Extraction of Cellulose from Stem and Bark of *Daphne* papyracea and *Grewia optiva* Plants and Its Characterization

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Highlights:

- Cellulose was extracted from bark & stem of Daphne papyracea and Grewia optiva
- Bark and stem of Daphne papyracea was found to consists of 28.4 % & 38.6 % cellulose
- Bark and stem of Grewia optiva was found to consists of 27.2 % & 35.23 % cellulose

Abstract

Cellulose, the most abundant biopolymer on the earth, has been successfully extracted from bark and stem of Daphne papyracea and Grewia optiva plants. Extraction of cellulose was performed by a series of sequences; pre-alkalization, alkalization, acetylation and acid hydrolysis processes. The extracted cellulose was dried and weighed. The yield % of the cellulose in the bark and stem of Daphne papyracea was found to be 28.43 % and 38.63%, respectively, and in the bark and stem of Grewia optiva was found to be 27.2% and 35.23%, respectively. Extracted cellulose was further characterized by FESEM, XRD and FTIR measurement method. The cellulose content in the stem of the plant is found higher than that of bark. From quantitative point of view, it is recommended as a raw material for paper making industries.

Keywords: Lokta, Daphne papyracea, Grewia optiva, pre-alkalization, acetylation, cellulose,

Introduction

Cellulose, hemicellulose and lignin are categorized as lignocellulose and are the main components of biomass [1]. Abundantly available agricultural wastes and low cost biomasses are the main sources of lignocellulose and is basically used for the production of lignocellulosic materials [2]. Recently, lignocellulosic materials have gained much attention because of their high performance, low cost sources and biodegradability with low or zero carbon emission [3]. Estimation and extraction of lignocellulose are in the forefront of research because of the wide range of applications of lignocellulosic materials.

Biomass-derived cellulose enables multiple functions and transformative applications due to its specific structure and versatile applications [4]. Not limited to this, cellulose resembles unique advantageous properties such as biodegradability, recyclability, renewability, biocompatibility, relatively high resistance and fitness [5, 6]. Nevertheless, cellulose has some characteristics like; incompatible with hydrophobic polymer, aggregation during processing and high water absorption, which limits its uses in some of the area of applications. But it has wide range of application in textile and fiber industries [7, 8].

Cellulose is a water-insoluble, white crystalline structural polysaccharide of glucose monomer unit with a general formula of

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Amrit Research Journal, Dec 2023, Vol. 4

 $(C_6H_{10}O_5)_n$ which makes up about 30 % of the plant cell wall [9]. It contains apparently simple linear chains of glucose residues and has great importance in living systems [10, 11]. The structural formula of cellulose is given in scheme 1.



Scheme 1: Molecular structure of cellulose (n = degree of polymerization)

Humans cannot digest cellulose, but it is important in the diet as fiber. Cellulose is important for the development of reduced calorie emulsion based functional food products [5]. Cellulose can be used to make paper, film, explosives and plastics, in addition to having many other industrial uses. The inorganic paper produced by raw materials has many applications such as fire resistance, pollutant treatment, flexible electronics and energy storage [12, 13]. Most plant materials contain nonfibrous elements or cells and these also are found in pulp and paper [14]. Wood cellulose is a versatile and renewable natural resource that has potential for use in the place of synthetic organic polymers [15]. Cellulose paper and derivatives of cellulose are the most abundant and widely used biopolymers for packaging [16]. The use of cellulose is not only limited for the production of fibers, textiles made of cotton, printing, packaging, decorating, security paper and a number of industrial processes [17-19]. Different studies have been done previously to extract nanocellulosic entities like cellulose nanofibres (CNF) and cellulose nano crystals (CNC) from sisal [20], hemp [21] and flax fibers [22] by special chemical treatments including extractives, pre-alkalization, alkalization, acetylation and acid-hydrolysis. Cellulose extracted from some of the most commonly used softwood trees like spruce, pine, fir, larch, and hardwood trees such as eucalyptus, aspen and birch are used in paper making factory.

In the context of Nepal, *Eulaliopsis binata, Bambusa vulgaris, Danphe panachea,* etc. are most commonly used plants in the production of Nepali paper/Nepali Kagaj [23, 24]. Distribution and availability of such plants have been studied but the cellulose ingredients in the different parts of plant have not been studied yet. Therefore, the extraction of lignocellulosic components from different plant species is not limited due to the wide applicability of lignocellulosic materials. So, in this study, stem and bark of *Daphne papyracea* and *Grewia optiva* have been selected.



Fig 1. (A) Daphne papyracea (B) Grewia optiva

Daphne papyracea is commonly known as Lokta plant in Nepal. It is branched, erect, evergreen shrub of Thymelaeaceae family. It has smooth grey bark with the dull green narrow lanceolate to oblanceolate leathery leaves and has scented white or greenish—white flowers [25] as shown in Figure 1 (a). The genus Daphne is one of the largest genera in the Daphne group, well-known ornamental plants distributed many parts of the world. The shows that the genus Daphne provides the source of raw materials for the production of handmade paper in Nepal. This paper describes the development of a Daphne biomass table to be used in the management of the crop in mixed age, natural forests in the hills of Nepal [26].

