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Novel Method for Extraction of Lignin Cellulose & Hemicellulose from *Pinus roxburghii* Needles

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ABSTRACT

Lignocelluloses are becoming a major area of attraction for the researchers for their sustainability and cost effectiveness. The ease of functionalization of these matrices, remarkable physical and chemical properties, and tunable functional sites make them incredible materials for tissue engineering and drug delivery among other applications. The present study focuses on extracting biomaterials lignin, cellulose and hemicellulose from the *Pinus roxburghii* (PR) needles following a single source procedure. A green chemistry approach ensures minimum wastage with maximum output from the raw material.The *Pinus roxburghii* (PR) needles were collected from the local area, washed thoroughly, dried in the oven at 45°C for few days and grounded to powder. The PR powder was subjected to treatment with acetic acid: formic acid in the ratio of 1gm/10ml mixture at different concentration to study the yields, for 1hr at low temperature followed by 3hrs treatment at 110°C. The concentrated mother liquor thus obtained was filtered, diluted and left undisturbed for 48h. Lignin precipitates obtained were separated and dried in oven at 30°C. Subsequently, the residue from the extracted lignin was washed to neutralise the pH and dried in oven. By using 5% NaOH reflux treatment at 90-110°C for 3h, hemicellulose was recovered from the pre-treated pine needles mother liquor from which the lignin had been extracted. The mother liquor was treated with ethanol and acetic acid to precipitate hemicellulose in the freezer. The residue obtained after the removable of hemicellulose was washed again to and subsequently bleached to get cellulose. An excellent yield of the desired products was obtained. The extracted products were subjected to characterization studies namely FTIR and HRMS etc to get evidence for successful extraction. The analysis revealed that the PR leaves contained about 1.5wt% to 3.0wt% lignin, 15-30wt% hemicellulose and rest is cellulose.Further, aim is to use the extracted material to prepare the nano-hydrogels for the drug delivery and other applications.

INTRODUCTION

Recently, the utilization of the bio-waste for value added products has received solemn attention of the researcher's world over. With the eminent threat of the natural resources being depleted at an alarming rate various studies have been preformed to replace the petroleum based products by agro-based polymers products such as cellulose, hemicellulose, chitosan and lignin (Dhumal, C. V., *et al*., 2019). These biomaterials have huge potential for varied applications and pose an efficient method for utilization of bio wastes. The organic wastes such as shed leaves, bark of trees etc. have a considerable potential for the extraction of lignocellulose material as renewable feed stocks for the production of a spectrum of biomedical materials, chemical and other sustainable materials.

Lignocellulosic materials (lignn+cellulose+hemicellulose) are one of the most important natural sources for the production of high value added materials for biomedical industries or biopolymers for agro based industries and are very attractive research candidates due to their biodegradability, low density, easy availability, excellent mechanical properties, eco-friendly and socio-economic nature. Lignocelluloses, a low cost and renewable feedstock for producing bio fuels, bio-based chemicals, bio-medical materials and bio-degradable materials, have received considerable attention these days. The three dimensional network of lignin with cellulose and hemicellulose in these entities provides strength and stiffness to the plants.

In nature, lignin is the most abundant aromatic material with methoxyl, hydroxyl, phenolic, carbonyl and carboxylic groups on aromatic rings as phenypropane polymer 3-D structure. The aromatic and hydroxyl groups are crucially important in the anti-microbial (Raj, T., *et al*., 2022) and anti-oxidant activities. Lignin has become a smart choice for use in the paper and pulp industries due to its easy availability, low cost, eco-friendly nature, high aromatic content and good pest resistant nature (Garcia, A., *et al*., 2017). Along with that lignin is also being explored as a suitable candidate for applications such as for sensor making (Kumari, S., *et al*., 2014) antimicrobial agent (Yang, W., *et al*, 2016), food packaging material (Domenek, S., *et al*., 2013), drug delivery (Alqahtani, M. S., *et al*. 2019), gene delivery, tissue engineering (Kumar, R., *et al*., 2021) , as a binder (Farhat, W., *et al*., 2017, Lu. H., *et al*., 2016) several laboratory aromatic reagents (Wang, H., *et al*., 2019, Rinaldi, R., *et al*., 2016), as Scaffold materials (Salami, M. A., *et al*, 2017), matrix for drug loading and release and many health care products (Gordobil, O., *et al*., 2018, Dominguez-Robles, *et al*., 2020). The lignocelluloses

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material is organized as micro-fibrils linked together to form cellulose fibres. These fibres once extracted have been reportedly used for the production of ethanol (Hallac, B. B., & Ragauskas, A. J., 2011) , raw material for paper industries, manufacturing flexible electronic film, coating for packaging, receptacles for drug delivery and optical digital storage media (Abitbol, T., *et al.*, 2016). The properties of cellulose fibers are affected by many factors such as source, climate, harvest, maturity, retting degree, decortications, disintegration (mechanical, steam explosion treatment), fibre modification, textile, and technical processes (spinning and carding). (Van de Velde K., 2001). Cellulose and hemicellulose get associated with lignin through covalent bonds to form lignin-carbohydrate complexes in the primary and secondary cell-wall of wood. The hemicellulose has been used to produce alcohol by fermentation and sorbitol and also have important applications in cosmetics, papermaking, explosive, antimicrobial and drug delivery (Zhao, J., *et al.*, 2014). Hemicelluloses can be used in value-added industrial applications including hydrogels (Hu, L. S., *et al.*, 2018), thermoplastics, blends composites and nano-composites (Farhat, W., *et al.*, 2017, Azhar, S., *et al.*, 2015). Hence these materials are of utmost importance and we need to look for novel methods for the extraction of these materials.

