Synthesis and characterization of nanocellulose and wood fibers maceration in *Adina cordifolia* (Roxb.) Brandis

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Abstract - *Adina cordifolia (*Roxb.) Brandis (Rubiaceae) contains a superior source of pulp for manufacturing paper of the utmost quality. It is a huge deciduous tree that grows wild in Bangladesh's rocky terrain. It can withstand drought and is fire resistant. The tree's bark has antibacterial properties and medicinal properties. An economical method for full maceration of its cellulose was developed during the process of fiber analysis. Wood core samples of *Adina cordifolia* were collected from the hills of Muthumalai and their fibers were macerated using various concentrations of macerating agents (Nitric acid) 40% and 50%. The separation of all the fibers with 50% nitric acid was found to be quite effective. All of the compact fibers are resolved clearly by this maceration process, making their measurement simple. In addition to wood maceration, Nanocellulose was also extracted by using acetic acid and sodium hypochlorite. The technique could be utilized to produce a high-quality paper as well as research of wood fibers for the purpose of discovering better populations.

***Index Terms*: *Adina cordifolia,* Nitric acid, Acetic acid, Sodium hypochlorite, Wood fiber, Maceration.**

I. INTRODUCTION

Wood from numerous trees has been used for high-quality furniture, pulp, and paper industry repeatedly since the dawn of time. Sustainability and strength of items based on the mechanical strength of wood fibers. Sapwood usually has longer cellulosefibers than heartwood and contributes 40–45% the dry weight of the wood's fiber wall, making it appropriate for pulping. Processing mechanically and chemically is required for pulp production. While fibers can easily blend chemically without breaking, they cannot cut in a mechanical process. Fiber length is one of the quality criteria for pulpwood, and it has undergone substantial research in connection to tree age and within-tree position. The separation method is one technique that makes cellular structure visible in cells.Chemicals are given to the target plant, dissolving the intermediate lamella and enabling the separation of the cells and threads. These fiber aggregations have the impression of being a single fiber. The maceration technique, sometimes known as "test-tube pulping," is actually small-scale pulping. Certain procedures cause bleaching as well as maceration. **[4]**

The production of cellulose results from the biosynthesis of plants, animals, or bacteria, whereas the term "nano cellulose"generally refers to cellulosic extracts or treated substances with specified nanoscale structural dimensions. Nanocellulose is classified into three types of materials: (I) cellulose nanocrystals (CNCs), also known as nanocrystalline cellulose (NCC) and cellulose nano whiskers (CNWs), (II) cellulose nanofibrils (CNFs), also known as nano-fibrillated cellulose (NFC), and (III) bacterial cellulose (BC). Nanoparticles are extracted from cellulose sources using a variety of methods. **[2]**

FTIR spectroscopy may be applied to good advantage in such specialized areas as micro analysis where high sensitivity isrequired, in the analysis of aqueous solutions or dark, solid-state samples that require the use of special reflectance techniques, in investigations placing emphasis on quantitative evaluation, and in experiments where analysis time is a limiting factor, e.g., in process or quality control measurements. **[8]**

Adina cordifolia which is also known as Haldu is a deciduous tree that can grow well over 20 meters high. Oppositely arranged leaves are broadly oval in shape, heart-shaped at the base, and pointed at the tip. The flowers may be insignificant individually but are very pretty when they bloom together in balls with a circumference of 2 to 3 cm. They are usually yellow in color often tinged with a shade of pink. Haldu is at its blossoming best during winter. The bark of the tree acts as an antiseptic.
A fluorescence microscope is used to study organic and inorganic samples. Fluorescence microscopy uses fluorescence and phosphorescence to examine the structural organization, and spatial distribution of samples. It is particularly used to study samples that are complex and cannot be examined under the conventional transmitted-light microscope. Fluorescence microscopy images helps to study substances present in low concentrations where high sensitivity is crucial to detect them.

II. MATERIALS AND METHODS

MACERATION

The wood core samples of *Adina cordifolia* were collected from the hills of Muthumalai. In a 37% formaldehyde solution, the collected wood core samples were submerged. In order to prevent further evaporation of fumes, the samples were drained from the formaldehyde solution before beginning the maceration procedure. Nitric acid in concentrations of 40% and 50%were taken. Wood core samples were placed in test tubes, fully submerged in nitric acid solution, and heated in a water bath to 70ºC. After being taken out of the water bath, test tubes containing macerated fibers were allowed to cool to room temperature. After cooling, nitric acid was removed, and the macerated fibers were cleaned three times with distilled water before being filtered through Whatman Grade 1 filter paper. Before and after immersion in nitric acid, precautions for a well-drained formaldehyde solution are required due to the presence of nitrogen oxide, carbon dioxide, and nitric acid in the fumes (when formaldehyde-preserved samples are immersed in nitric acid, the exothermic reaction occurs due to the oxidation of formaldehyde). Fibers were then stained with a 20% safranin solution for slide preparation, and they were then washed once more with distilled water to remove any remaining safranin stain. Placed some amount of fiber suspension on a standard glass slide with the help of an ink/medicine dropper and allowed for air drying. Mounting was done in Canada balsam using a cover glass. The use of glycerol enhances the visibility of fibers.**[4]**

