Bull. Chem. Soc. Ethiop. **2019**, 33(1), 61-68. ISSN 1011-3924
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DOI: https://dx.doi.org/10.4314/bcse.v33i1.6

ENVIRONMENTAL FRIENDLY METHOD FOR THE EXTRACTION OF CELLULOSE FROM *TRIFLOLIUM RESOPINATUM* AND ITS CHARACTERIZATION

Noor Rehman[1*], Sultan Alam[2], Inamullah Mian[2] and Hidayat Ullah[3]

[1]Department of Chemistry, Shaheed Benazir Bhutto University (18000) Sheringal, Dir (Upper) Khyber Pakhtunkhwa, Pakistan
[2]Department of Chemistry, University of Malakand (18800) Khyber Pakhtunkhwa, Pakistan
[3]Institute of Chemical Sciences, University of Peshawar (25000) Khyber Pakhtunkhwa, Pakistan

(Received May 31, 2018; Revised January 7, 2019; Accepted February 4, 2019)

ABSTRACT. The leaves of *Triflolium resopinatum* were collected from the mountains of Malakand division, Khyber Pukhtoonkhwa, Pakistan and was grinded into smaller particles and converted into powder. The ground biomass was treated with different solvents in the Soxhlet apparatus for the removal of soluble extractive like pectin, cutin and wax substances. For bond breaking the alkaline substance was kept in the autoclave. Ethylene diamine tetra-acetate (EDTA) and hydrogen peroxide (H_2O_2) were used for the removal of most polar substances like pectin, cutin, waxes and other extractives. Furthermore, raw cellulose was purified through acetic acid and nitric acid. Double distilled water was used for the neutralization of pH. The analysis of purified cellulose was carried out through different procedures such as X-ray Diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). The extracted cellulose has high degree of purity and crystallinity (72%) and thermal stability indicating that the process for the extraction of cellulose is quite adequate.

KEY WORDS: *Triflolium resopinatum*, Cellulose, FTIR, XRD, TGA, SEM

INTRODUCTION

Biopolymers of cellulose are existed abundantly in the universe. The structure of cellulose holds straight chains of D-glucose which is linked by β-1,4-glycosidic. D-anhydroglucopyranose delivers OH groups at carbon 2, 3 and 6 for further reactions [1]. Cellulose crystals are closely arranging through Vander wall and intra and intermolecular hydrogen bonding. The properties of cellulose by its structure contain degradability, hydrophylicity and chirality. Cellulose is insoluble in water because it contains extended chain and superior molecular mass [2, 3]. Initially it has been used as a raw material for the paper and textile industries. It is used a buffer additive to decrease electro osmotic flow in capillary electrophoresis [4, 5]. It is also applicable for making shirts, knobs, uniforms, fabrics, toothpaste, purges, food pills, soaps and water based dyes [6, 7]. Moreover, the derivatives of cellulose have been widely used in various areas like pharmaceutical, cosmetics and plastic industries. Recent research indicates on increasing demand like, the production of bioethanol from cellulose is very important [8]. Cellulose is the essential component of plant which produce high amount of ethanol [9]. Internationally in 2008 manufacturing of bioethanol is exceeded over 39 billion. Manufacturing of bioethanol from sugarcane in Brazil is above over 17.2 billion to facilitate about 20% of the country necessities [10].

Nowadays, it's very hot topic to investigate isolation of cellulose from biomass which takes excessive effect on the environment. Literature displays the cellulose isolation from altered sources like, soft wood, hard wood, agriculture waste and residue [11, 12]. Derivatives of cellulose consist of methyl, ethyl and propyl groups. The key reason of isolation was to find out

*Corresponding author. E-mail: noorrehman@sbbu.edu.pk
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their applications on medical aspect such as, for the treatment of hemorrhoids, diverticulosis, diarrhea and irritable bowel syndrome. Dryness can be preventing to take the enough quantity of it because it has strong attraction towards water adsorption [13, 14]. Furthermore, methyl cellulose in lubricating form is used for the treatment of dry eyes [15].

Cellulose has numerous applications in the constructions zone. In construction materials it is used for the purpose of additive performance. Additionally, in grout mixture it increases the assessment of workability, water maintenance, thickness in cement and gypsum based industries [16, 17]. Particularly methyl cellulose is used in culture cell virology to detect virus-related duplications. Simply infected virus is capable to extent in the cells wherever tissues close together [18, 19].