Till date many successful methods such as alkali extraction, steam explosion, hot water, enzymatic hydrolysis, bioconversion method, and the hydrothermal method (Sun, B., *et al.*, 2014, Chen, W., 2011) have been employed for the extraction and isolation of lignocelluloses material. In its native area, *Pinus roxburghii* generates a bulk a quantity of needles waste after the shedding. A significant quantity of this waste remains un-utilized and catches fire, generating a large quantity of smoke that severely impacts human health and the environment. Due to current demands and wide range of applications the researcher have focused more on the isolation of lignin, cellulose and hemicellulose from different resources such as pines Needles, (Raj, T., *et al.*, 2022), pines cones, (Rambabu, N., *et al.*, 2016) rice Husk, (Johar, N., *et al.*, 2012), straw of wheat, jute, banana stem, hemp and flex straws, sun flower stalk (Albishi, T., 2019, Fortunati, E., *et al.*, 2016) pine oil residue, pine apple leaf fibers and sisal fibers (Siqueira, G., *et al.*, 2010) etc. Cellulose and hemicellulose interactions in the cell wall play an important role in the excellent flexibility of wood-based materials. At present, the methods of extracting hemicellulose (Geng, W., *et al.*, 2019, Zhang, J., *et al.*, 2018, Zhao, J., 2014) from plants can be divided into physical pre-treatment and chemical pre-treatment. The former includes steam explosion, hot water extraction, ultrasound, and microwave-assisted methods (Perez, J., *et al.*, 2002, Mehanny, S., *et al.*, 2020).

In the present study we report another method for extracting these three plant backbone forming materials from the *Pinus roxburghii* needles. We aim to develop a method that ensures have maximum extraction of the desired products with minimum waste and apply and hence extracted and characterized materials for speckled applications. The *Pinus roxburghii* (PR) needles were employed for the extraction of lignin, cellulose and hemicellulose following previously reported methods and formulating a novel eco-friendly procedure. The lignin, cellulose and hemicellulose yield was checked and found with highest yield of cellulose 60-70wt%, Hemicellulose 15-30wt% followed by Lignin (1.5wt% to 3.5wt%) and subjected to FT-IR and HRMS characterisations to get evidence for successful isolation.

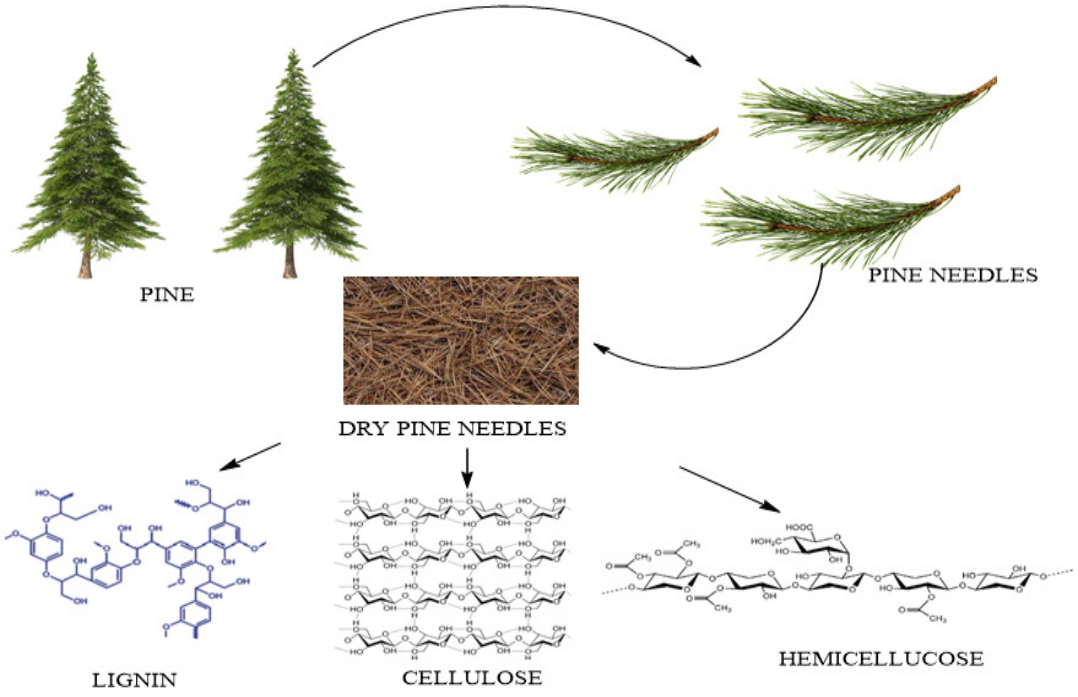


Figure 1: Graphical Abstract

METHODOLOGY

Raw Material

Pinus roxburghii (PR) needles were collected from forest near Palampur India. The collected needles wash properly and cut into 10mm pieces. Sample was air/oven dried and stored in the room temperature.

Chemical Used

All chemicals and reagents utilized are of standard quality. The formic acid (assay 85%) (Sisco Research Laboratory Pvt Ltd), NaOH flakes (central Drug House (P) Ltd Daryaganj New Delhi), Ethanol (Changshu Yangyuan Chemical, China, Acetic Acid (RDC Limited Delhi), Double distilled (DD) water (Lab prepared) and Sterilising Water (Sun Rise Pharma) were used as received.

Extraction of Lignin, Cellulose and Hemicellulose

Step 1

Extraction of lignin from PR needles was carried out following an amalgamated approach with steps from a standard method (Raj, T., *et al.*, 2022) along with formulation of novel steps. PR needles were collected, separated from the branch, washed thoroughly with distilled water and dried under sunlight followed by grinding them to powder stage using a mixer grinder. Acetic acid (AcA), Formic acid (FA) and water were used in the ratio of 5.5:3.5:1 by volume for extraction of lignin. One part of the powdered pines needle was combined with the 20 parts of solvent (AcA: FA: Water in 5.5:3.5:1) in a round bottom flask. The resultant mixture was heated to 70-80°C for one hour, and subsequently next 3hrs at 110°C. The reaction mixture was allowed to cool and filtered. Thus obtained solid residue was washed with 1:1.5 ratio of dilute acetic acid (3.0 wt%) solution at 60°C keeping 20-25min incubation time for three to four washes and the decanted solutions were mixed with mother liquor to left for evaporation under constant stirring to reduce the volume to 10ml at 30-40°C and diluted to 300ml to precipitate the lignin. Precipitated lignin was filtered by using Whatman filter paper number 42 and dried. The work has been reported by Raj, T., *et al.*, 2022. The yield of the extracted lignin was determined using the following formula:

$$\%Wt = \frac{Wi-Wr}{Wi} \mathbf{X\ 100}$$

Where, W_i = initial weight of Pinus needles. W_r = Weight of residue left after the Lignin precipitation. $\%W_t$ = Weight %age of component.

The left over pine waste post lignin extraction was employed for separation of cellulose and hemicelluloses bagasse and mother liquor respectively. This paper focuses on the same.