A. Rajaure et al., 2023

Grewia optiva is commonly named as Bhimal in Nepal It is a small multipurpose tree found in Nepal and India. Bhimal is found up to an elevation of 1800-2000 m altitude in the north-west Himalayas and in the hills [27]. It is a multifunctional Himalayan tree that is most typically employed as an animal feed source. Furthermore, fibres extracted from the *Grewia optiva* plant have been used by local communities to make ropes, mats, packs, boots, and other items, contributing significantly to the state economy. It is expected to have high lignocellulose content in these parts. However, the detailed lignocellulosic content in the plant stem and bark has not been estimated in both of these plants. This work aims to find the cellulose content of the plants qualitatively.

Materials and Methods

Chemicals and Instruments

All the required reagents and chemicals were taken from the chemistry laboratory, Department of Science, Mahendra Multiple Campus, Dang. The used reagents and chemicals were of reagent grade and were used directly without purification. Chemicals like Ethanol (99.9%, Global India Ltd.), Benzene (98%, Thermo electron), NaOH (97%, Nike Chemical India), Acetic acid (99.5%, RFCL limited), Nitric acid (71%, sp.gr. 1.41, Fine Chemicals Ltd.), and Sulphuric acid (98.0%, sp.gr. 1.84, RFCL Ltd.) were used in the research work. Glass wares like funnel, beakers, weighing machine (KERN WAAGEN), pH meter (Hanna instruments) and grinder were used to complete this project.

Collection of Plant Material

The stem and bark of *Daphne papyracea* was collected from Jaljala, Rolpa (Latitude: 28.04, Longitude: 82.51), Nepal whereas the stem and bark of *Grewia optiva* was collected from Kotgaun, Rolpa (Latitude: 28.32, Longitude: 82.56). The geographical location of the sample collected area is shown in figure 2(a) and 2(b).



Fig 2. Geographical map showing sample collection site A) Jaljala, Rolpa and B) Kotgaun, Rolpa (Source: Google Maps).

Preparation of Powder

About 1 kg of the plant stem and bark was collected and shade dried. The dried sample was then ground into powder by herbal disintigrator. The powder of the sample was then stored in the moisture free desiccator prior to use.

Extraction of Cellulose

The extraction of the cellulose was carried out in the following order: extractives, pre-alkalization, alkalization, acetylation and acid hydrolysis [5]. First, the materials were subjected to solvent mixture of ethanol/ benzene (1:2 v/v) for the extractives. For this, a mixture of 20 mL ethanol, 40 mL benzene was used for each sample and dissolved for 24 hours. The resulting mixture was then filtered, the filtrate was discarded and the residue was taken for further analysis. After successful completion of extractive step, pre-alkalization step was done in next step. In pre-alkalization, the residue from previous step was treated with 2% NaOH in a round bottom flask for 12 h at 40 °C. The mixture was then filtered. The filtrate was discarded and the residue was taken. The residue obtained from pre-alkalization step was then subjected for alkali treatment.

Alkali treatment (alkalization) was done by refluxing the material with 7.5% NaOH in a round bottom flask arranged with condenser system for 90 minutes to remove hemicellulose and lignin. The mixture after reflux was cooled to room temperature,

Amrit Research Journal, Dec 2023, Vol. 4

and filtered. The filtrate was discarded and the residue was taken. The obtained residue was washed with distilled water until the pH of the mixture reached to 6. Then the residue was subjected for acetylation. The acetylation was done by refluxing the obtained material with 60 mL acetic and 10 mL nitric acid (6:1 v/v) with continuous refluxing for 2 h. The acetylated mixture was filtered. The filtrate was discarded and the residue was taken. The residue (acetylated material) was treated with 32% sulphuric acid at 45 °C for 45 minutes under continuous stirring. The action was stopped and the suspension was washed using repeated centrifuge cycle until suspension becomes turbid. The turbid suspension was dialyzed in deionized water for 4 days, and freeze-dried.

Characterization

The extracted cellulose was characterized by various ways. The quantitative determination of cellulose content was performed by collecting white gelatinous cellulose fiber followed by drying in hot air oven and weighing. Similarly, the Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD) and field emission scanning electron microscopy (FESEM) were performed.

Results and Discussion

Yield

The collected cellulose mass was dried in hot air oven below 100 °C. And the weight of the dried sample was measured using a digital balance (PH2204C) at the Department of Chemistry, Amrit Campus, Kathmandu, and tabulated in table 1. The weight of the cellulose collected from bark and stem of the plant sample was measured from each consecutive experiment and yield % was calculated.