EXTRACTION OF NANOCELLULOSE

In a nutshell, sodium hydroxide (4%), an alkali, was used to treat the cellulose after it had been treated to remove lignin and hemicelluloses. Acetic acid, sodium hypochlorite, and distilled water were added for the bleaching process and allowed to runfor 4 hours at 60° C. With the help of extra distilled water, the mixture was allowed to cool and filtered. The dried filtrate was then processed for 40 minutes with constant stirring in 10 mol-1 L of H_2SO_4. In order to achieve a pH of between 5 and 6, the above-mentioned mixture was washed with distilled water at 10,000 rpm, 10 °C, for 10 minutes. The resulting suspension underwent 30 minutes of sonication and freeze-dried to obtain solid nanocellulose.[3] This method used to extract nanocellulose was identical, with a few minor adjustments which is derived by Johar **[9].**

FTIR ANALYSIS OF ADINA CORDIFOLIA

The FTIR instrument sends infrared radiation of about 10,000 to 100 cm^{-1} through nanocellulose (sample), with some radiation absorbed and some passed through. The absorbed radiation is converted into rotational and vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm^{-1} to 400 cm-1, representing a molecular fingerprint of the sample. In the present study nanocellulose was used for FTIR (Fourier Transform Infrared Spectroscopy) analysis.

XRD ANALYSIS OF ADINA CORDIFOLIA

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector.
X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being Kα and Kβ. Kα consists, in part, of Kα1 and Kα2. Kα1 has a slightly shorter wavelength and twice the intensity as Kα2. The specific wavelengths are characteristic of the target material (Cu). Filtering, by foils or crystal monochromators, is required to produce monochromatic X-rays needed for diffraction. Kα1and Kα2 are sufficiently close in wavelength such that a weighted average of the two is used. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or a computer monitor.

III. RESULTS AND DISCUSSIONMACERATION

The wood samples of *Adina cordifolia* were employed in partial maceration using 40% and complete maceration using 50% ofnitric acid. *Adina cordifolia* wood maceration fibers were observed under fluorescent microscopy, which shows better results in 50%of nitric acid than in 40% of nitric acid **[Fig. 1].**

Nitric acid acts as an easy and fast resolving agent to break down the middle lamella for separating the cells. Boiled nitric acid separates organs/cells much faster. Results reveal that 50% nitric acid is not only convenient for maceration in hot conditions but dissolves other extractives also. This protocol helps to reduce the time and chemicals' cost. The central lamella is easily and quickly broken down by nitric acid, allowing the cells to be separated. Organs and cells separate significantly faster when nitric acid is heated. According to the present findings, 50% nitric acid dissolves other extractives in addition to being practical for maceration in hot conditions. This procedure aids in reducing the expense and time of the chemicals.

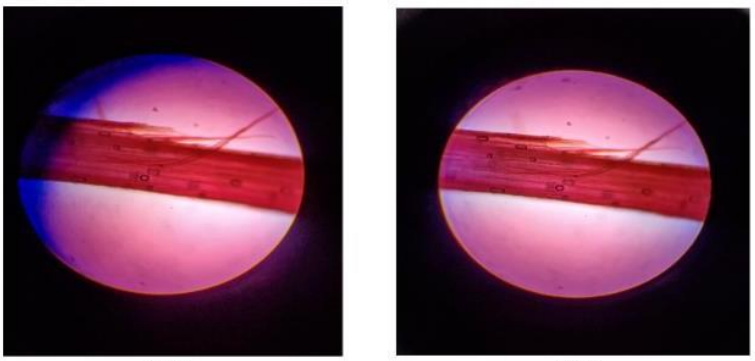

Figure 1: Macerated fibers of Adina cordifolia

Franklin (1945) used acetic acid and hydrogen peroxide but only partial maceration was observed in samples incubated at 70°Cfor 3 hrs. But in the present results the method was not found efficient to macerate *Adina cordifolia* fibers and was comparatively time-consuming and costly also. According to Jeffrey (1917), maceration by using a mixture of equal portions of freshly combined8 to 10% nitric acid and chromic acid was not efficient to present concordance maceration techniques.
The present studies reveal that the wood samples of *Adina cordifolia* were employed in partial maceration using 40% and complete maceration using 50% of nitric acid. *Adina cordifolia* wood maceration fibers were observed under fluorescent microscopy, which shows better results in 50% of nitric acid than in 40% of nitric acid.

