



Research Paper

Environmental friendly method for the extraction of cellulose from *Trifolium resopinatum* and its characterization

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ABSTRACT

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The leaves of *Trifolium resopinatum* were collected and converted into powder. The ground biomass was treated with different solvents in the Soxhlet apparatus for the removal of soluble extractive and wax substance. For bond breaking, the alkaline substance was kept in the autoclave. Ethylenediaminetetraacetic acid (EDTA) and hydrogen peroxide was used for the removal of most polar substances. Furthermore, raw cellulose was purified through acetic acid and nitric acid. Double distilled water was thereafter used for the neutralization of pH. The analysis of purified cellulose was carried out through different procedures such as X-ray Diffraction (XRD), Fourier Transfer Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA) and Scanning Electron Microscopy (SEM). The extracted cellulose has high crystallinity, thermal stability and good mechanical properties.

Keywords: *Trifolium resopinatum*, EDTA, cellulose,

INTRODUCTION

The biopolymers of cellulose exist abundantly in the universe. The structure of cellulose holds straight chains of D-glucose linked by beta-1, 4-glycosidic bond. D-anhydroglucopyranose delivers OH groups at carbon 2, 3, and 6 for further reactions (Klemm et al., 1998). Cellulose crystals are closely arranged through Van der Waals and intra and intermolecular hydrogen bonding. The properties of cellulose by its structure contain degradability, hydrophilicity and chirality.

Cellulose is insoluble in water because it contains extended chain and superior molecular mass (Lu and Hsieh, 2010; Habibi et al., 2010). Initially, it was used as a raw material for the paper and textile industries. Presently, it is used as a buffer additive to decrease electro osmotic flow in capillary electrophoresis (Haafiz et al., 2013; Leitner et al., 2007). It is also applicable for making shirts, knobs, uniforms, fabrics, toothpaste, purges, food pills, soaps and water based dyes (Rosa et al., 2012; Lorenzo et al., 2014). Moreover, the derivatives of cellulose are widely used in various areas. Recent research indicates an increasing

demand like, the production of bioethanol from cellulose being very important (Karapatsia et al., 2014). Cellulose is the essential component of plant which produce high amount of ethanol (Lorenzo et al., 2014). Internationally in 2008, manufacturing of bioethanol exceeded over 39 billion L. Manufacturing of bioethanol from sugarcane in Brazil is above 17.2 billion L to facilitate about 20% of the country necessities (Tang et al., 1996).

Currently, the isolation of cellulose from biomass which takes excessive effect on the environment is very vital. Literature displays the cellulose isolation from altered sources like, soft wood, hard wood, agricultural waste and residue (Costa et al., 2013; Iskalieva et al., 2012). Derivatives of cellulose consist of methyl, ethyl and propyl. The key reason of isolation was to find out their applications on medical aspect such as, for the treatment of hemorrhoids, diverticulosis, diarrhea and irritable bowel syndrome. Dryness can be prevented by taking enough quantity of it because it has strong attraction towards water adsorption (Diedericks et al., 2012; Rosa et al., 2012).

Furthermore, methyl cellulose in lubricating form is used for the treatment of dry eyes (Bogati, 2011).

Cellulose has numerous applications. 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 (Miranda et al., 2013; Serrano et al., 2011). Particularly, methyl cellulose is used in culture cell virology to detect virus-related duplications. Infection by virus is possible to some extent in cells wherever two tissues are close together (Jahan et al., 2011; Abraham et al., 2011).

Ethyl cellulose is used for the protection of foodstuff worked as an emulsifier. Meanwhile, it is applicable in film base photography, as a border substance for eyeglasses, cigarette and playing cards (Li and McHugh, 2004). Due to pressure and heat plasticizers it can be simply bonded with acetate of cellulose because acetate is destabilized by strong alkaline and oxidizing agents (Krishnamachari et al., 2011).

Nitrocellulose is an adhesive sheath used for immobilization of nucleic acid. Furthermore, it is used to control proteins in Western blots and Atomic Force Microscopy. Usually, its usage causes maintenance in investigative tests where the binding of antigen-antibody occur including pregnancy tests and U-Albumin tests (Zimmermann et al., 2011).

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

MATERIALS AND METHODS

Cellulose isolation

The low cost and easily available biomass was selected for the cellulose isolation. Biomass was grinded into minor units and sieved using 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 lower polar ingredients, 3 h with ethanol (96%) to eliminate the polar constituents and finally, deionized water was used to eliminate the supreme polar extractives and waxy materials. The free extractive biomass was dried in an oven at 80°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°C in 2 atmospheric pressure, with 1:100 g /ml for half an hour. The paste was filtrated and eroded with double distilled water to attain pH 7.