Ethyl cellulose is used for the protection of foodstuff worked as an emulsifier. Meanwhile, it is applicable in film based photography, as a border substance for eyeglasses, cigarette and playing cards [20]. Due to pressure and heat plasticizers can be simply bonded with acetate of cellulose because acetate is destabilized by strong alkaline and oxidizing agents [21].

Nitrocellulose is adhesive sheath used for immobilization of nucleic acid. Furthermore, it is used to control proteins in Western blots in atomic force microscopy. Usually it usage cause the maintenance in investigative tests where the binding of antigen-antibody happen, pregnancy tests, U-albumin tests [22].

Triflolium resopinatum is used as precursor in this paper for the isolation of cellulose. The raw biomass was treated with numerous solvents with a different ratio, temperature, and time. The sample was examined with modern analytical techniques for the confirmation.

The aim of this study is to suggest a simple and low cost method for the extraction of cellulose from *Triflolium resopinatum* straw by means of an environmental-friendly multistep procedure. This procedure efficiently removes extractives like pectin, cutin, waxes and other extractives from *Triflolium resopinatum*. The techniques of infrared absorption spectroscopy (ATR-FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and X-ray diffraction (XRD) were used for characterization of cellulose, showing that the overall process is adequate to obtain cellulose.

EXPERIMENTAL

Cellulose isolation

The low cost and easily available biomass was selected for cellulose isolation. Biomass was grinded into minor units and allows 80-mesh. The sample was treated in Soxhlet apparatus through various solvents based on increasing polarity such as, n-hexane (96%) for 3 h to eradicate the low polar ingredients, 3 h with ethanol (96%) to eliminate the polar constituents and lastly deionized water was used to eliminate the supreme polar extractives and waxy materials. The free extractive biomass dried in an oven at 80 ^{o}C. Furthermore, it was reacted with the 5% (w/v) sodium hydroxide (99%) solution for bond breaking and fiber parting and reserved in an autoclave (model Stermax 20EHD), at 121 ^{o}C in 2 atm of pressure, with 1:100 g/mL for half an hour. The paste was filtered and eroded with double distilled water to attain pH 7.

Maximum polar ingredients were detached through bleaching which was reported in our previous work [23]. The sample was reacted with 1:25 for 12 h at 48 ^{o}C in a solution of 2% (v/v) H_2O_2 (99%) and 0.2% (w/v) EDTA solution under vigorous stirring. The filtered raw cellulose was washed with double distilled water until pH become 7. Further purification of cellulose was carried out under mechanical stirring for 30 min with 80% (v/v) acetic acid (99.8-100%) in a 1:33 and with 65 % (v/v) of nitric acid (65%) in a 1:4, at 120 ^{o}C. The sample was passed through filter paper and splashed with ethyl alcohol and double distilled water for neutralization of pH.

CHARACTERIZATION

FT-IR analysis

FTIR was accomplished to know various functional groups present on the surface of raw source and cellulose were checked after each stage. The recorded spectra were taken through Fourier Transform Infrared Spectrophotometer "IR Prestige, Shimadzu Japan" through KBr pallet. Spectra were recorded under atmospheric pressure at 25 ^{o}C in the range of 800-2000 cm^{-1} [24]. The main constituent of the sample is cellulose, hemicellulose and lignin [25]. Lignin has characteristic peak in the range of 1500-1600 cm^{-1}, which corresponds to aromatic ring vibrations. From the spectra of crude *Eucalyptus lenceoleta* and extractive free straw it is confirmed that lignin is present in these samples due to the absorption at 1514 and 1604 cm^{-1}. The (C-O) group stretching bands were observed at 1150, 1172 and 1188 cm^{-1} [26, 27].

The assignment of FTIR spectra at various stages were recorded which were shown in Table 1. The detected bands in a spectrum of the crude sample are in the area of 1636 cm^{-1} which represent H-O-H bending which is available in the cellulose which is already present in the market because it specifies the adsorbed water. The peak present at 1315 cm^{-1} related to aromatic ring vibration which shows the presence of lignin the sample and 1031 cm^{-1} specifies the symmetric alcohols [28]. The bands of the raw sample were changed from others due to disappearance some groups which were appears in raw sample. After bleaching-I the peak were appeared at 1420 cm^{-1} and a solid peak seems at 1031 cm^{-1} specified C-O stretching bond which occurred in the bands of pure cellulose [29, 30] as shown in Figure 1.