Name of Plant	Plant Part	Obtained % of cellulose
Daphne papyracea	Stem	38.63
Grewia optiva	Bark	28.43
	Stem	35.23
	Bark	27.20

Table 1. Cellulose content in stem and bark of the Daphne papyracea and Grewia optiva

The result in table 1 shows that the cellulose content in the stem of the plant sample is higher than that of bark. However, the cellulose content in bark is not bad to report as the primary raw material for paper industries. Generally, the plant materials with cellulose content more than 30 % are reported as efficient raw materials [28]. Based on the data, the plant materials are found to be as rich source of cellulose content and can serve as potential resource for paper making industries.

Visual Characterization

At the end of the extraction procedure, the white gelatinous cellulose in acidic medium was obtained. The gelatinous cellulose mass on filtration and drying gave a white solid mass. The solid mass was washed repeatedly, freeze dried and stored for further characterization. The cellulose fiber in solution form and in dry form is shown in figure 3 (A) and 3 (B).



Fig 3. (A) White gelatinous cellulose fiber in acidic solution, and (B) White cellulose fiber after drying

XRD Characterization

Crystallinity of extracted cellulose was determined using XRD diffractometer using x-ray source of CuKa (λ =1.54Å) at the range 10-80° with scan rate 5°/minute. The diffraction pattern of all cellulose shows highly intense peak around 23° is present which the characteristic peak of cellulose crystal. Similarly, the supporting peaks around 16° and 34° resembles the cellulose crystals. The intense peaks at 16, 23 and 34° corresponds to the crystal planes (110), (200), and (004) respectively, [29, 30]. Similar findings are reported in others work also [31].



Fig 4. XRD pattern of extracted cellulose of Daphne papyracea (DA in figure) and Grewia optiva (GO in figure).

FTIR Characterization

Characterization of cellulose fiber by spectrometric measurement is major technique for the identification of surface functional group. FTIR spectrometric determination of cellulose was carried out at the Department of Chemistry, Amrit Campus, Kathmandu, Nepal. Spectra of cellulose extracted from bark and stem of lokta plant was recorded using Perkin Elmer Spectrometer 10.6.2 version. The background correction was carried out using isopropanol. All the spectral data were collected from 450-4000 cm⁻¹ cutoff range with 4 cm⁻¹ resolutions.

The recorded spectra of stem and bark are shown in figure 5. In both of the case, the absorbance peaks are at the same position, however there is slight difference in peak splitting pattern. All the observed peaks in the range of 2850-3650 cm⁻¹ are the characteristic peaks for stretching vibration of O-H and C-H bonds in polysaccharides [32-34]. The broad beak at 3391 cm⁻¹ is characteristic for stretching vibration of the O-H group on polysaccharides. The peak at 2957 cm⁻¹ in stem and splitted peak at 2957 cm⁻¹ in bark are due to C-H stretching vibration of all hydrocarbons in polysaccharides.



Fig 5. FTIR spectra of cellulose extracted from stem and bark of Daphne papyracea (DA in figure) and Grewia optiva (GO in figure) plant.

The peaks in the region of 1750-900 cm⁻¹ is typical for cellulose identification. The peak located at 1635 cm⁻¹ is due to unsaturated double bond carbon. The strong peaks at 1164, 1159, 1034 and 897 cm⁻¹ is due to stretching and bending vibrations of $-CH_2$, -C-H, -O-H and C-O bonds in cellulose [35-37]. Presence of these peaks ensures the cellulose.

FESEM characterization

The surface morphology of the as-extracted cellulose was studied in terms of field emission scanning electron microscopy (FESEM). The FESEM images of cellulose extracted from stem of *Daphne papyracea* and *Grewia optiva* with corresponding EDX mapping are shown in the figure 6. Images show the fiber nature of cellulose mass. The EDX mappings show the presence of carbon and oxygen which are the essential constituents of cellulose. In addition, the presence of Sulphur in the EDX mapping could be associated with the dopant Sulphur obtained from the sulphuric acid used in acid hydrolysis process.

Fig 6. FESEM image of cellulose extracted from stem of (A) *Daphne papyracea* and (B) *Grewia optiva* with corresponding EDX mapping (C and D, respectively)

Conclusions

The lignocellulosic material, cellulose was successfully extracted from bark and stem of *Daphne papyracea* and *Grewia optiva* plants. Extracted cellulose was characterized by XRD, FTIR and SEM techniques. The cellulose content in the plant sample has been quantified and can be listed in the high lignocellulose containing materials. XRD characterization revealed the pure crystalline nature of extracted cellulose. Similarly, FTIR spectra best resembled with the cellulose material. FTIR spectra of cellulose extracted from bark and stem were found similar indicating similar polymeric cellulose content in both of the cases. Based on the quantity and chemical characterization it can be recommended as best raw material for paper making industries.

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