Italian Journal of Food Science, 2024; 36 (4): 17–25

Extraction and characterization of cellulose from agricultural waste of hemp (*Cannabis sativa)* and parthenium (*Parthenium hysterophorus)*

Muhammad Usman[1], Afia Zia[1]*, Muhammad Nauman Ahmad[1], Sahib Alam[1], Niamat Ullah[2], Muhammad Baseer Us Salam[1], Tariq Aziz[3,4]*, Majid Alhomrani[5], Walaa F. Alsanie[5], Abdulhakeem S. Alamri[5]

[1]Department of Agricultural Chemistry and Biochemistry, The University of Agriculture, Peshawar, Pakistan; [2]Department of Human Nutrition, University of Agriculture Peshawar; [3]Institute of Molecular Biology and Biotechnology, The University of Lahore, Punjab, Pakistan; [4]Laboratory of Animal Health Food Hygiene and Quality, University of Ioannina, Arta, Greece; [5]Department of Clinical Laboratory Sciences, The Faculty of Applied Medical Sciences, Taif University, Taif, Saudi Arabia

***Corresponding Authors:** Afia Zia, Department of Agricultural Chemistry and Biochemistry, The University of Agriculture, Peshawar, Pakistan. Email: afia.zia@aup.edu.pk; Tariq Aziz, Institute of Molecular Biology and Biotechnology, The University of Lahore, Punjab, Pakistan; Laboratory of Animal Health Food Hygiene and Quality, University of Ioannina, Arta, Greece. Email: tariqckd@uoi.gr

Received: 16 June 2024; Accepted: 23 July 2024; Published: 26 September 2024

ORIGINAL ARTICLE

Abstract

The current study focused on the extraction of cellulose from two selected plants, hemp (*Cannabis sativa*) and parthenium (*Parthenium hysterophorus)*. The research successfully isolated high-purity cellulose from both plants using a chlorination and alkaline extraction process. A higher yield (%) (38.4 ± 0.18) was obtained from hemp compared to parthenium (22 ± 0.82). Characterization techniques were used to probe the structure and properties of the extracted cellulose. Fourier transform infrared spectroscopy analysis revealed functional groups characteristic of cellulose, while X-ray diffraction confirmed its highly crystalline structure in both samples. Scanning electron microscopy provided valuable insights into the cellulose morphology, indicating a smoother surface and reduced fiber diameter after treatment due to the removal of noncellulosic components. The research paved the way for the development of eco-friendly bioproducts utilizing cellulose from hemp and parthenium, promoting a more sustainable future.

Keywords: circular economy; eco-friendly bioproducts; hemp's cellulose; parthenium's cellulose

Introduction

One of the Earth's most abundant renewable resources is the lignocellulosic biomass. Annually, an estimated 200 billion tons of this material are generated globally from sources like forestry residues, crops, and agricultural waste streams (Aziz *et al*., 2023; Khan *et al*., 2021; McKendry, 2002; Sanchez and Cardona, 2008). Natural products are becoming increasingly popular as a priority research area globally, driven by their potential as sustainable sources of fuel, energy, and various value-added products. Biofuels, in particular, are recognized as a highly promising alternative in the contemporary world. This has spurred an intensive global research effort focused on identifying efficient chemical conversion methods to transform biomass into valuable products. The ultimate goal is to develop economically viable processes that can be implemented on a commercial scale to fight climate change and develop sustainable agricultural products (Boutheina *et al*., 2022; Naik *et al*., 2010).

Lignocellulosic biomass is a complex bio-composite comprised primarily of cellulose, hemicellulose, and lignin, along with other minor components. Notably, cellulose is

the most abundant renewable organic material on Earth. It is ubiquitous in higher plants and found to a lesser extent in various marine animals, algae, fungi, bacteria, invertebrates, and even amoeba. Chemically, cellulose is a polysaccharide, a type of molecule consisting of β-1,4-linked D-glucose units. Due to its inherent properties, cellulose holds immense promise as a sustainable feedstock for the production of various valuable chemicals, including cellulosic ethanol, hydrocarbons, and precursors for polymers (Cheng *et al*., 2024; Das *et al*., 2016; Kim *et al*., 2024).

