PROJECT REPORT

On

SYNTHESIS AND CHARACTERIZATION OF NANOCRYSTALLINE CELLULOSE DERIVED FROM PINEAPPLE CROWN FIBERS

Submitted by

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In partial fulfillment for the award of the

Bachelor's Degree in Chemistry

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

2023-2024

DEPARTMENT OF CHEMISTRY AND CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)

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This is to certify that the project **"SYNTHESIS AND CHARACTERIZATION OF NANOCRYSTALLINE CELLULOSE DERIVED FROM PINEAPPLE CROWN FIBERS"** is the work done by **EMY MARIA.**

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CERTIFICATE

This is to certify that the project work entitled **"SYNTHESIS AND CHARACTERIZATION OF NANOCRYSTALLINE CELLULOSE DERIVED FROM PINEAPPLE CROWN FIBERS"** is the work done by **EMY MARIA, SANDRA JAMES, THENNAL P S** and **LIYA M G** under my guidance in the partial fulfilment of the award of the Degree of Bachelor of Science in Chemistry at St. Teresa's College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

DR. JAYA. T. VARKEY

PROFESSOR

PROJECT GUIDE

DECLARATION

I hereby declare that the project work entitled "S**YNTHESIS AND CHARACTERIZATION OF NANOCRYSTALLINE CELLULOSE DERIVED FROM PINEAPPLE CROWN FIBERS**" submitted to Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, is a record of an original work done by me under the guidance of **DR. JAYA. T. VARKEY, PROFESSOR**, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam and this project work is submitted in the partial fulfilment of the requirements for the award of the Degree of Bachelor of Science in Chemistry.

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Acknowledgement

We are immensely grateful to God Almighty for his unceasing blessings for the successful completion of our final year project.

We sincerely thank Rev. Dr. Sr. Vinitha CSST, Provincial superior and Manager, and Dr. Alphonsa Vijaya Joseph, Principal of St. Teresa's College, Ernakulam for their assistance and for providing excellent facilities for academic and personal growth of the students.

We thank Dr. Saritha Chandran A, HOD of the Chemistry Department, for providing an opportunity to do the project and giving all support and guidance which helped us to complete the project duly.

We would like to express our profound sense of gratitude to Dr. Jaya T Varkey, Professor and Research guide, the Department of Chemistry, St.Teresa's College for her valuable guidance, personal attention, significant recommendations, help, and encouragement.

We sincerely thank Associate professor Dr. Ushamani M, for her valuable guidance.

We would also like to express our gratitude to Mrs. Sicily Rilu Joseph, Research Scholar, Department of Chemistry, St. Teresa's College, for her continuous guidance and support throughout the work.

We would also like to thank Mrs. Tiya K J, Teresian Instrumentation and Consultancy Center (TICC) for helping us to do the antibacterial studies and Mrs. Priya, former faculty of Department of Chemistry and Ms. Sophiya, senior M.Sc. student for being with us.

We would also like to express our sincere gratitude to all our teachers and non-teaching staff for their wholehearted help throughout our project.

We heartily thank STIC (CUSAT) and Bharat Mata College for providing all the spectroscopic assistance needed for the characterization of the samples within the time limit.

Last but not least, We express our heartfelt thanks to our loving family and friends for their concern, care, and support while we were pursuing our passion.

EMY MARIA
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Chapter 1

Introduction

In recent times, there has been a growing inclination towards replacing conventional materials with sustainable alternatives, prompted by the imperative to mitigate the detrimental impact on the environment. Simultaneously, extensive investigations have been undertaken to explore the potential utilization of cellulosic-rich wastes[1].

Hemicellulose, a complex polysaccharide polymer, is characterized by its branched structure and consists of various sugar types such as glucose, xylose, galactose, arabinose, and mannose. On the other hand, lignin is a phenolic polymer that exhibits a high degree of cross-linking. While hemicellulose and lignin are both amorphous polymers, cellulose, in contrast, possesses a semi-crystalline nature. The linkages between hemicellulose groups and lignin can be classified into two distinct types.