Step 2

The residue obtained after the removal of Lignin was neutralised by washing with DD water and dried in oven followed by treating with an alkali solution (5% wt) of NaOH (Kumari S., *et al.*, 2014). The mixture was transferred to round bottom flask and heated to 60°C for 2 hrs followed by reflux for 4 h at reflux temperature. The solid was then filtered and washed thrice with DD water to get cellulose fibrils. These cellulose fibrils were bleached by aqueous chlorite thrice at 100-130°C for 4 h. Thus obtained sample was of Cellulose. The yield of the extracted cellulose was determined using the following formula:

$$\%Wt = \frac{Wr-Wp}{Wi} \mathbf{X\ 100}$$

Where, W_r= Residue weight that left after the removal of Lignin. W_p = Weight of hemicellulose precipitates formed.

$\%W_t$ = Weight %age of Cellulose. W_i = initial weight of Pinus needles.

Step 3

The filtrate obtained in the First step was treated with 25% acetic acid and transferred to 96% ethyl alcohol to maintain a pH of 4.5 at 4°C. The mixture was left for two

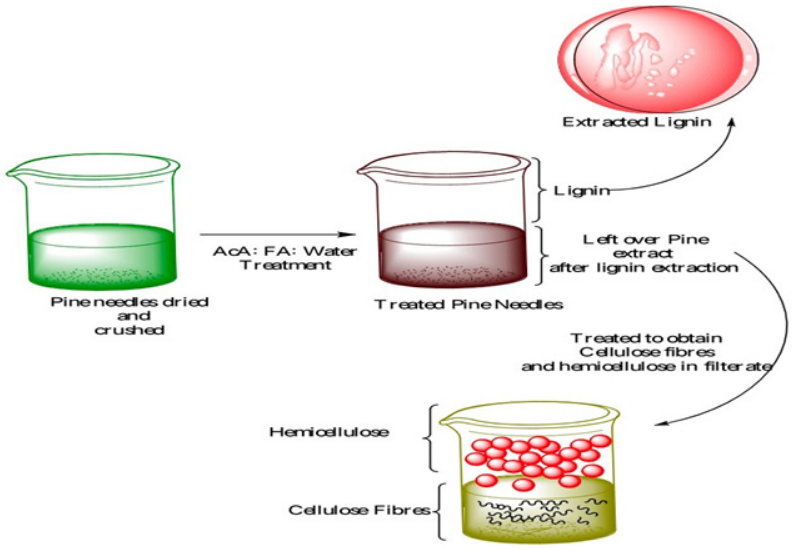


Figure 2: Representation of procedure for the extraction of lignin, cellulose and hemicellulose from *Pinus roxburghii* needles.

days to allow the hemicellulose to precipitate and settle to the bottom. The clear layer above the precipitate was removed by vacuum suction. The precipitates obtained were washed with 60% ethanol and frizzed- dried to get the hemicellulose powder. Figure 1 gives the graphical representation of the extraction process. The yield of the extracted hemicellulose was determined using the following formula:

$$\%Wt = \frac{Wp}{Wi} X\ 100$$

Where, Wp = Weight of precipitate formed.
%Wt = Weight %age of Hemicellulose. Wi = initial weight of Pinus needles.
On applying the above mentioned approach the obtained yield for extracted lignin, cellulose and hemicelluloses indicated that PR leaves contained about 1.5wt% to 3.0wt% of lignin, 15-30wt% of hemicellulose and remaining is Cellulose. Figure 2 represents the images of the as extracted samples of lignin, cellulose and hemicelluloses from *Pinus roxburghii* needles.

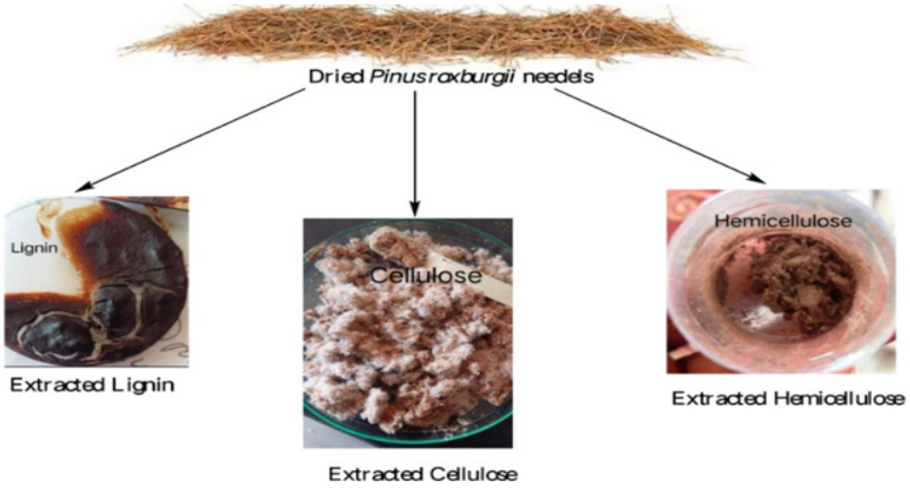


Figure 3: Represents the Images of The As Extracted Samples of Lignin, Cellulose and Hemicelluloses from *Pinus roxburghii* Needles.

Characterization

The extracted lignocelluloses material was characterised by various techniques such as FT-IR (spectrum 100 Perkin Elmer with HATR & DRIFT) for vibrational analysis of samples, and HRMS (Xevo XS QTof mass spectrometer, Waters ACQUITY UHPLC, Mass Range 20-4000amu, Resolution 40000 FWHM Mass Accuracy: Typically <1ppm, Sensitivity: Full Scan Sensitivity up to 500fg on column with S/N> 100:1 in ESI MS mode for reserpine, Ionisation Method: API ESI positive & negative, APCI positive & negative, Direct infusion for Mass Analysis (MS, MS,/MS), UHPLC with PDA detector to get the evidence of successful extraction.
The FTIR and HRMS data indicated that the extracted lignin, cellulose and hemicellulose are of excellent purity.

RESULTS AND DISCUSSION

The FTIR results indicate the successful separation of all lignin, cellulose and hemicellulose from the PR needles (Shi, Z., *et al*., 2019, Sheltami, R., 2012). The stretching observed at 1510cm^{-1} represents the –C=C– groups of the aromatic ring of lignin compounds. The absence of this characteristic peak in the spectrum of cellulose and hemicellulose points towards the successful separation of lignin form the other two components. The prominent peak at 1734cm^{-1} of the residue is attributed to either acetyl or an ester group of hemicelluloses indicating the successful isolation of the three components (Mohammed, & Saber, 2022).