Driven by the growing need for environmental protection and the development of sustainable societies, industries and government organizations are actively pursuing the exploration of natural resources. Their focus lies in creating novel, environmentally friendly, bio-based, and degradable materials for diverse engineering applications. (Chandrahasa *et al.*, 2014; Fortunati *et al*., 2012; Johar *et al*., 2012; Nasreen and Ashraf, 2020; Zameer *et al*., 2023). Among these promising biopolymers readily available in nature are cellulose, chitosan, collagen, and soy protein isolates (Lewandowska, 2017). These materials share the valuable properties of being biodegradable, environmentally compatible, and nontoxic. Cellulose emerges as the most attractive biopolymer among this group due to its exceptional abundance, biodegradability, and renewability (Rouf and Kokini, 2018; Saba *et al.*, 2014; Sakthivel and Ramesh, 2013). On a global scale, cellulose production is estimated to reach approximately 1.5 × 10^11 tons annually, with plant fibers being the primary source (Rouf and Kokini, 2018; Saba *et al*., 2014; Sakthivel and Ramesh, 2013). Notably, cellulose is the principal crystalline component of plant cell walls, while hemicellulose and lignin are amorphous constituents. The presence of cellulose is responsible for the high modulus and strength of these fibers (Chakraborty *et al*., 2013; Han *et al*., 2014; Jabbar *et al*., 2017; Shi *et al*., 2024; Wang *et al*., 2009). The percentage composition of cellulose varies significantly across different plant waste materials, including banana, ramie, jute, hemp, sugarcane bagasse, and bamboo (De France *et al*., 2017).

Hemp (*Cannabis sativa*), a versatile lignocellulosic crop with a long history, has traditionally been cultivated for its bast fibers and seeds. However, the remaining stalk, often discarded as waste, holds significant potential (Fike, 2016; Manaia *et al*., 2019). Recent research has focused on unlocking the value of this previously underutilized resource. The hemp stalk boasts a complex structure. The outer bark (epidermis) encloses a core divided into two distinct regions: the bast fibers (outer layer) and the woody inner core (hurd). Notably, hemp stalks are composed of roughly 65% hurd and 35% bast fibers (Stevulova and Schwarzova, 2014). The chemical makeup of hemp fibers is equally intricate, containing varying amounts of cellulose, hemicellulose, lignin, and other cell wall components (waxes, pectins, and minerals). These elements significantly influence the extracted fibers' properties. On average, hemp fiber (combining bast and hurd) contains 55% cellulose, 16% hemicellulose, 18% pectic substances, and 4% lignin (Rehman *et al*., 2013). However, significant variations exist within the stalk. Bast fibers boast higher cellulose content (57–77%) and lower lignin content (5–9%) compared to the hurd (40–48% cellulose, 21–24% lignin). Additionally, the hurd has a higher hemicellulose content (18–24%) than the bast fibers (Areeba *et al*., 2024; Asma *et al*., 2024; Rehman *et al*., 2021). Studies indicate that *Parthenium hysterophorus*, a fast-growing weed, is a promising source for the development of eco-friendly materials. This plant holds a significant amount of cellulose (78%), a key component in biodegradable products (Naithani *et al*., 2008; Varshney *et al*., 2011).

Cellulose, the most abundant polysaccharide on Earth, is a naturally occurring polymer with a wide range of applications (Harini *et al*., 2018; Kono *et al*., 2003; Nishino *et al*., 2004). Its structure consists of long chains of sugar molecules (β-D-glucopyranose units) linked together by specific 1-4 glycosidic bonds) (Kono *et al*., 2003; Nishino *et al*., 2004). Compared to other natural polymers such as starch and protein, cellulose offers several advantages and has been a popular choice in biomass utilization for over 150 years due to its versatility (Harini *et al*., 2018; Reddy *et al*., 2016). Plants such as *Parthenium heterophoria* demonstrate a particularly high content of cellulose, making them ideal candidates for biomaterial production (Eichhorn *et al*., 2010; Azizi Samir *et al*., 2005). In addition to cellulose, plant cell walls also contain hemicelluloses and lignin. While cellulose is semi-crystalline, these other components are amorphous (Sheltami *et al*., 2012). This study investigates the extraction and characterization of cellulose from the aerial parts of hemp (*C. sativa*) and *P. hysterophorus* to assess their suitability for biomaterial production.

