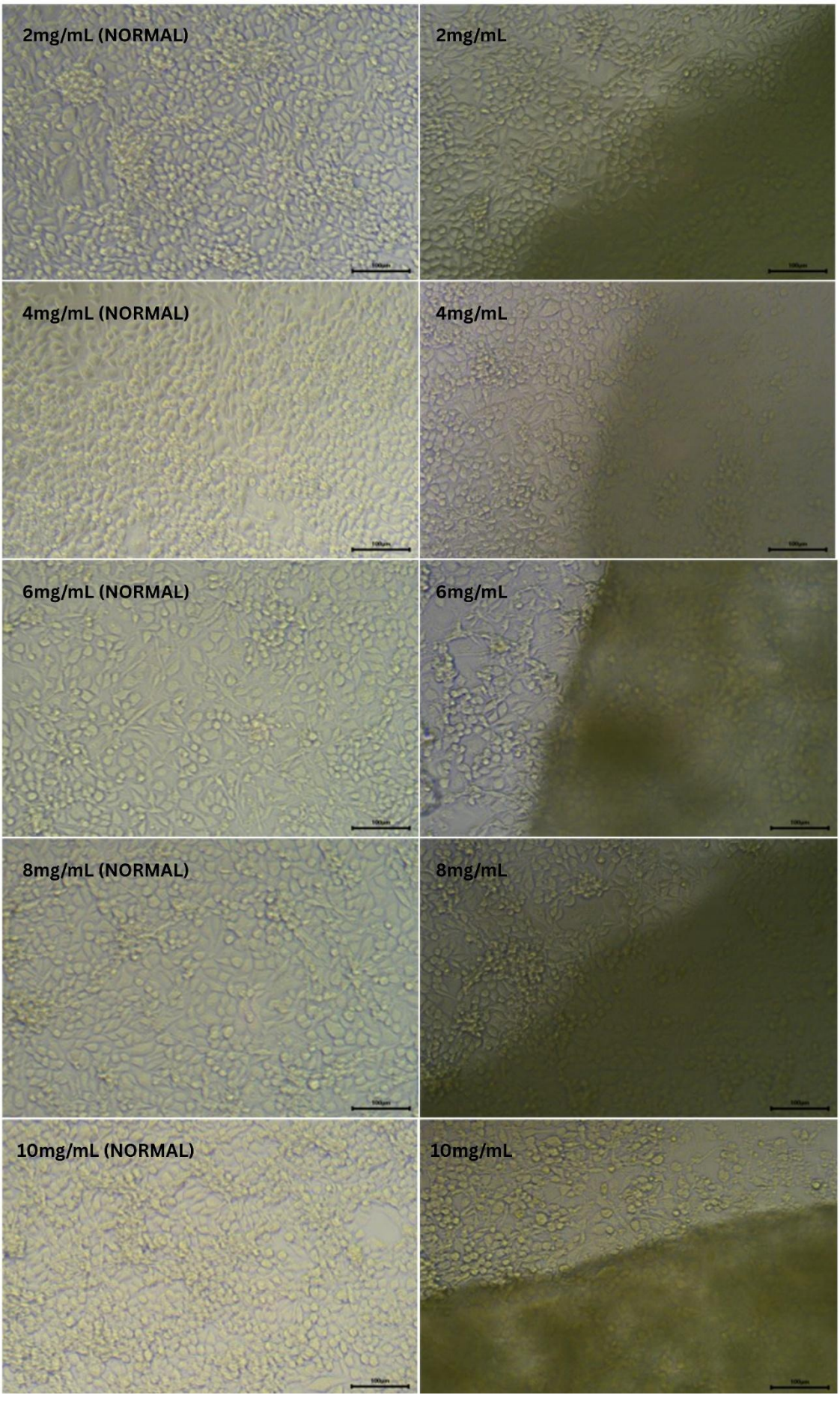
3.9.*In vitro* analysis

3.9.1. Morphological Analysis

After 24 h exposure to the control and *D. quercifolia* extract-infused cellulose films, the cells exhibited similar morphology across conditions. Cells maintained a round to slightly polygonal shape with no elongation, cytoplasmic spreading, membrane blebbing, or cellular debris. These observations suggest that the tested concentrations (2-10 mg/mL) do not induce cytotoxic or activation-related morphological changes. The morphology aligns with the previous reports showing that the extract possesses anti-inflammatory activity without eliciting cellular stress or cytotoxic responses. The absence of toxic morphology in the study reinforces the extract's cytocompatibility, an essential criterion for biomedical applications. However, further functional assays (e.g., cytokine expression and nitric oxide modulation) would be necessary to detect potential biochemical or transcriptional changes. The morphology is shown in **Figure 6** below. (Modak et al. 2021; Jegan et al. 2025)