Maximum polar ingredients were detached through bleaching which was reported in our previous work (Mian

et al., 2017). The sample was reacted with 1:25 for 12 h at 48°C in a solution of 2% (v/v), H₂O₂ (99%) and 0.2% (w/v) EDTA solution under vigorous stirring. The filtrated raw cellulose was washed with double distilled water until the pH becomes 7. Further purification of cellulose was carried out under mechanical stirring for 30 min with 80% (v/v) of acetic acid (99.8 to 100%) in a ratio 1:33 and with 65% (v/v) of nitric acid (65%) in a ratio 1:4, at 120°C. The sample was then passed through filter paper and splashed with ethyl alcohol and double distilled water for neutralization of pH.

Characterization

FT-IR Analysis

FTIR (FTCOM-1, 8201PC Shimadzu Fourier) 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 procedure. Spectra were recorded under atmospheric pressure at 25°C in the range of 800 to 2000 cm⁻¹ (Rehman et al., 2014).

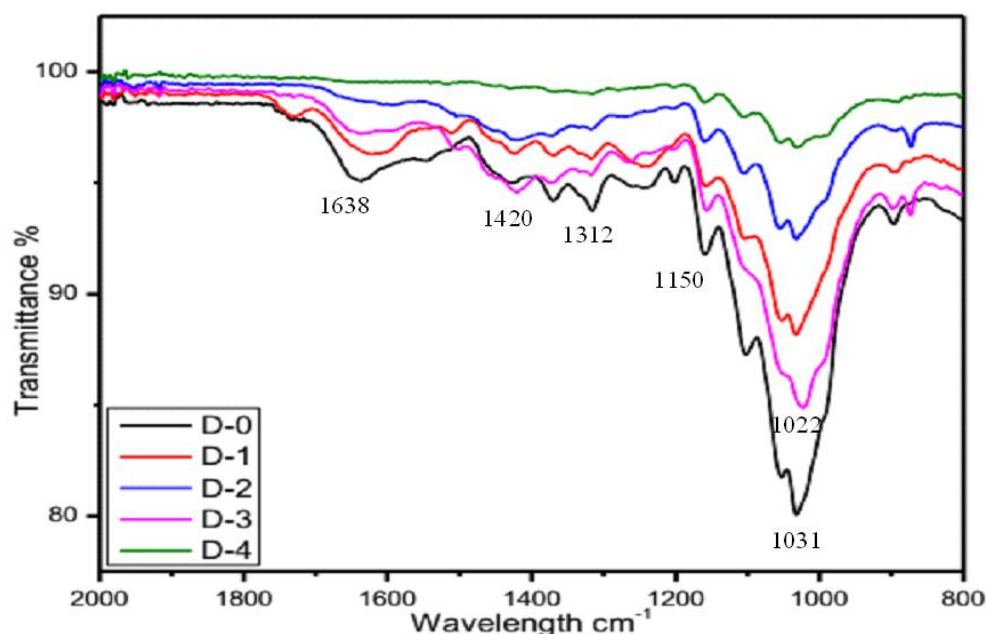
The main constituent of the sample is cellulose, hemicellulose and lignin (Abraham et al., 2011). Table 1 shows the assignment of FTIR spectra at various stages recorded. The detected bands in a spectrum of the crude sample are in the area of 1,636 cm⁻¹ which represent H-O-H bending available in the cellulose which is already present in the market because it specifies the adsorbed water. The peak present at 1,315 cm⁻¹ is related to aromatic ring vibration which shows the presence of lignin and the sample, while 1,031 cm⁻¹ specifies the symmetric alcohols (Sun et al., 2005). The bands of the raw sample changed from others due to disappearance of some groups which appeared in the raw sample. After bleaching-I the peak appeared at 1,420 cm⁻¹ and a solid peak at 1,031 cm⁻¹ specified C-O stretching bond which occurred in the bands of pure cellulose as shown in Figure 1 (Alemdar and Sain, 2008; Jonoobi et al., 2009).

X-Rays diffractometer

XRD (X-ray diffractometer Rigaku D/Max-II, Cu Tube, JAPAN) is an important apparatus used to calculate crystallinity. X-rays indicate accumulated properties of cellulose. X-rays are scattered when focused 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 the greatest technique used to calculate the crystallinity of cellulose because it only needs highest and lowest peak (Bansal et al., 2010; Sheltami et al.,

Table 1: Assignment of infrared adsorption bands of the *Trifolium resopinatum* as designated in table wave length (cm⁻¹).

Raw	1st	2nd	3rd	4I	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 *Trifolium resopinatum* at various phases of cellulose isolation.