Materials and Methods

Samples of hemp and parthenium were collected from the fresh fields of Tirah Valley after reaching the harvesting stage. The samples were brought to the Laboratory of the Department of Agricultural Chemistry and Biochemistry, University of Agriculture, Peshawar, where all the necessary analyses were made.

To remove surface impurities, the hemp and parthenium samples were subjected to repeated washes with distilled water to remove all the dirt. After washing with water, the desired aerial part of the plant was kept in a hot air oven at 50°C, and the weight of the samples was recorded at an interval of 30 minutes. Following this step, the desired

aerial parts for both samples were crushed using mortar and pestle followed by an electric blender. The fine powder was obtained with a nominal mesh aperture of 250 µm. The dried powder was referred to as "Hemp" and "Parthenium" biomass.

To achieve all the key objectives, the following steps were carried out:

Cellulose extraction from hemp and parthenium

To extract the biomass from samples for cellulose extraction, the procedure by Bian *et al.* (2012) and Nigam *et al*. (2021) was followed. Samples were subjected to chlorination and alkaline extraction method. In the first step, 10 g of samples from each plant were taken and impurities like waxes and pectin were removed using a Soxhlet apparatus with a solvent mixture of toluene and ethanol for 6 h. The resulting dewaxed powder was then subjected to filtration followed by washing with distilled water and ethanol, and later, dried in an oven at 50°C until its weight remained constant. Five grams of each sample were then taken for hemicellulose removal through treatment with 3% sulfuric acid at 90°C for 2 h. After cooling and filtering, the remaining solid residues were washed and dried. The samples were then delignified with sodium hypochlorite solution at 90°C for 2 h, followed by sodium bisulfite solution at 30°C for 1 h. This process yielded holo-cellulose, a mixture of cellulose and residual hemicelluloses. The holo-cellulose was then filtered, washed, and dried again. Finally, to obtain pure cellulose, the holo-cellulose was then treated with sodium hydroxide solution at 90°C for 2 h. The purified cellulose was thoroughly washed and dried at 60°C.

Determination of cellulose qualitatively

To confirm the presence of cellulose, hemicellulose, and lignins, the residues were subjected to standard qualitative tests, that is, Phloroglucinol Test (Davidson *et al*., 1995), Bial's test (Sumner, 1923) and Wiesner Test (Nakano and Meshitsuka,1992), respectively.

Phloroglucinol test

One gram of sample was taken to which 1% of phloroglucinol solution was added. A drop of concentrated sulfuric acid was added to phloroglucinol, and the solution was carefully added to the sample. A red coloration indicated a positive result, while no or faint yellow color indicated a negative result.

Bial's test

One gram of sample was taken in a test tube followed by adding and mixing the sample with a few drops of Bial's reagent (prepared by dissolving 1.25 g of orcinol in 100 mL of ethanol and 50 mL of concentrated hydrochloric acid). After this, the test tube was heated. The formation of a greenish-blue color indicated positive result, while yellow to slightly greenish-yellow color indicated negative results.

Wiesner test

One gram of sample was taken followed by moistening the sample with phloroglucinol solution in hydrochloric acid (prepared by dissolving 1 g of phloroglucinol in 100 mL of concentrated hydrochloric acid). The red color indicated positive results, while yellow to a faint yellow color indicated negative results.

Estimation of cellulose in hemp and parthenium

After the confirmation of cellulose in both samples, the percentage of cellulose was calculated as per the below calculation:

$$\%\,\text{Cellulose Estimation} = \frac{\text{Final Weight of the Plant Residues Biomass}}{\text{Initial Weight of Plant Sample Biomass}} \times 100 \quad (1)$$

Characterization of cellulose

The extracted cellulose from both samples was subjected to the following analysis.

Fourier Transmission Infrared (FTIR)

FT-IR spectroscopy analysis was used to determine the functional groups in cellulose.

FTIR Model Cary630 (Agilent Technologies, USA) was used for all the analyses recorded in the region from 4000 to 500 cm^{-1}.