One type of bond found in hemicellulose is an ester bond between the hydroxyl of lignin and the carboxyl of uronic acid. This bond is sensitive to alkali solutions, which can be used to remove hemicellulose, soluble mineral salts, and other components from raw fibers. Another type of bond is an ether bond between the hydroxyls of lignin and carbohydrates. Acid hydrolysis can be used to disrupt other types of bonds, releasing individual cellulose nanocrystals (CNCs) with high mechanical strength, surface area, and aspect ratio. CNCs are non-toxic, biocompatible, and biodegradable, making them ideal for pharmaceutical applications such as composite materials, regenerative medicine, and drug delivery. Recent studies have focused on extracting CNCs from food industry wastes and natural sources such as wood, sisal, coconut husks, agave fibers, bananas, rice husks, soy hulls, mango seeds, sweet potato residue, garlic skins, and pineapple leaves [2-4].

Pineapple, a widely consumed fruit, generates approximately 3 billion tons of by-products annually, including pineapple leaves, which contribute to environmental pollution and agricultural land issues. Pineapple crown leaves (PCL) consist of cellulose, hemicellulose, and lignin, with cellulose comprising 79-83% of the material. Recently, PCL has been utilized in the production of textiles, paper, and as a reinforcement in polymers. However, the burning of PCL in large quantities causes environmental pollution, necessitating the need to reduce waste and utilize PCL as a source of cellulose. Cellulose, a biodegradable and renewable polymer material, can be processed into various forms, including nanocrystalline cellulose (NCC). NCC is a new material with unique physical and chemical properties, such as biocompatibility, non-toxicity, hydrophilicity, increased crystallinity, dispersion ability, and biodegradation. These properties make cellulose widely applicable in various fields, including polymer reinforcement, biodegradable product additives, and membrane reinforcement, and hold great potential for the development of renewable biomaterials in chemistry, food, pharmacy, and other fields [5-7].

1.1 PINEAPPLE

Pineapple, scientifically referred to as Ananas comosus, is a highly sought-after tropical fruit that is renowned for its delectable taste and widespread consumption across the globe. This fruit is recognized for its composition of numerous volatile organic compounds (VOCs) present in different concentrations. Extensive efforts have been dedicated to comprehending the specific VOC that contributes significantly to the sensory aroma characteristics exhibited by this fruit. It is a short-lived perennial monocot, thrives in tropical climates, and is the third most cultivated tropical fruit after bananas and citrus. The 'Cayenne' or 'Smooth Cayenne' variety is the most commonly grown pineapple for consumption. Pineapple plants can grow up to 2-4' tall and have spiky, thick long leaves that form a tight rosette. Optimum growth is achieved in temperatures ranging from 68-86 degrees Fahrenheit. In temperate areas, pineapples can be grown as an attractive houseplant in potted containers or as an outdoor ornamental plant, but require consistent moisture, acidic soil, bright, indirect sun, and high humidity. Due to its shallow root system, a 3–7-gallon pot is sufficient for container growth. The components of a pineapple plant comprise the crown leaves, peduncle, slip, second ratoon sucker, unfruited first ratoon sucker, old mother plant peduncle, fruiting first ratoon sucker, and mother plant [8-9].

The pineapple industry is unique in that only one fruit can be obtained from each harvested plant, unlike other fruits and vegetables. This single fruit is the sole source of revenue for pineapple producers, making it a crucial component of the industry. Unfortunately, the pineapple plant leaves, which make up 75% of the harvested product, are typically discarded and considered waste. As a result, every producing company discards approximately one ton of pineapple leaves daily. Furthermore, it is noteworthy that the pineapple industry lacks a proper waste management system, resulting in the accumulation of waste in production fields for natural degradation or disposal through garbage trucks. This uncontrolled approach poses various challenges, including the emission of greenhouse gases, alteration of soil pH, proliferation of pests that harm neighbouring crops, and the spread of infectious diseases that can potentially harm workers[10].