FTIR of Lignin, Cellulose & Hemicellulose

FTIR of extracted lignin sample

The FTIR data obtained for the extracted samples indicated successful extraction of the same from the PR needles. The characteristic functional groups with their values are mentioned in the Tables-1, 2 & 3 for lignin, cellulose and hemicelluloses respectively. The band for -OH was observed at 3414.00 cm^{-1} (3500-3100 cm^{-1}), -C=O group conjugation appeared at 1716.65 cm^{-1} and 1631.78cm^{-1}, -C-O stretching for S-type of lignin (Figure-4 & Table-1) was also observed. The -C-O-H group was indicated by the stretching at 1029.99 cm^{-1} (Figure-5 & table-2) and –C-O-C bridge between sugar units was justified by stretching at 1045.41cm^{-1} (Figure-6 & Table-3). The spectra obtained for lignin, cellulose and hemicellulose, exhibited major differences in the region of 1800-600 cm^{-1}. The stretching peak at 1122.57 cm^{-1} is designated to aromatic C-H stretching characteristic of S-type unit of lignin, whereas at 1273.08 cm^{-1} the characteristic stretch bands is comparable to ether & G-type unit in lignin in the pines needles(Figure-4 & Table-1). The stretching observed in the range of 1850–1450 cm^{-1} represents the functional groups generally originating from lignin and at 650 cm-1 for cellulose and hemicelluloses components. (Brinchi, L., *et al*., 2013, Chirayil, C. J., *et al*., 2014). The characteristic peak of lignin's aromatic ring vibration presents at wave number 1200–1400 cm^{-1} almost disappeared after chemical and mechanical treatment.

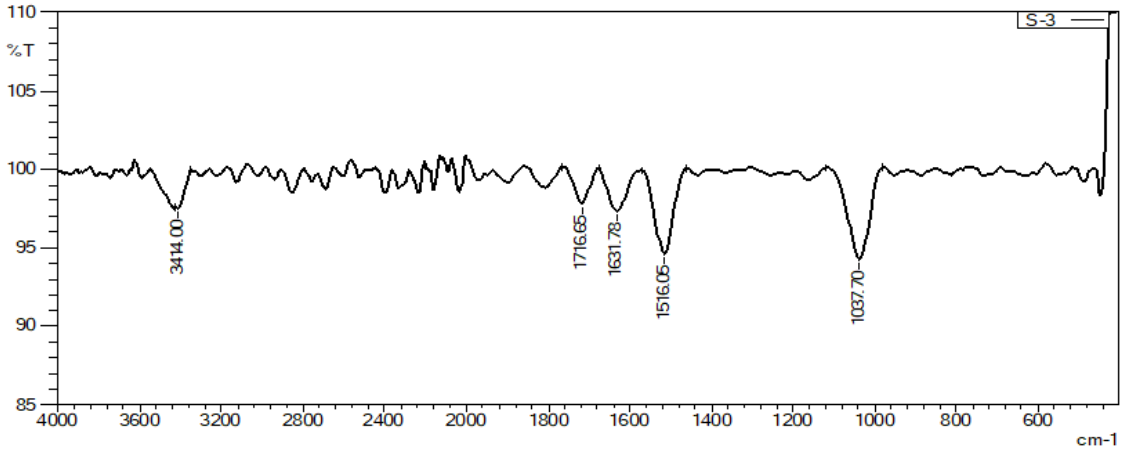


Figure 4: The FTIR spectra of the extracted lignin.

Table 1: The FTIR spectral peaks of lignin extracted from *Pinus roxburghii* needles.

S. NO.	Reference Frequency Cm-	Peaks appears in the present study (cm-)	Functional groups	Compounds
1	3450-3300, 3500-3100	3414.00	-OH polyphenols -OH str Ar. Alipha.	phenolic group
2	3500-3100	3502.72, 3174.83	-OH str	Ar. Aliphatic grp
3	2960-2925, 2950-2850	2958.80, 2924.08	Str -C-H, -CH3.-CH2	Alkyl, aliphatic grp
4	2500-2100	2503.60, 2102.40	C=C conj.	Benzene ring
5	2500-2100	2499.74	C=C conj C=C Str.	Benzene ring
6	2500-2100, 2156	2156.41	C=C conj. C=C str.	Benzene ring
7	1720-1715	1718.85	Str. C=O unconj.	Carbonyl group
8	1650-1600, 1647	1631.78	Str. C=O conjugation.	Carbonyl group
9	1600-1500, 1515	1516.05	Ar. rings of S -type	S-type ring
10	1450-1600,	1450.46	Ar. Stretching of Lignin, OH Str	Aromatic, aliphatic alchohol
11	1450, 1460	1458.18	-OH Str Ar,	Aromatic grp
12	1272-1220, 1200-1215	1273.08	-C-O stre,phenol,	Ether & G-type grp
13	1115-1125, 1121	1122.57	Ar. C-H stretching S-type unit	S-type unit
14	1030, 1041, 1040	1037.70	C-O bond in aromatic S-type	S-type unit
15	825, 835	825.53	S-type aromatic C-H bonding	S-type unit
16	800-785	786.93	C-H out of plane defor S-type	S-type unit
17	627-500	628.79	C-H Str	Aromatic Hydrogen

FTIR of extracted cellulose sample

The FTIR spectra for extracted cellulose exhibited various characteristic peaks such as ones at 3525 cm^{-1} and 1650 cm^{-1} indicative of the non-aromatic moieties. A weak peak around 2900 cm^{-1} was observed (Figure 5) for alkali treated extracts indicating complete removal of hemicellulose moiety from the extract. The stretching frequency for untreated cellulose fibre was observed around 1423.47cm^{-1} for -C=O group. The detailed analysis of the data obtained is presented in the table 2 which strongly indicates successful extraction of cellulose form the pine needles (Moran. J. I., *et al.*, 2008, Gadzama, S. W., *et al.*, 2020).