X-ray diffraction (XRD)

The crystalline structure of the extracted cellulose from both samples was analyzed using a high-resolution X-ray diffractometer (Model: JDX-3532, JEOL, Japan) at the facility available at Centralized Resource Laboratory (CRL), the University of Peshawar, Pakistan, after which the data was analyzed further using software such as PANalytical, X'Pert HighScore, and Origin24. The crystalline particle size (µm) of cellulose was determined from X-ray diffraction curves based on the Scherrer equation (Eq. 1), while the equation of the Segal Method (Segal *et al*., 1959) was used for determining the crystalline index (%) as given in Equation 2.

$$D(\text{nm}) = \frac{K\lambda}{\beta \cos\vartheta} \quad (2)$$

where, D is the crystalline particle size (nm), K = 0.9 (Scherrer constant), λ = 0.15406 nm, β = FWHM (radians), ϑ = Peak position (radians)

$$\text{Crystallinity Index } (\%) = \frac{II_{002} - I_{AM}}{I_{002}} \times 100 \qquad (3)$$

where, I_{002} is the peak height at 22.4 (2θ) and I_{AM} is the peak height of amorphous cellulose to 19.5 (2θ).

Scanning Electron Microscopy

Surface morphological characteristics of the samples were studied using a Scanning Electron Microscope (Model JSM-IT-100) at the facility at the National Center of Excellence in Geology, Peshawar. The instrument used was SEM-EDS JEOL JSM-6360LA, Japan.

Results and Conclusions

Cellulose extraction

Crude cellulose extracted from each sample was subjected to confirmatory tests. The results were positive for cellulose while negative for hemicellulose and lignin. Hence, all further analyses were based on the derived crude cellulose from the samples. Additionally, the yield estimation for the hemp sample was observed as 38.4%, while it was 22% for parthenium, as shown in Table 1.

The current study aligns with previous research highlighting their potential as sustainable sources of this valuable biomaterial. The studies by Shubhaneel *et al*. (2013) and Singh *et al*. (2014) have reported the cellulose content in parthenium to be around 28%. However, it is crucial to acknowledge potential variations within this range. Seasonal variations in Pakistan, for instance, can significantly impact the biochemical composition of plant samples, including cellulose content. Similar observations have been documented for hemp by Tutt and Olt. (2013), where cellulose content fluctuated based on the plant's maturity stage and harvesting season. This underscores the importance of considering Pakistani-specific varieties and their responses to the country's diverse climatic conditions.

Fourier Transform Infrared (FTIR) of cellulose samples

The structural and physicochemical properties of the extracted celluloses were studied under FTIR, and the findings are shown in Figure 1.

FTIR results for cellulose extracted from both samples revealed a prominent peak around 3400 cm^{-1} in all samples, which signifies the presence of hydroxyl (O-H) groups, a hallmark of polysaccharides. Similarly, the C-H stretching vibrations appeared at 2900 cm^{-1}. This indicated stable organic components of the samples. Interestingly, small peaks at 1742 cm^{-1} and 1510 cm^{-1} were observed, which suggests either the presence of minute impurities from hemicellulose and lignin, respectively or the formation of bonds at the time of chemical treatment at the time of extraction of cellulose from the samples. Furthermore, minute peaks at 1636–1612 cm^{-1} signify O-H bending vibrations, likely due to moisture absorption in all samples. The peaks at 1058 cm^{-1} and 897 cm^{-1} point toward C-O-C and C-O stretching within the β-glycosidic linkages, the building blocks of cellulose. The significant peaks in the 1200–890 cm^{-1} range for cellulose samples further confirm the enrichment of cellulose content after the chemical treatment. The current findings were compared and were in line with the findings of Avolio *et al*. (2012), Alemdar *et al*. (2008), Rashid *et al*. (2020), and Romruen *et al*. (2022).