1.2 THE DETRIMENTAL CONSEQUENCES RESULTING FROM AGRICULTURAL WASTE

Agricultural waste, when present in significant quantities, can exert detrimental effects on the environment and habitat. This is primarily manifested through the release of greenhouse gases, the generation of unpleasant Odors, and the potential contamination of water sources by toxic liquids. During the early autumn season, the burning of agricultural waste on a large scale across the globe leads to the occurrence of smog. The World Health Organisation (WHO) has identified the burning of agricultural waste as a significant contributor to ambient air pollution, thereby making it one of the primary sources of smog. In addition, the combustion of agricultural waste, often employed to prepare fields for cultivation, can result in the emission of substantial quantities of greenhouse gases and particulate matter into the surrounding atmosphere. Furthermore, the disposal of agricultural waste contributes to environmental degradation by releasing heavy metals and other hazardous substances[11].

1.3 NANOCHEMISTRY

Nano chemistry is a sub-field of chemical and material science that deals with the study of innovative techniques for producing materials at the nanoscale. In 1992, Ozin coined the term "nano chemistry". Nano chemistry focuses on solid-state chemistry that is less concerned with matter formation and more with the synthesis of building blocks that depend on features such as size, shape, defect, and surface area [12-13].

The degrees of freedom of the atoms in the periodic table are the primary focus of atomic and molecular properties. Nevertheless, through the conversion of materials into solutions, nano chemistry added more degrees of freedom. The limited tininess of nanoscale objects leads to the emergence of unique material characteristics. Several chemical alterations on structures at the nanoscale validate size-dependent effects.

Applications in engineering, biology, medicine, and physical, chemical, and materials science all make use of nano chemistry. Iron oxide, or rust, can be converted via nano chemistry into the most potent MRI contrast agent, which can both identify and eradicate cancers in their early stages. Lights can be bent or stopped in their tracks with silica, or glass. Silicone is also utilized in developing nations to create circuits for fluids used in pathogen detection. Through the process of nano-construct synthesis, the building blocks self-assemble to form functional structures that could be applied to bioanalytical, photonic, electronic, or medical issues[14].

1.4 NANOCELLULOSE

Nano-structured cellulose is known by the term nanocellulose. This can be either bacterial nanocellulose, which is a term for nano-structured cellulose made by bacteria, or cellulose nanocrystal (CNC or NCC), cellulose nanofibers (CNF), also known as nano fibrillated cellulose (NFC).

Utilized as a rheological modifier, reinforcing agent, and additive in various high-performance materials and applications, nanocellulose is a high-performance additive. Through homogenization and high-power ultrasonic milling, nanostructured fibrils can be effectively isolated from any cellulose-containing source. Higher fibrillation, a greater yield of nanocellulose, and thinner fibers can all be achieved with sonication. Because of the extremely high cavitation shear forces, ultrasonic technology outperforms traditional methods of producing nanocellulose.

Due to the production process's excessive cost in terms of energy and economy, it was initially difficult to market; however, in recent years, a great deal of research and experimentation has allowed for a significant reduction in costs [15-16].