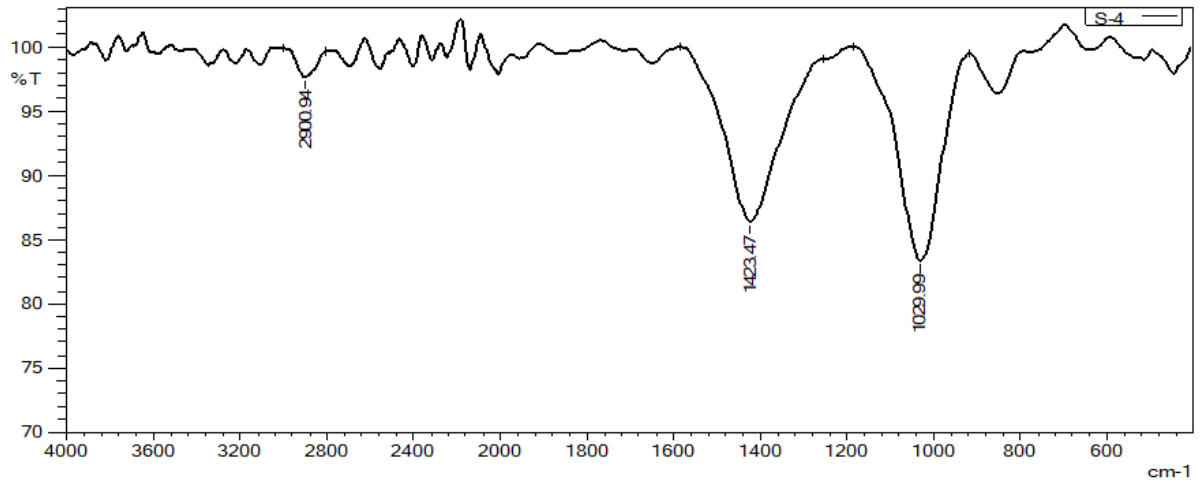


Figure 5: The FTIR spectra of the extracted Cellulose.

Table 2: The FTIR spectral peaks of Cellulose extracted from *Pinus roxburghii* needles.

S. NO.	Reference Frequency Cm-	Peaks appears in the present study (cm-)	Functional groups	Compounds
1	4,000-2,995	3996.50	-OH Acid, methanol	Alcoholic group
2	2950.85	2900.94	Asymmetric and the Symmetric Stretching of Methylene (-CH2-) groups	-CH2 group
3	2,890	2889.36	H-C-H Alkyl, aliphatic	Aliphatic group
4	1440-1400	1423.47	-OH bending	Acid
5	1,640	1612.49	Fiber-OH Adsorbed water	
6	1,270-1,232	1546.05	C-O-C	Aryl-alkyl ether
7	1,170-1,082	1029.99	C-O stretching	Pyranose ring Skeletal
8	1,108	1107.14	-OH, C-OH	Alcoholic groups

FTIR of the extracted hemicellulose sample

In the FTIR of the extracted hemicellulose it was observed that the ring vibrations overlapped with stretching vibrations of side group -C-OH bonds and -C-O-C glycosidic bond vibrations. Asymmetric and symmetric stretching of the carboxylate ions was seen at 1600 cm^{-1} and 1380 cm^{-1}. The stretching frequencies at 1060.84 cm^{-1}, 1037.70 cm^{-1} and 898.82 cm^{-1} were indicative of the mannose and glucose units. The detailed analysis and spectrum is represented in figure 6 and table 3 (Wei, L., *et al.*, 2018, Farhat, W., *et al.*, 2017).

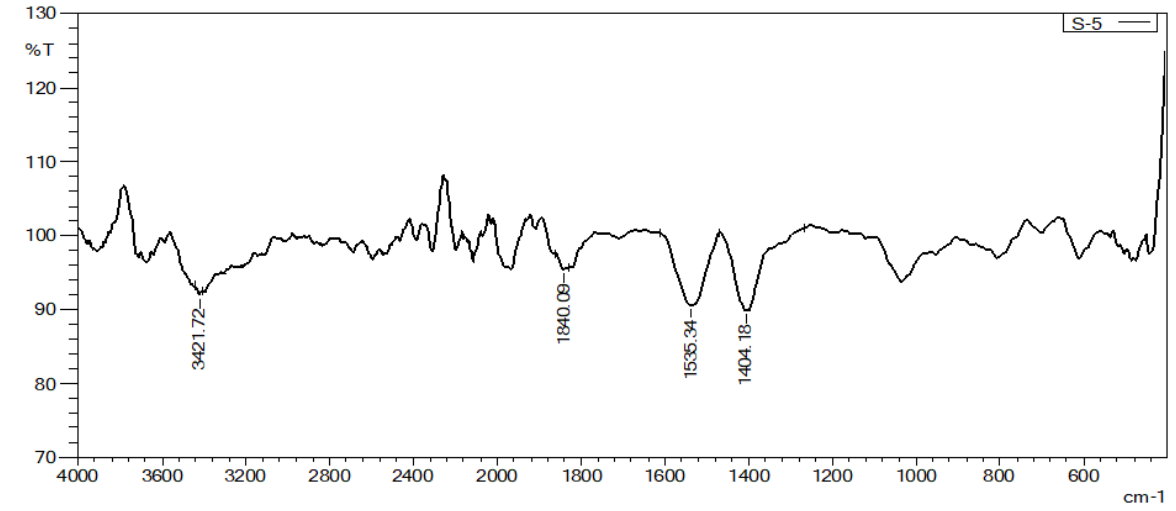


Figure 6: The FTIR spectra of the extracted Hemicellulose.

Table 3: The FTIR spectral peaks of Hemicellulose extracted from *Pinus roxburghii* needles.