FLUORESCENCE MICROSCOPE ANALYSIS OF ADINA CORDIFOLIA

It shows the image of nanocellulose extraction of *Adina cordifolia* by magnification after being treated with the chemo-mechanical method. *Adina cordifolia* cellulosic fiber has been observed in a size range of 40-200 nm in diameter **[Fig 2].**

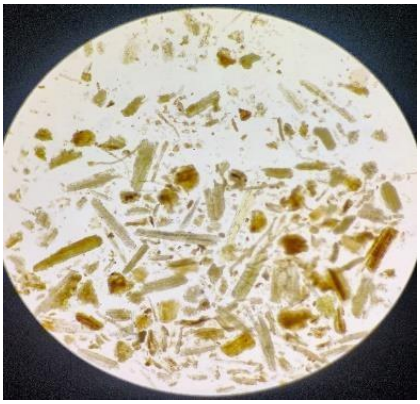

Figure 2: Fluorescence microscopic view of nanocellulose

According to Babi *et al.*, (2022), the visualization of naturally derived cellulose nanofibrils (CNFs) and nanocrystals (CNCs) within nanocomposite materials partially shows the nano structural features of cellulose through super-resolution microscopy, but in current observation, the nanocrystals show the better nano structural features of cellulose through fluorescent microscopy.

FTIR ANALYSIS OF ADINA CORDIFOLIA

The extracted nanocellulose was passed into the FT-IR, and the functional groups of the components were separated on its peak radiation were represented in the graph. The present investigation results of FT-IR analysis is confirmed the presence of alcohol, alkenes, carboxylic acid, ethers, sulfonyl chloride, and alkyl halides, and these functional groups are clearly shown with bonds in the graph **[Fig 3].**

The FT-IR spectroscopic analysis showed the presence of phytoconstituents. It gives a broad peak of **3350.25** (alcohol), **2922.10** (alkenes), **1641.42** (carboxylic acid), **1230.37** (ethers), **1031.92** (sulfonyl chloride), and **688.59** (alkyl halides). Using these peak values, the functional groups of the active components present in the nanocellulose were identified in the region of IR radiations, which confirmed the production pulp of *Adina cordifolia.*

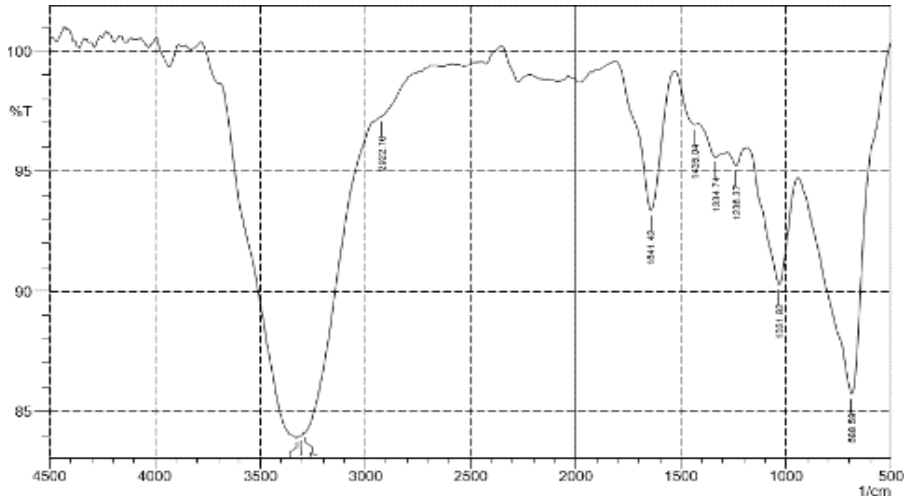

Figure 3: FTIR Analysis of *Adina cordifolia*

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Nataraj *et al*., (2022) confess that, the reduction in the intensity of the peaks suggests that cellulose loses its crystallinity during regeneration and successive dispersion to form nanocellulose. The peaks around 3400 cm^{-1} are due to C-H and O-H group vibrations.

In the present analysis, it manifests that the presence of phytoconstituents gives a broad peak of 3350.25. Using these peak values, the functional groups of the active components present in the nanocellulose were identified.