2012). The formula used is given as:

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

I_{200} is the top peak which designates the crystalline substituent, while, (I_{AM}) signifies the amorphous zone which is in the range of 200 and 110.

The present study specifies that cellulose isolated from *T. resopinatum* which contains 72% of crystallinity is considered from the maximum value of 894 and 250 is the minimum value as displayed in Figure 2.

For the purpose of comparison, crystallinity of the isolated cellulose from different biomass available in the literature include 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 *T. resopinatum* is much closer to the literature according to Segal's method and the result is quit efficient (Abe and Yano, 2009).

Thermogravimetric analysis

TGA (TA Instruments model TGA Q5000 IR) 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 and was analyzed in an inert media at from 25°C to 600°C (Morán et al., 2008). 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 (Vila et al., 2011; Yang et al., 2007; Hosoya et al., 2007).

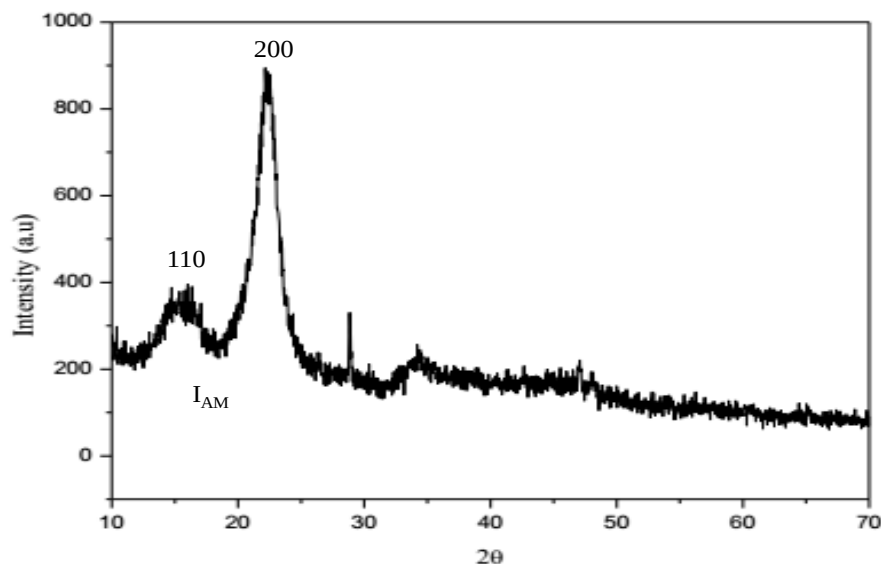


Figure 2: XRD spectrum of *Trifolium resopinatum* for cellulose isolation.

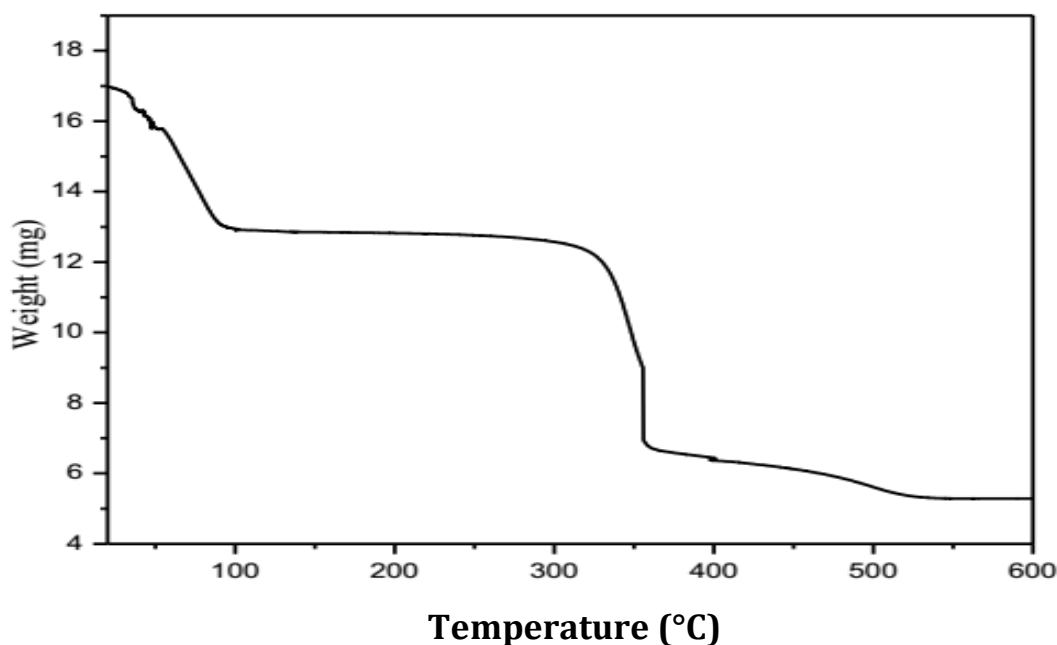


Figure 3: TGA spectrum of *Trifolium resopinatum* for cellulose isolation.