S. NO.	Reference Frequency Cm-	Peaks appears in the present study (cm-)	Functional groups	Compounds
1	4,000-2,995		-OH, Acid, Methanol	Acidic group
2	3000-3640 Farhat W, 2017	3421.72- 3001.23	Stretching vibrations of –OH	Alcoholic group
3	2889, 2890	2889.36	Symmetric and Asymmetric vib. Of C-H	Alkyl group
4	1765-1715	1770.05	C=O ketone and Carbonyl	Carbony group
5	1505	1535.34	C=O streaching	Carbonyl group
6	1483	1404.18	CH2 bending	
7	1108	1107.00	-OH, C-OH Group	
8	1045	1045.41	-OH, C-OH Group Asymmetric stretching of C-O-C bridge between the sugar units.	Pyranose ring structure
9	985	983.69	C-O stretching characteristics of sugar st.	Sugar units
10	898	898.82	Characteristics of β-glucosidic linkage between the sugar units	Glucose & Mannose units

High Resonance Mass Spectrometry (HRMS) of Extracted Lignin, Cellulose and Hemicellulose

The HRMS data pointed towards the cross-linked nature of the lignin exhibiting various characteristic chemical linkages and consequently, unique functional groups appearing at predictable regular position relative to the others in the mass spectra (Raj, T., *et al.*, 2022, Prothmann, J., & Turner, P., *et al.*, 2018, Pikovskoi, I. I., *et al.*, 2020). The corresponding values at 105.0694 to 130.0 indicate the presence of the methoxy groups. Mass to charge ratio (m/z) at 157.1003, 179.0844 and m/z 209.1316 with standard error are ascribed the presence of 4-methoxybenzaldehyde, 4-methoxycinnamic acid and 3, 4-dimethoxycinnamic acid respectively, that constituted major peaks in the spectra of lignin (Figure 7). Other signal at m/z 271.0956 corresponds to the signal obtained in the (Raj, T., *et al.*, 2022) (Figure 7). These are very close to the values reported for the groups present in lignin. The m/z values obtained in the HRMS of cellulose at m/z 158.9638 (Figure 8) and m/z at 158.9638 (Figure 9) correspond to the value of single monomer unit of cellulose and hemicellulose respectively (Lunsford, K. A., *et al.*, 2011, Ma, M.-G., *et al.*, 2012, Mahardika, M., *et al.*, 2018). Large number of glucose units attached through glycosidic bonds to form linear chain of cellulose with high degree of crystallinity.

The hydroxyl groups attached with carbon atoms of cellulose monomer units makes a internal hydrogen bonding network. The calculated mass value for each of glucosyl units ($C_6H_{10}O_5$) is 162m/z. After the comparison, the peaks m/z values with calculated mass are as: m/z 974.8090 (6 glucosyl units) 1178.7690 (7 glucosyl units) 1240.7610(8 glucosyl units) 1450.7228(9 glucosyl units) 1586.6989(10 glucosyl units) (Figure 9). The difference with the calculated value is almost equivalent to the number of water molecules removed during the bond formation. (Jung, S., *et al.*, 2010) These values show the successful extraction of cellulose from the needles (Figure 9). The major constituent of hemicellulose in soft wood is O-acetyl-(4-O-methylglucurona)-xylan to gather with small amount of glucomannan in alkaline condition. From the lower mass peaks of the spectra indicate that the cellulose and Hemicellulose obtained is depolymerised completely. The main subunits of Hemicellulose obtained after the extraction are xyloglucan, Xylan, β-glucose & Glucomannans, which are shown in peaks as: m/z 873.6373 indicates the five units of Hexose, m/z895.5160 indicates hexose-5units & 1 acetyl group and m/z 917.6600 indicates hexose-5 units & two acetyl groups attached (Figure 9).

The low mass spectral ranges predominately contain peaks of monomeric glucose, xylan, phenols and aromatic acids, the most important of which are the representative of C_6-C_3 units (phenyl propane type) of hydroxycinnamic acids and m/z 158.9638 representative of cellulose. These values provide strong evidence for the presence of G-unit coniferyl alcohol and S-unit Synapyl alcohols in the extracted lignin and the high m/z 1110.7838 to m/z 1654.6804 represent the presence of cellulose polymeric structure of glucose units (Wei, L., *et al.*, 2018) (Figure 8). These units are the key components in the 3D structure of the coniferyl lignin, cellulose and Hemicellulose. Similarly, the m/z values obtained for hemicellulose namely at 289.1737, 377.2256, 425.1477, 513.2003, 585.4064, 713.4513 and 873.6373 m/z indicates the partially de-polymerization of hemicellulose (Figure 9) & support the successful extraction of hemicellulose from the *Pinus roxburghii* needles (Zhao, W., *et al.*, 2015).