2mg/mL (NORMAL)
2mg/mL
4mg/mL (NORMAL)
4mg/mL
6mg/mL (NORMAL)
6mg/mL
8mg/mL (NORMAL)
8mg/mL
10mg/mL (NORMAL)
10mg/mL

513


2mg/mL (NORMAL)
2mg/mL
4mg/mL (NORMAL)
4mg/mL
6mg/mL (NORMAL)
6mg/mL
8mg/mL (NORMAL)
8mg/mL
10mg/mL (NORMAL)
10mg/mL

514

Figure 6 *Morphological Analysis of RAW264.7 cells in the presence of (a) control film and (b) extract-infused cellulose film*

3.9.2. MTT Assay

The cytotoxic potential of the extract-free (control) and the extract-loaded cellulose films was evaluated using the EZcount^{TM} MTT Cell Assay Kit (HIMEDIA labs) on RAW 264.7 macrophages. Due to unexpected microbial contamination of the cell culture facility, the assay was conducted in a single replicate, and the graphical representation was omitted. However, the obtained percentage of viability values provides preliminary insight into the fabricated films' biocompatibility.

For the extract-free films, the cell viability ranged from 55.15% (2 mg/mL) to 91.91% (4 mg/mL), indicating a concentration-dependent increase in the cell viability. The extract-loaded films demonstrated consistently higher viability, ranging from 73.71% to 81.44%. This suggests the cytoprotective effect of the incorporated *D. quercifolia* phytoconstituents. The results imply that the plant extract-infused film does not elicit cytotoxic effects and may promote cellular metabolic activity at lower concentrations. The viability can be due to the flavonoids and glycosides reported to produce antioxidative and anti-inflammatory cellular effects. While the preliminary values support the non-toxic effects, further validation through repeated experiments is essential to confirm the statistical validity and eliminate variability due to uncontrollable experimental failures. (Padhy et al. 2017; Modak et al. 2021; Jegan et al. 2025)

3.9.3. Cell Migration Assay

Cell migration was assessed qualitatively using a scratch assay model following 24 h exposure to extract-loaded and control cellulose films at 1-3 mg/mL concentrations. Due to the film's physical placement, precise imaging was partially obstructed under the microscope, limiting the ability to perform accurate quantitative measurements of scratch closure. However, the presence of cells and their morphology were observed in the previously scratched region after 24 h, suggesting active migration across the scratched gap. This qualitative evidence supports the notion that the infused films do not inhibit the macrophage mobility, but support it. Although exact chemotactic pathways were not measured due to the assay's limitations, the visible migration suggests that the films are biocompatible and do not serve as physical barriers to macrophage migration, which is critical for biomaterial evaluation in wound contact applications. Together with the control film and the extract-infused film, **Figure 7** shows the scratched area and the cell-migrated area under the concentration of 2mg/mL. (Anuja et al. 2014; Noda et al. 2022)