XRD analysis

The XRD spectra of crude cellulose extracted from both hemp and parthenium are presented in Figure 2. The mean calculated crystallinity participle size is shown in Table 2. The Average Crystallinity Index* for hemp and parthenium was 73.27 nm and 58.61 nm, respectively (Table 2). Furthermore, the crystallinity index (%) was calculated using the Segal method, which resulted in 87.70 and 82.83 % for hemp and parthenium, respectively. These high CI values indicate a highly crystalline structure for the cellulose in both samples. The XRD pattern for hemp exhibited sharp diffraction peaks at 2θ = 17° (d-spacing: 5.180) and 23.02° (d-spacing, 3.45), in line with the characteristics of cellulose (I). Similarly, parthenium showed major peaks at 2θ = 24.84° (d-spacing: 3.58) and 26.63° (d-spacing: 3.34), all corresponding to the crystallographic structure of cellulose (I). These narrow

Table 1. Sample weight analysis for the selected samples.

Sample name	Initial weight	Final weight	Difference	%
Hemp	10	3.84 ± 0.01	6.16 ± 0.02	38.4 ± 0.18[a]
Parthenium	10	2.2 ± 0.08	7.8 ± 0.08	22 ± 0.82[b]

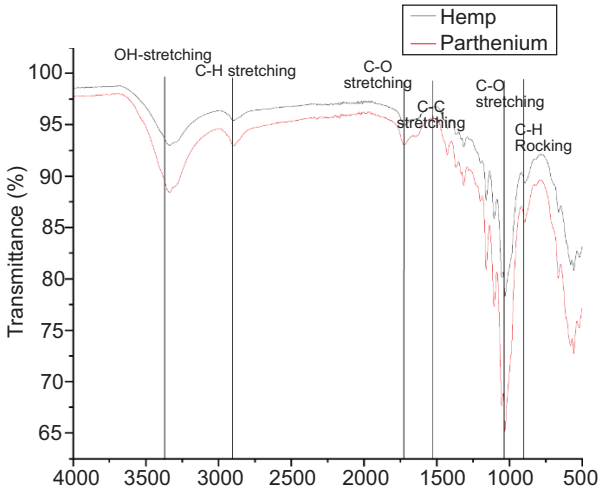

Figure 1. FTIR spectra of cellulose extracted from selected plants.

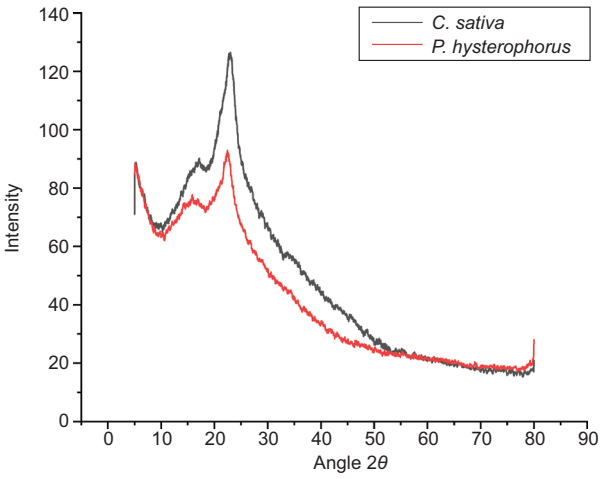

Figure 2. XRD peaks for both samples (black line: Hemp, red line: Parthenium).

peaks, as evidenced by their FWHM (Full Width at Half Max) values, shown in Table 2, suggest a relatively large crystallite size of cellulose in both hemp and parthenium. This aligns with the findings of previous studies, where extracted cellulose from various agricultural waste materials displayed high crystallinity (Chi *et al*., 2019; Perumal *et al*., 2018; Romruen *et al*., 2022). In XRD, FWHM (Full Width at Half Max) is a measure of a peak's width in the diffraction pattern. It tells about the crystallite size and imperfections in the material. A wider peak (larger FWHM) suggests smaller crystallites or more strain/defects and vice versa. It is a key parameter to understand a material's crystal structure and quality. The numbers/values shown in Table 2 were machine-generated. XRD data has variability due to sample preparation and instrument fluctuations, making statistical interpretations (P-values, confidence intervals) less reliable. Hence, statistical analyses on XRD data are not performed as per SoP.