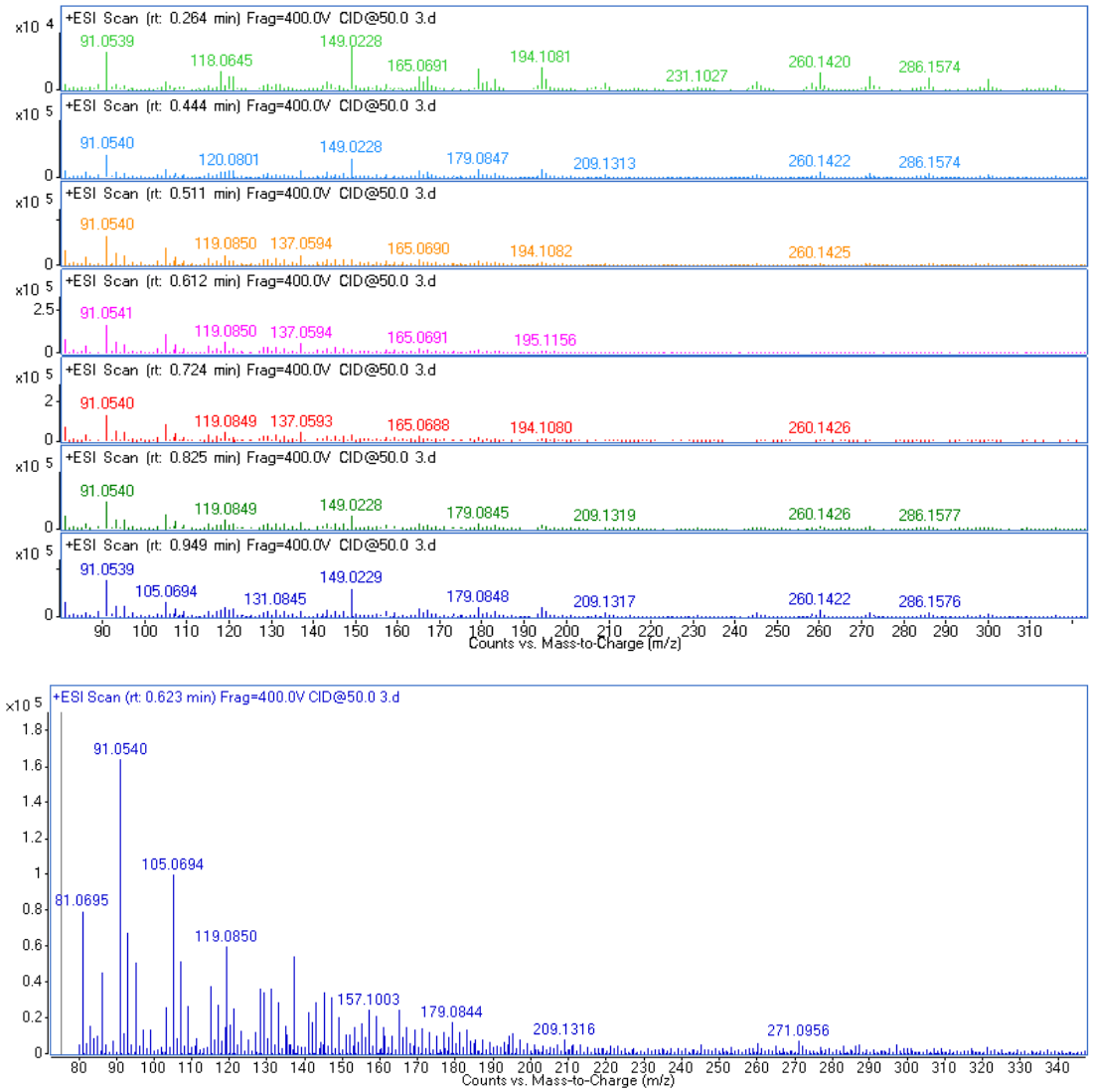
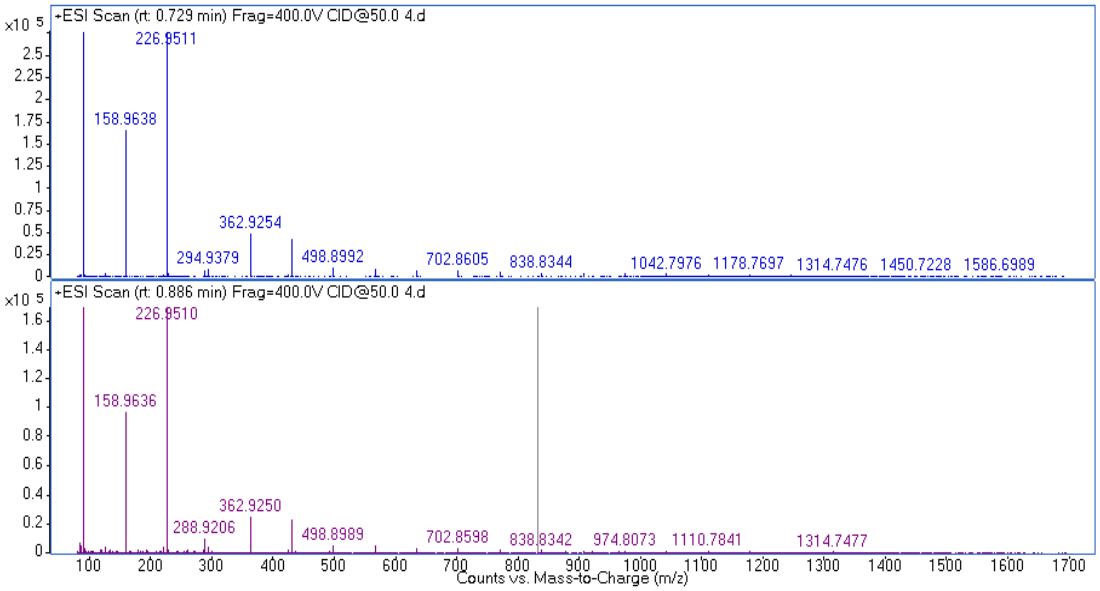


Figure 7: The HRMS spectra of the extracted lignin.