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

Scanning electron microscopy

SEM (JSM 5910, JEOL, JAPAN) is the analytical technique. Through SEM altered properties on the sample surface can be identified according to the periods of pre-extraction and pulping. The changes that occurred in the outer epidermis indicate the chemical occurrence agonized by the substance at various phases (Chandrasekhar et al., 2003).

Scanning electron microscopy was used to determine surface morphology of the isolated cellulose from the biomass of *T. resopinatum*. SEM images were taken at

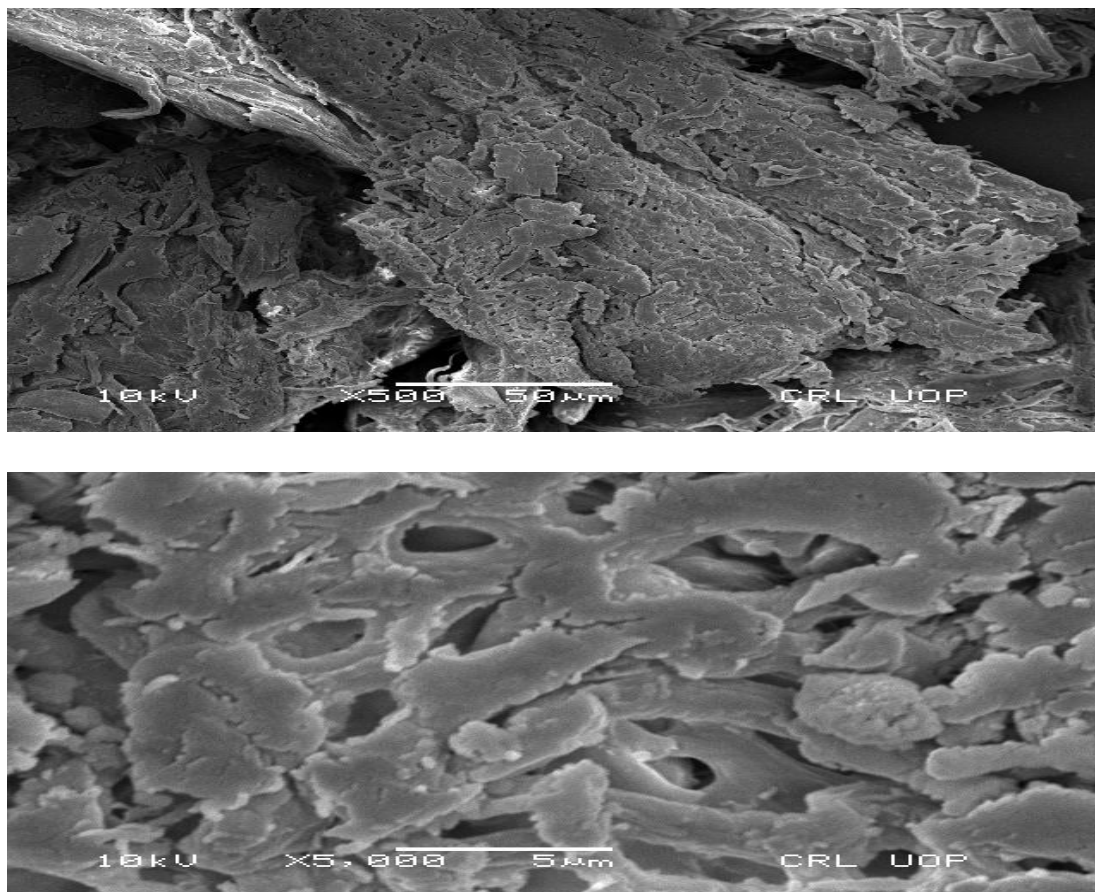


Figure 4: SEM micrograph of *Trifolium resopinatum* for cellulose isolation at a resolution of (X500 and X5000).

various magnifications that visibly exposed the elimination of pectin, hemicellulose and lignin from the sample surface. SEM analysis shows that the surface possesses pores of divergent forms and sizes (Figure 4). Maheswari et al. (2012) 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

T. resopinatum is less expensive, supportable and a 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 aspects and properties. Thermogravimetric (TGA) analysis specified that the degradation soluble extracted at temperature 250°C and lignin/hemicellulose at 320 to 370°C occurred. FTIR was done at every stage of cellulose isolation, a determinant of different functional groups. XRD signified that the cellulose has 72% crystallinity which is very close to the marketable cellulose. Furthermore, SEM was 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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