Where, Pos. [°2Th.] refers to the angle at which the X-rays diffract after interacting with the crystal planes; Left FWHM (Full Width at Half Maximum) of a peak in the XRD pattern specify the left-hand side of the peak's maximum intensity; Crystalline size (nm) refers to the size of the coherent crystalline domains within a material; d spacing (Å) refers to the distance between adjacent parallel planes of atoms within a crystal structure; and Rel. intensity (%) represents the intensity of an XRD peak compared to the strongest peak in the pattern.

Surface morphology

The morphology of the samples was examined using Scanning Electron Microscopy (SEM). Figure 3 illustrates the SEM of the extracted cellulose from both samples. The alkaline treatment effectively removes noncellulosic components such as natural fats, pectin, waxes, and lignin, consistent with the findings of Kabir *et al*. (2013). The mechano-chemical treatment resulted in a more irregular structure, indicating that the treatments disrupted the orientation of microfibrils. This observation suggests that the surface of hemp and Parthenium fibers became smoother after treatment due to the removal of noncellulosic materials and impurities. Consequently, there was a noticeable reduction in the fiber diameter, which aligned with the results reported by Obi Reddy *et al*. (2012). The process generated microfibrillated cellulose, comprising aggregates of cellulose nanofibers with both amorphous and crystalline regions. However, the alkaline ions can cause undesirable reactions, potentially leading to the cleavage of cellulose chains and loss of interfibrillar morphology. Notably, the dimensions of fibers ranged from 120 to 132 nm.

The studies by Terinte *et al*. (2011), Ju *et al*. (2015), and Gong *et al*. (2017) also confirmed similar trends in surface morphology.

Conclusion and Recommendation

It was evident from our findings that the cellulose extraction process was successful in acquiring cellulose from both hemp and parthenium with high crystallinity indices (%) of 88 and 82, respectively. Similarly, a crystallite size (nm) of 58.61 was observed for parthenium and 73.27 for hemp, signifying a high degree of structural order. Analysis of yield (%) revealed a statistically significant difference between *Cannabis sativa* (hemp) and *P. hysterophorus* (parthenium). Hemp exhibited a notably

Table 2. XRD Analysis for Cannabis sativa (hemp) and parthenium.

Hemp					Parthenium				
Pos. [°2Th.]	FWHM Left [°2Th.]	Crystalline size (nm)	d-spacing (Å)	Rel. Int. [%]	Pos. [°2Th.]	FWHM Left [°2Th.]	Crystalline size (nm)	d-spacing (Å)	Rel. Int. [%]
5.07	0.11	67.00	17.38	100	5.08	0.15	50.27	17.36	100
12.23	0.05	131.59	7.22	23.23	24.8423	0.07	91.60	3.58	43.03
17.10	0.94	8.04	5.18	40.68	26.6356	0.11	60.13	3.34	53.19
19.01	0.05	127.30	4.66	44.04	29.102	0.05	117.65	3.06	88.77
25.77	0.05	121.25	3.45	95.12	31.1023	0.07	86.43	2.87	1.69
27.44	0.07	89.59	3.24	62.41	31.6567	0.07	85.92	2.82	19.01
28.43	0.05	118.41	3.13	41.41	41.548	0.05	100.77	2.17	64.92
29.76	0.07	87.63	2.99	47.26	45.807	0.39	14.07	1.97	15.62
31.25	0.05	115.10	2.85	63.68	46.4053	0.23	23.19	1.95	37.3
32.43	0.31	21.29	2.75	3.72	49.8987	0.07	71.07	1.82	78.98
33.96	0.94	6.98	2.63	20.7	53.9054	0.07	59.47	1.69	51.79
36.39	0.07	81.26	2.46	48.7	54.2499	0.09	47.17	1.68	53.99
49.89	0.07	71.08	1.82	66.01	55.0218	0.07	57.87	1.66	36.19
50.44	0.07	70.27	1.80	24.47	73.4712	0.07	31.39	1.28	53.58
51.23	0.15	31.60	1.78	17.08	75.3388	0.12	16.76	1.26	20.3
53.40	0.19	24.07	1.71	14.65			24.07		
		Average 73.27*					Average: 58.61*		