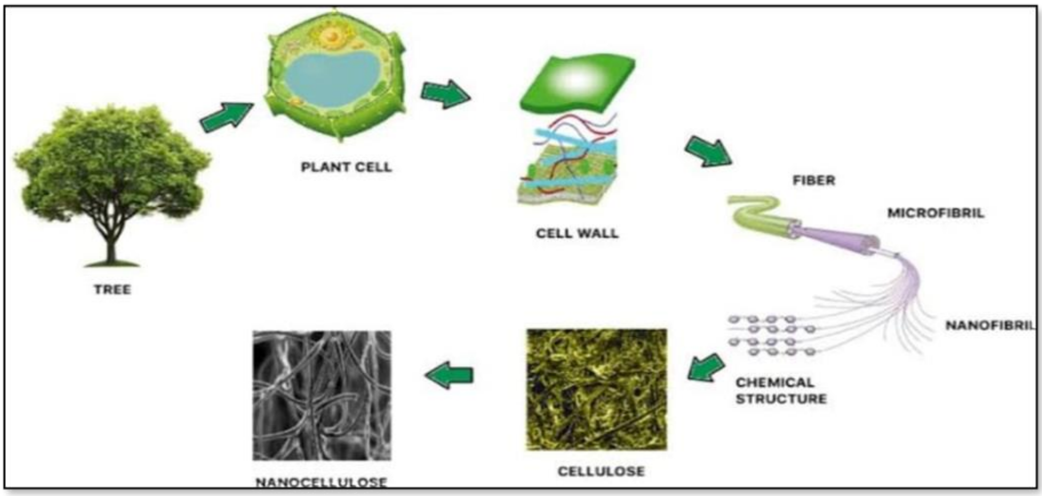

Fig.1 Nanocellulose

1.4.1 CELLULOSE NANOCRYSTAL

A unique combination of structural and physicochemical properties, including renewability, low density, biocompatibility, adaptable surface chemistry, optical transparency, nontoxicity, biodegradability, and improved mechanical properties, has made cellulose nanocrystals (CNCs) a highly sought-after sustainable bio-based nanomaterial globally.

Biomedicine, pharmaceuticals, electronics, barrier films, nanocomposites, membranes, and supercapacitors are just a few of the industries were

CNCs show promise for use. New resources, new extraction techniques, and new functionalization processes have been developed as a result of the growing industrial demand for novel forms of CNC-based nanomaterials. CNCs can be functionalized for desired applications with enhanced functionality due to their large surface area and abundance of surface hydroxyl groups. Cellulose, the most prevalent and nearly limitless natural polymer, is the source of cellulose nanocrystals, which are special nanomaterials. The mechanical, optical, chemical, and rheological properties of these nanomaterials have sparked a lot of interest. For the majority of applications, cellulose nanocrystals—which are mostly derived from naturally occurring cellulose fibers—serve as a sustainable and environmentally friendly material because they are renewable and biodegradable in nature. These nanocrystals are essentially hydrophilic by nature, but they can be surface functionalized using hydrophobic polymer matrices to satisfy a variety of demanding needs, like the creation of high-performance nanocomposites.

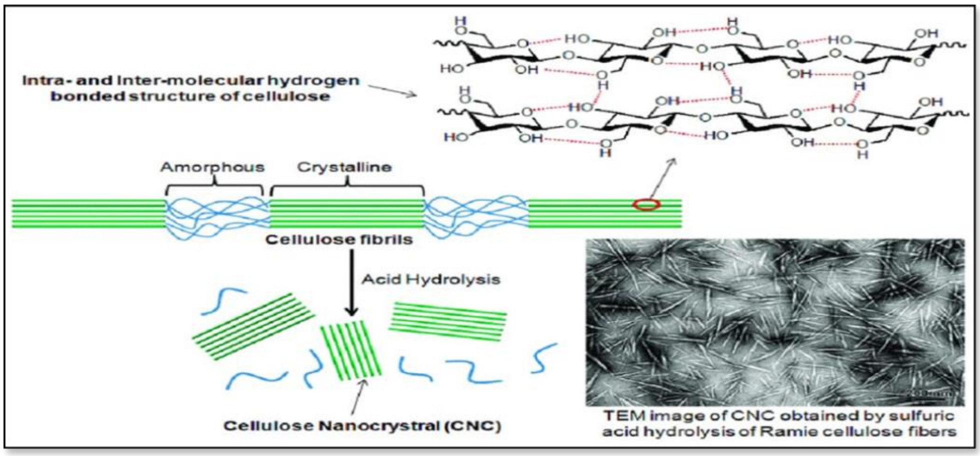

Fig.2 Graphical depiction of a CNC (cellulose nanocrystal) derived from cellulose chain.