Table 1. Assignment of infrared adsorption bands of the *Triflolium resopinatum*.

Raw	1st	2nd	3rd	4th	Assignment
1031	1032	1031	1022	1031	C–O stretching
1150	1153	1151	-	1150	C–O–C anti-symmetric bridge stretching
1315	1315	1315	1312	-	CH_2 deformation
1425	1420	1420	1420	-	CH_2 bending
1638	1630	1638	-	-	Adsorbed water

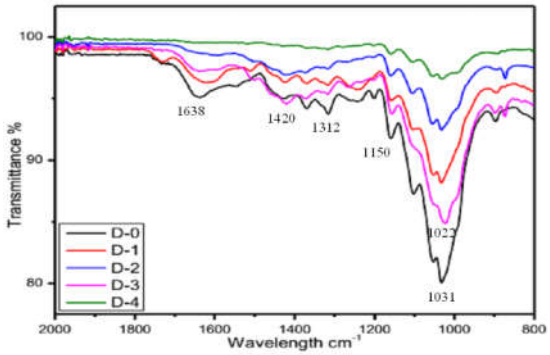

Figure 1. FTIR spectrum of *Triflolium resopinatum* at various phases of cellulose isolation.

X-Rays diffractometer

XRD is important analysis to calculate crystallinity. X-rays are indicated to accumulate the properties of cellulose, and are scattered when focus on the targeted sample. Here are two opportunities one is positive or the second is negative interference. Crystallinity of cellulose was examined by X-rays diffraction. Previous reports show that Segal's equation is a greatest technique to calculate the crystallinity of cellulose because it only needs highest and lowest peak [31, 32].

$$X_C = 100(I_{200} - I_{AM})/I_{200} \quad (1)$$

I_{200} is the top peak which designates the crystalline substituent. Whereas (I_{Am}) signify the amorphous zone which is in the range of 200 and 110.

Present study specifies that cellulose isolated from *Triflolium resopinatum* contain 72% of crystallinity is considered from the maximum value of 894 and 250 is the minimum value displayed in Figure 2.

For the purpose of comparison crystallinity of the isolated cellulose from different biomass which is available in the literature is straw of rice 68%, *Eucalyptus lenceolata* 74%, *Acacia modesta* 71%, *Cedrus deodara* 69%, *Ficus palmate* 63% and *Platanus orientalis* 73%. Our investigation revealed that the crystallinity of *Triflolium resopinatum* is much closer to the literature according to Segal's method and the result is quit efficient [33, 34].

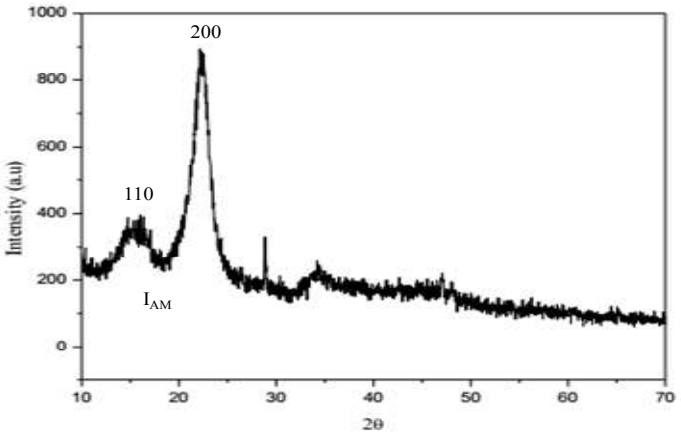

Figure 2. XRD spectrum of *Triflolium resopinatum* for cellulose isolation.

Thermogravimetric analysis

TGA is modern process in which degradation of the sample is detected as a function of temperature or time. The characteristics TG (in weight %) curves of cellulose was analyzed in an inert media at from 25 ^{o}C to 600 ^{o}C [35]. Biomass contains 3 main components such as, (hemicellulose, cellulose and lignin). The complex activity of hemicellulose in thermal decay might be ascribed to its chemical configuration. Hemicellulose contains random amorphous structure with little strength. Furthermore, cellulose is a long polymer of glucose units without any branches which is strong, and showing resistant to hydrolysis. Lignin is altered from

cellulose and hemicellulose, which are composed of polysaccharides. It is very challenging to degrade lignin due to its thermal stability. In thermal decay, the three constituents are in the order of easiest to the problematic to reduce are hemicellulose > cellulose > lignin [36-38].