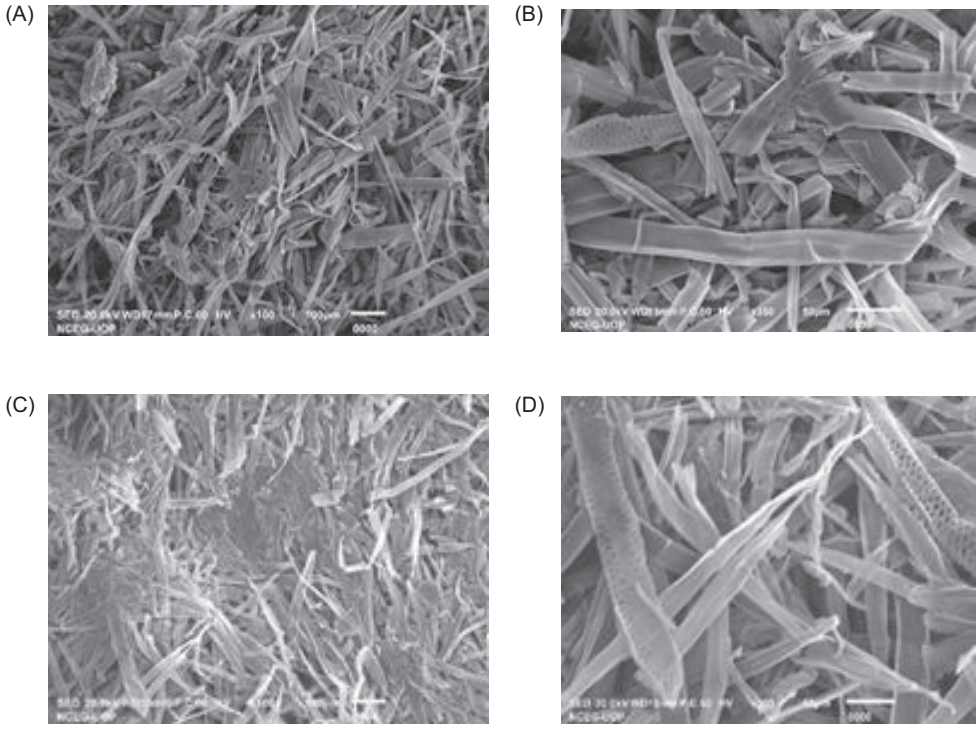

Figure 3. SEM images of (A, B) Hemp; (C, D) *Parthenium hysterophora*.

higher yield (38.4 ± 0.18) compared to parthenium (22 ± 0.82). This disparity is likely attributable to fundamental variations in plant biochemistry between the two species. FTIR analysis confirmed the presence of cellulose with signature peaks for O-H and C-H stretching, while minor peaks suggested minimal presence of impurities such as hemicellulose and lignin. These findings were further supported by XRD analysis, which revealed characteristic peaks for cellulose (I) and high crystallinity index. Scanning electron microscopy provided valuable insights into the cellulose morphology. The micrographs indicated that the alkaline treatment successfully removed non-cellulosic components, resulting in smoother and thinner fibers. These findings suggest that hemp and parthenium hold significant promise for the development of eco-friendly bioproducts. Further research is warranted to explore and optimize cellulose extraction processes from these readily available plant materials. This could pave the way for the development of a new generation of sustainable biomaterials, contributing to a more environmentally friendly future. This research gave an insight into the development of eco-friendly bioproducts utilizing cellulose of hemp and parthenium, promoting a more sustainable future.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgment

The authors extend their gratitude to the Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-15).

Authors Contributions

Conceptualization, Afia Zia; methodology, Muhammad Usman and Nureen Zahra; software, Majid Alhmorani and Walaa F Alsanie; validation, Muhammad Nauman Ahmad; formal analysis, Muhammad Baseer ul Salam, investigation, Muhammad Usman and Tariq Aziz; resources, Sahib Alam.; data curation, Niamat Ullah; writing—original draft preparation, Muhammad Usman; writing—review and editing, Abdulhakeem S Alamri and Walaa F Alsanie; visualization, Muhammad Numan Ahmad; Supervision, Afia Zia.; project administration, Sahib Alam; Funding Acquisition: Tariq Aziz

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