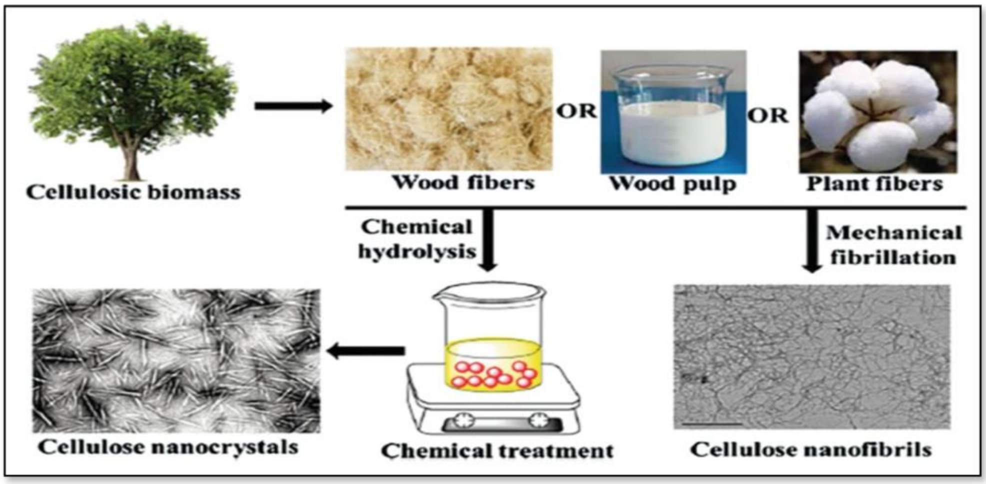

Fig.3 Synthesis of Cellulose Nanocrystals

USES OF CELLULOSE NANOCRYSTAL

1. BIOMEDICAL ENGINEERING: The application of CNCs in biomedical engineering is quite common. CNCs are modified on the surface and employed as scaffolds for tissue engineering, drug delivery vehicles, biomarkers or sensors, gene vectors, antibacterial and antiviral agents, and biocatalyst scaffolds.

2. WASTE WATER TREATMENT: For use in a variety of water treatment processes, including adsorption, absorption, flocculation, membrane filtration, catalytic degradation, and disinfection, CNCs have been the subject of intensive research. The high specific surface area, high specific strength, hydrophilicity, and biodegradability of CNCs make them desirable candidates.

3. ENERGY AND ELECTRONIC SECTOR: Organic electronics and energy storage have drawn more and more attention to nano cellulosic materials. While CNCs are electrical insulators, wood-based CNCs have

intriguing piezoelectric qualities and a powerful dipole that enables them to align in an intense electric field. Furthermore, they provide strength by using templating to produce a conductive composite.

4. EMERGING APPLICATIONS OF CELLULOSE NANOCRYSTAL: Research has expanded the applications of cellulose nanocrystals beyond the industrial sectors mentioned above to include the oil and gas, personal care, food additives, and packaging industries. They can be used as reinforcing food packaging films, rheological modifiers, Pickering emulsion stabilizers, and free radical scavengers.

1.4.2 CELLULOSE NANO FIBERS

Cellulose Nanofibers (CNFs) are cellulose structures at the nanoscale, exhibiting a diameter ranging from approximately 5 to 60 nm and a length spanning multiple microns. These fibrils encompass both crystalline and amorphous segments. In contrast, cellulose nanocrystals display a needle-shaped, highly crystalline structure with residual amorphous regions. Mechanical processes such as high-pressure homogenization, micro- .