Figure 3 shows TGA graph of cellulose prepared from the leaves of *Triflolium resopinatum*. The graph signifies that in the first stage 23% of mass loss occurred at 100 ^{o}C due to evaporation of water molecule. At temperature 320-360 ^{o}C the lignin and hemicellulose are decomposed with mass loss of 46%. The total degradation of mass is about 69% up to 600 ^{o}C. The remaining mass is 31% after heating up to 600 °C is due to char which is shown in Figure 3. So we conclude that the result of TGA are favorable for the degradation of cellulosic achieved from *Triflolium resopinatum.*

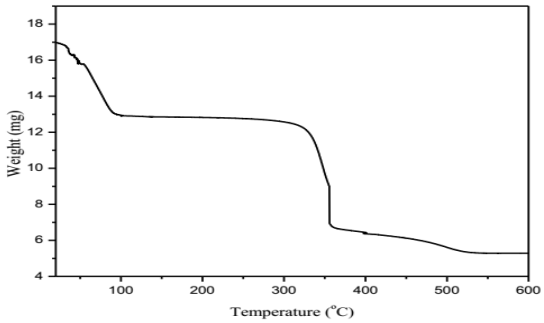

Figure 3. TGA spectrum of *Triflolium resopinatum* for cellulose isolation.

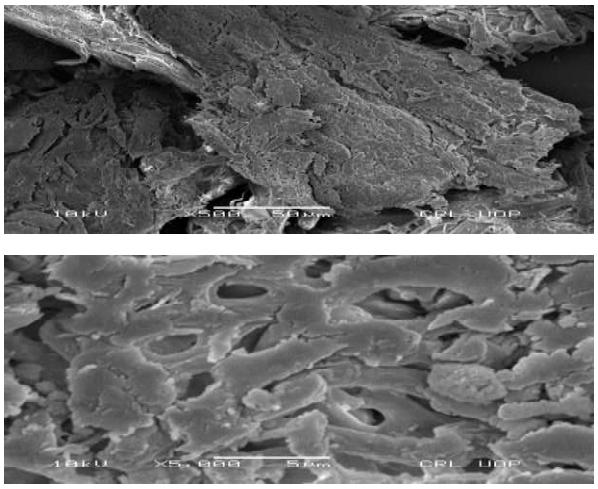

Figure 4. SEM micrograph of *Triflolium resopinatum* for cellulose isolation at a resolution of (X500, X5000).

Scanning electron microscopy

SEM is the analytical technique. Through SEM it is thinkable to identify altered properties on the sample surface according to the periods of pre-extraction and pulping. The changes occurred

in the outer epidermis show the chemical occurrence agonized by the substance at various phase [39].

Scanning electron microscopy was used to determine surface morphology of the isolated cellulose from the biomass of *Triflolium resopinatum*. SEM images were taken at various magnification visibly expose the elimination of pectin, hemicellulose and lignin from the sample surface. SEM analysis represents that the surface possesses pores of divergent forms and sizes which is shown in Figure 4. Maheswari *et al.* [40] extracted cellulose from agricultural residue. The archived sample was examined through SEM. The images displayed different properties such as, structure, morphology and size of cellulose sample.

CONCLUSION

Triflolium resopinatum is low cost, supportable and renewable source. The process of isolation of cellulose is eco-friendly growing concerns about current society and demands of energy. The achieved cellulose was analyzed by altered techniques such as, FTIR, XRD, TGA, and SEM which shows its different aspect and properties. The extracted cellulose has high degree of purity and crystallinity (72%). Thermogravimetric (TGA) analysis specifies the degradation of soluble substances at temperature 250 ^{o}C and lignin/hemicellulose at 320-370 ^{o}C was occurred. FTIR was done at every stage of cellulose isolation which decides different functional groups. XRD signify that the cellulose has 72% crystallinity which is very close to the marketable cellulose. Furthermore, SEM is used to analyze surface morphology and shape of the cellulose. Extremely observable pores with channels were noticed on the surface. The data collected have strong correlation with the literature survey.

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