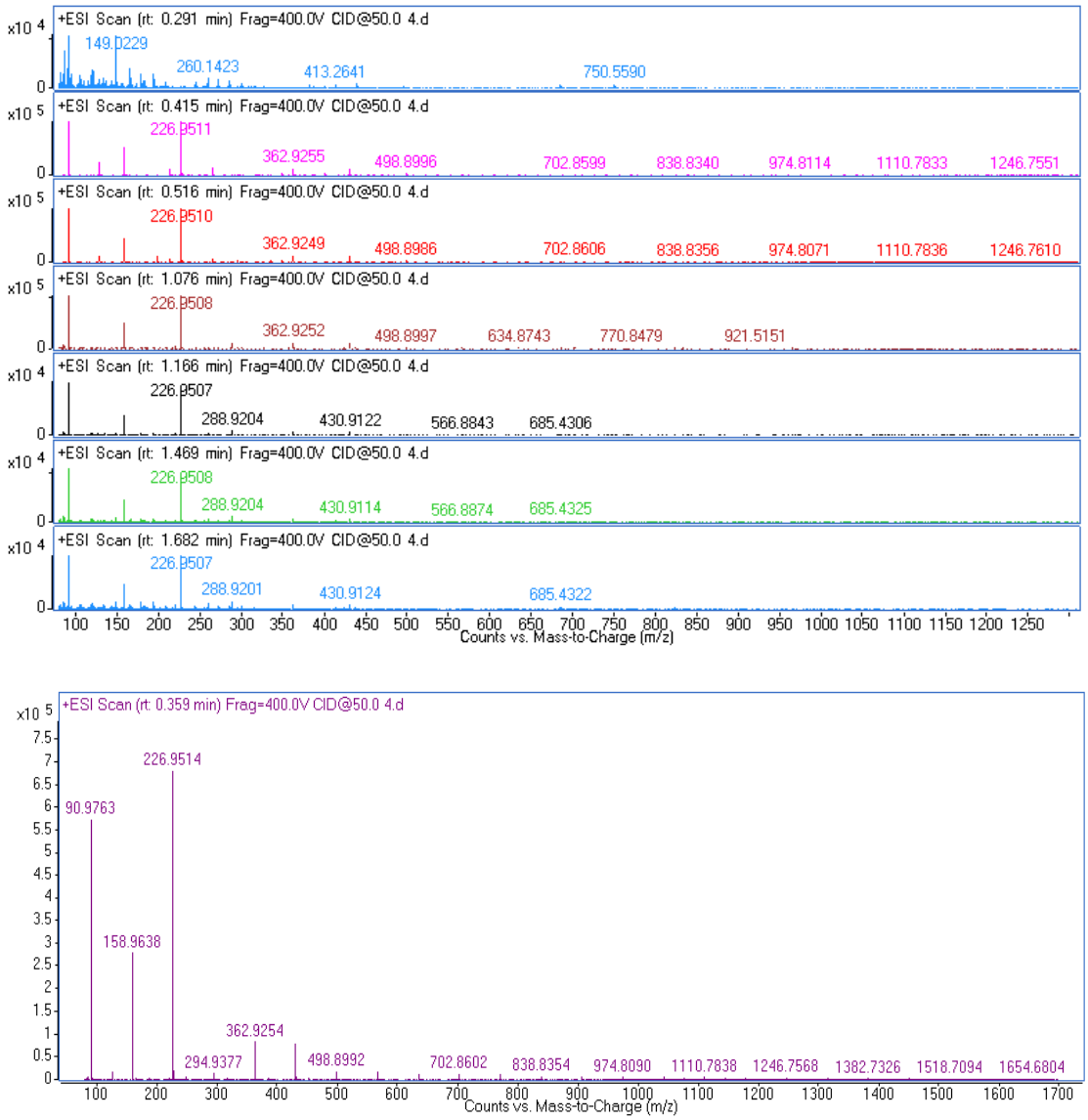
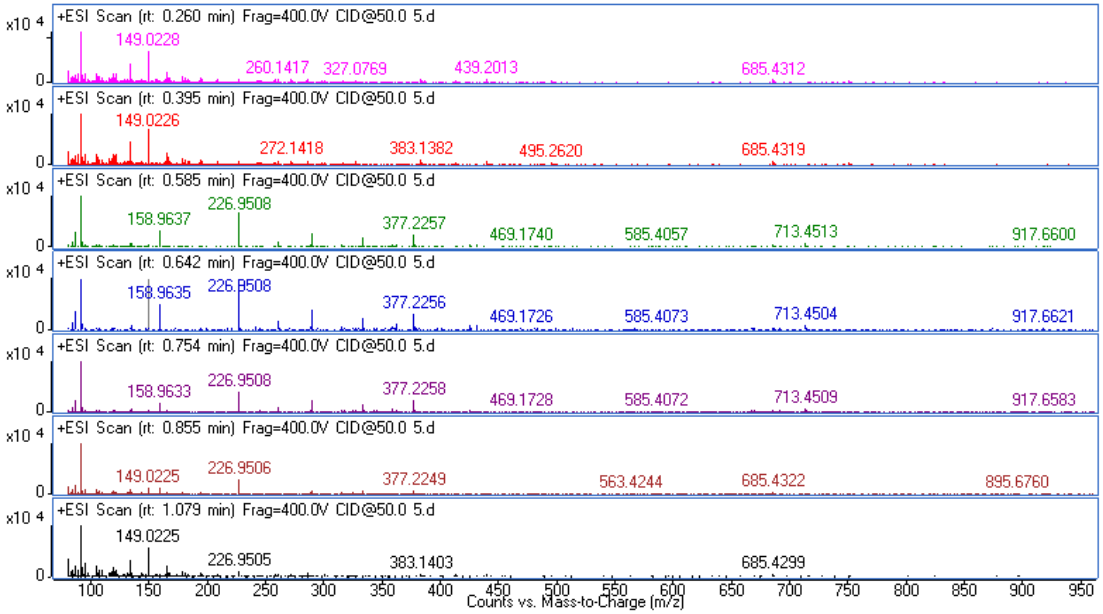


Figure 8: The HRMS spectra of the extracted Cellulose.



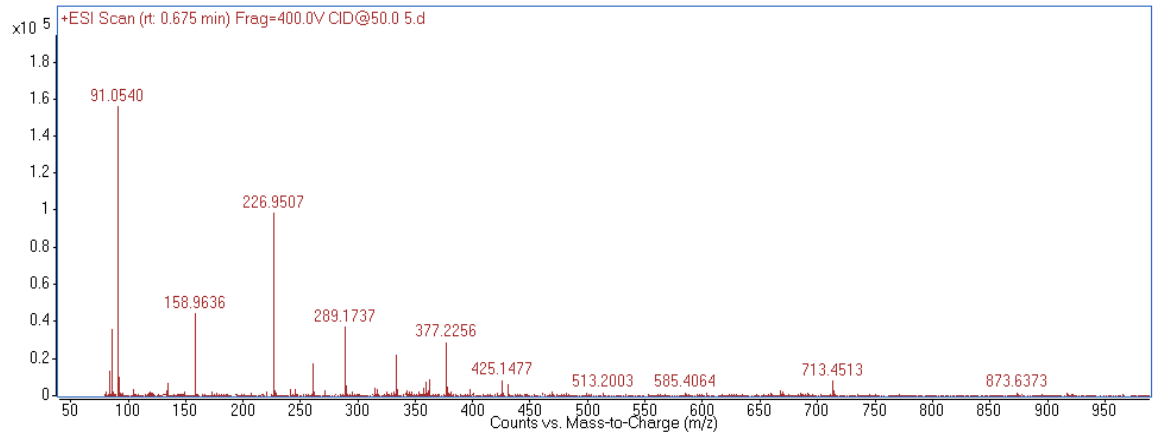


Figure 9: The HRMS spectra of the extracted Hemicellulose.

CONCLUSION

The aim of the study was to depict the versatility of the PR needles to act as a raw material for extraction of various natural products namely lignin, cellulose and hemicellulose. More specified and comprehensive approach was introduced to widen and simplify the existing synthesis methods to attain a good yield of the desired derivatives. The lignocelluloses products have great potential in the preparation of matrices for sensor, antimicrobial, and drug delivery applications. The present work is the second phase of the previously reported work of Raj, T., *et al*., 2022 wherein they reported the extracted of lignin from *Pinus roxburghii* needles and prepared its silver incorporated composites guar-gum and agar-agar that exhibited excellent antimicrobial properties. The pre-treated *Pinus roxburghii* needles from which the extraction of lignin had been carried out were employed for extraction of cellulose and hemicellulose subsequently. The characterization results indicated a good yield of the extracted products with excellent purity. This method follows a green chemistry approach with minimum wastage of the raw material. This method helps counter the scientific challenges such as waste, fire pollution, to prepare more and more economical and eco-friendly materials in the near future with sustainable development. The process opens up the doors for the further research to be carried out on extracted materials for preparation of lignin, cellulose or hemicellulose based nanogels/ hydrogels/composites/matrices for varied applications in industries as well as therapeutics.

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