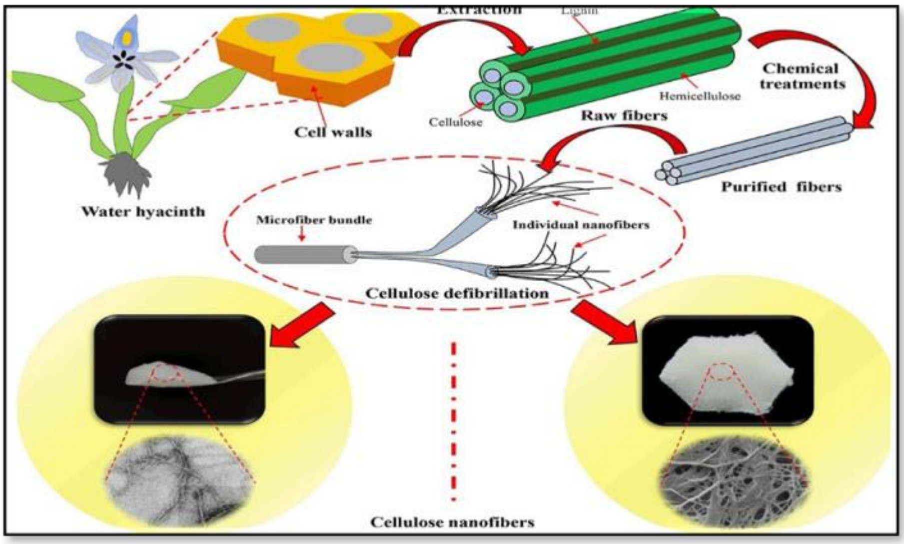

Fig.4 Synthesis of Cellulose Nanofibers

fluidization, refining, and grinding, along with less common methods like electro-spinning, ultra-sonication, cryo-crushing, or steam explosion, are employed for CNF production. However, the primary drawback lies in the high energy demand of these mechanical processes. To address this, various chemical or enzymatic pre-treatments, including cationization, hydrolysis, TEMPO-mediated oxidation, and acetylation, have been implemented on pulp. These pre-treatments aim to ease the mechanical processes, reducing energy consumption and achieving the desired surface chemistry of the product. CNFs, characterized by their light and robust nature, ultra-fine fibers, large specific area, low thermal expansion, high gas barrier properties, and being derived from environmentally friendly biomass, exhibit viscosity in the presence of water. Given these unique attributes and morphology, cellulose nanofiber emerges as a promising material with applications spanning diverse fields. These include its use in filter materials, high gas barrier packaging, electronic devices, foods, medicine, cosmetics, and healthcare [17-18].

1.4.3 BACTERIAL NANO CELLULOSE

Bacterial Nano-Cellulose (BNC) is a biomaterial crafted by various acetic acid bacteria species, boasting notable attributes such as elevated polymerization, crystallinity, superior water retention, purity, transparency, robust mechanical properties, and favourable biocompatibility. Its versatile applications span biomedical materials, functional paper, healthy foods, innovative materials, and textiles[19]. BNC combines the structural elements of plant cellulose with nanoscale material features, primarily produced by non-pathogenic bacteria like Rhizobium, Xanthococcus, Pseudomonas, Azotobacter, Aerobacter, and Alcaligenes, with

Komagataeibacter being the predominant BNC producer. The bacteria undergo a dual process of polymerization and crystallization, with glucose residues polymerizing into B-1,4 glucan linear chains within the bacterial cytoplasm. These chains are then extracellularly secreted and crystallized into microfibrils, forming a highly pure 3D porous network of entangled nanoribbons measuring 20-60nm in width. Noteworthy for its eco-friendly manufacturing, cost-effectiveness, robust mechanical strength, hydrophilicity, biocompatibility, and biodegradability, BNC has garnered increasing attention [20-21]. Bacterial cellulose stands out for its high purity, devoid of accompanying substances like hemicelluloses, lignin, or pectin, and an exceptionally high-water content of 90% or more[22].