S.NO	PEAK	BOND	FUNCTIONAL GROUP
1	3350.25	O-H STRETCHING	ALCOHOL
2	2922.10	C=C STRETCHING	ALKENES
3	1641.42	O-H BENDING	CARBOXYLIC ACID
4	1230.37	C-O STRETCHING	ETHERS
5	1031.92	S=O STRETCHING	SULFONYL CHLORIDE
6	688.59	C-Br STRETCHING	ALKYL HALIDES

Figure 4. FTIR Interpretation of compounds

XRD ANALYSIS OF ADINA CORDIFOLIA

The results obtained from the XRD analysis show that copper is the most common target material for single-crystal diffraction,with CuK_α radiation = 1.5418Å. These X-rays are collimated and directed onto the sample. It shows that the nanocellulose extractedfrom *Adina cordifolia* is amorphous in nature, which means the sample is without definite character or nature **[Fig 5].**

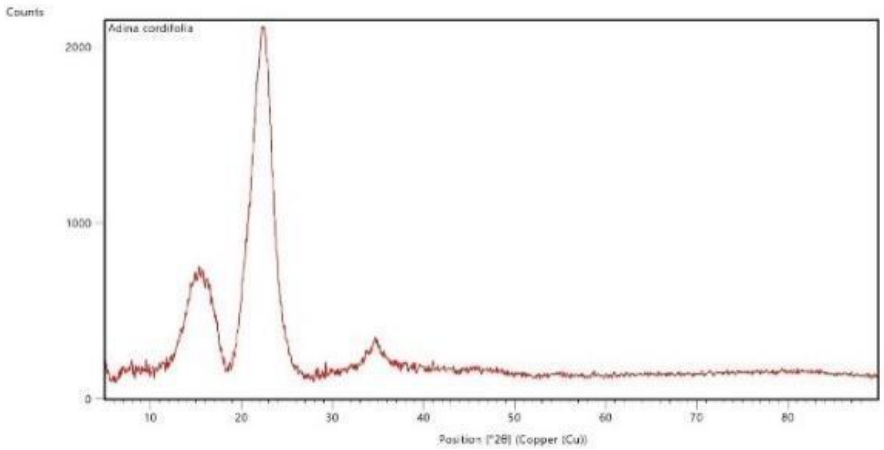

Figure 5: XRD Analysis of *Adina cordifolia*

Nataraj *et al*., (2022) explain that nanocellulose shows the characteristic crystalline peaks at 2 theta values of 12.03°, 20.19°, and 22.5°, corresponding to the (110), (−110) and (200) lattice planes, respectively, which is a characteristic of cellulose IIstructure. In my present studies, the nanocellulose extracted from *Adina cordifolia* shows the amorphous condition in nature.

IV. ACKNOWLEDGEMENT

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REFERENCE:

1. Frentrup, M., Lee, L. Y., Sahonta, S. L., Kappers, M. J., Massabuau, F., Gupta, P., ... & Wallis, D. J. (2017). X-ray diffraction analysis of cubic zincblende III-nitrides. Journal of Physics D: Applied Physics, 50(43), 433002.
2. Abitbol, T., Rivkin, A., Cao, Y., Nevo, Y., Abraham, E., Ben-Shalom, T., ... & Shoseyov, O. (2016). Nanocellulose, a tinyfiber with huge applications. Current opinion in biotechnology, 39, 76-88.
3. Nataraj, D., Hu, C., & Reddy, N. (2022). Extraction and characterization of nanocellulose from pongamia pinnata oil meal. Cellulose chemistry and technology, 56(1-2), 29-37.
4. Mahesh, S., Kumar, P., & Ansari, S. A. (2015). A rapid and economical method for the maceration of wood fibers in Boswellia serrata Roxb. Tropical Plant Research, 2(2), 108-111.\
5. Carlquist, S. (2013). Comparative wood anatomy: systematic, ecological, and evolutionary aspects of dicotyledon wood. Springer Science & Business Media.
6. Berthomieu, C., &Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. Photosynthesis research, 101,157-170.
7. Berthomieu, C., &Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. Photosynthesis research, 101,157-170.
8. Faix, O. (1992). Fourier transform infrared spectroscopy. Methods in lignin chemistry, 83-109.
9. Phanthong, P., Reubroycharoen, P., Hao, X., Xu, G., Abudula, A., & Guan, G. (2018). Nanocellulose: Extraction and application. Carbon Resources Conversion, 1(1), 32-43.
10. Franklin GL (1945) Preparation of thin sections of synthetic resins and woody resin composites and a new method for wood. Nature 155: 3924–3951.
11. Jeffrey EC (1917) The anatomy of woody plants. University of Chicago Press, Chicago, Illinois
12. Babi, M., Fatona, A., Li, X., Cerson, C., Jarvis, V. M., Abitbol, T., & Moran-Mirabal, J. M. (2022). Efficient labeling of nanocellulose for high-resolution fluorescence microscopy applications. Biomacromolecules, 23(5), 1981-1994.