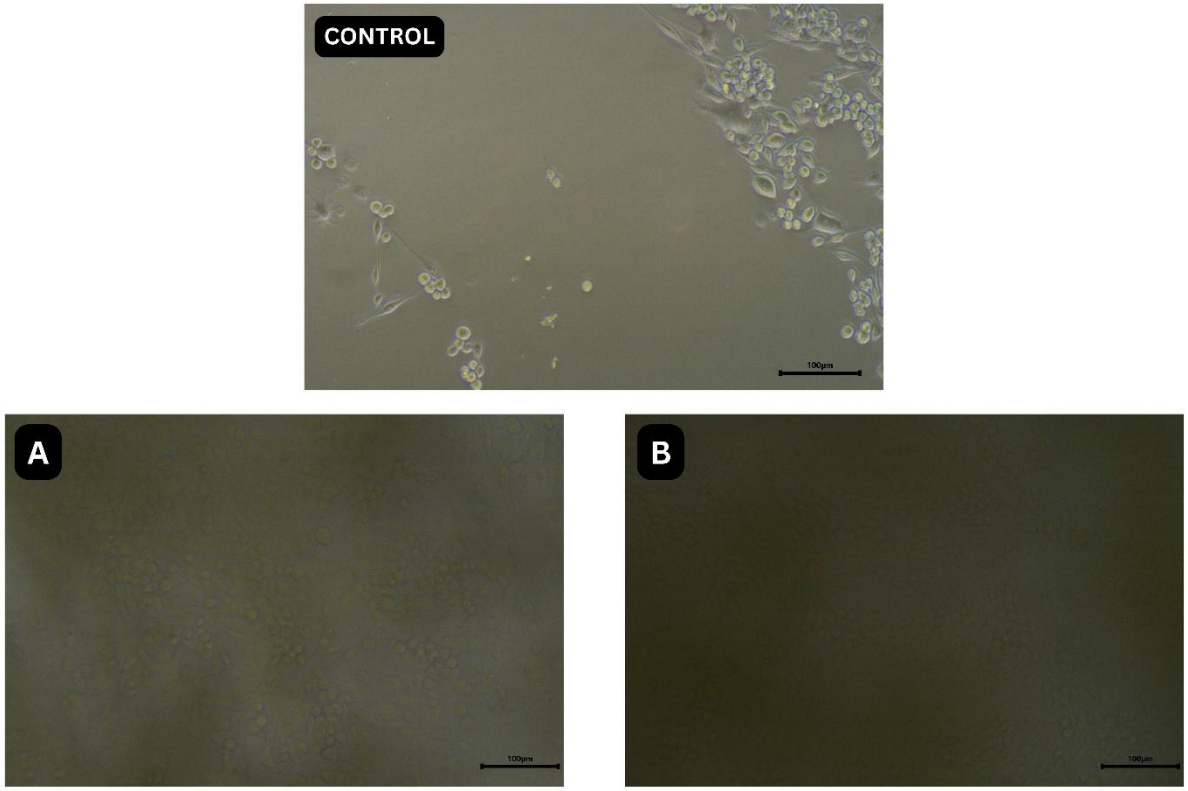


Figure 7 *Cell migration with the (A) plain cellulose sheet and (B) extract-infused film on the RAW264.7 macrophage cell line under the concentration of 2mg/mL*

4. Conclusion

The cellulose extraction from the rhizome of *Drynaria quercifolia* was performed through the alkaline peroxide treatment using NaOH and H_2O_2. The extraction yielded 27.20 ± 4.65% (%w/w), which was then subjected to various physicochemical, functional, and biological characterization. The functional property assessment shows good water retention capacity and settling volume compared to commercial cellulose, indicating its suitability for hydration and suspension stability applications.

Analytical characterization, like FTIR, SEM, XRD, TGA, and DSC, confirmed the presence of crystalline cellulose I, indicating successful isolation without non-cellulosic components. The infused cellulose film exhibits cytocompatibility with RAW 264.7

macrophages, maintaining cell viability above 70% across concentrations. Cell morphology remains unaffected, and qualitative cell migration assay observations confirmed the presence of migrating cells in the scratched gap. The film also exhibited sustained release of phytochemicals at physiological pH, with UV-Vis absorbance and qualitative phytochemical screening confirming the presence and diffusion of alkaloids, flavonoids, glycosides, and carbohydrates into the release media.

The study also highlights the potential of *D. quercifolia* rhizome as a source of biocompatible cellulose, capable of forming an extract-infused film with potential for biomedical applicability. Future studies should focus on optimizing release kinetics, performing in vivo wound healing models, and exploring pharma and cosmeceutical applications.

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5. Declarations

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Competing Interest

The authors declare no competing interests

Author Contributions

Zeno Vimalan A: Conceptualization, Methodology, Writing-Original Draft; **Arun K. B:** Conceptualization, Project administration, Supervision, Writing- Reviewing and Editing; **Soumya R. S**: Methodology and Supervision

Ethical Approval

This research work does not involve human and/or animal studies; thus, ethical approval is not applicable.

Data availability

The original data related to this research will be available to the authors on special request.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.docx
- OnlineReferenceFigure1.jpg
- OnlineReferenceFigure2.jpg