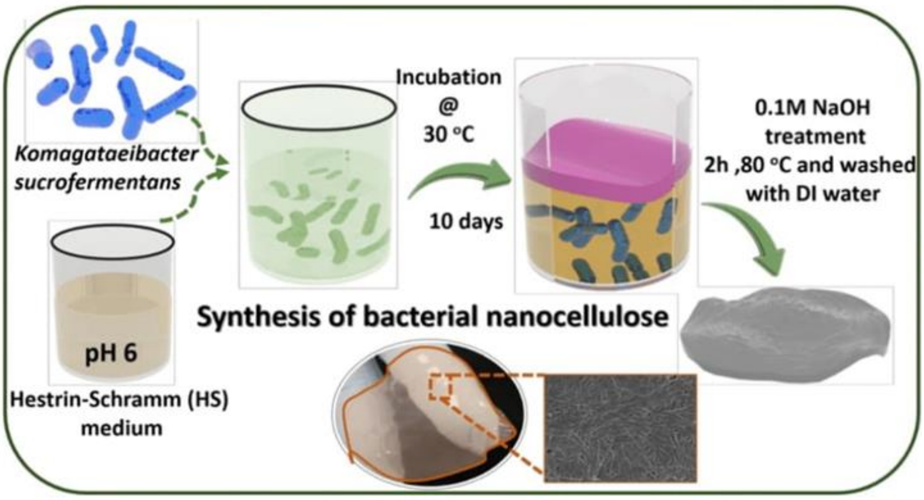

Fig.5 Synthesis of Bacterial Nanocellulose

1.4.4 ELECTROSPUN NANO CELLULOSE

Electrospinning is an alternative method for generating nanoscale/submicron cellulose fibers, gaining notable attention in recent times. To create Electrospun Cellulose Nanofibers (ECNF), cellulose is initially dissolved in a suitable solvent. Subsequently, a high voltage is applied to a cellulose solution droplet, overcoming surface tension and forming a jet. As the solution travels through the air, solvent evaporation occurs, resulting in a filament collected on a grounded target. Although this technique can produce fibers with diameters in the tens of nanometers, ECNF typically range from a few hundred nanometers to a few microns in diameter, still commonly referred to as nanofibers. Ongoing research in this field focuses on identifying suitable solvents for cellulose. Electrospinning is also employed for crafting polymer composite fibers that incorporate nano cellulose[23]. However, challenges such as low thermal stability and solubility limit the practical applications of electrospun cellulose. Recent efforts have aimed at improving cellulose's thermal stability and mechanical properties through polymer blending[21].

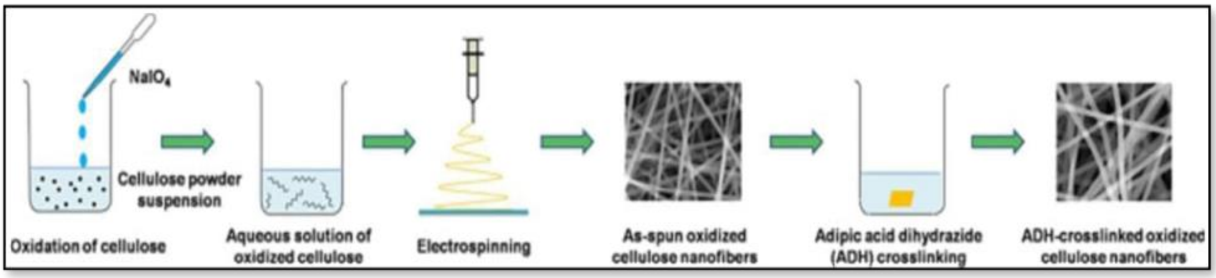

Fig.6 Synthesis of Electro-spun cellulose Nanofibers

1.5 APPLICATIONS OF NANOCELLULOSE

Nanocellulose can have many uses in a variety of industries, including advanced paper products. Preservatives, stabilizers and pigments in cosmetics; It is compact, light and highly resistant; Composites for construction, automobiles, furniture and consumer goods; New materials for electronic and medical applications. Water-based latex paints, industrial coatings, and suspensions benefit greatly from the high concentration of submicron-sized colloidal microcrystals found in industrial microcrystalline cellulose. Includes discussion of new applications for CNC in the catalysis, food, pharmaceutical, paper, and polymer industries[24].

Fig.7 Nanocellulose Applications

1.5.1 FOOD INDUSTRY

The further commercialization of nanotechnology depends heavily on nano cellulose. The food packaging industry is one such commercial sector. Using nanoparticles (Nano Cellulose) in polymer sheets, this packaging technique, known as Active Packaging, controls the microbial surface contamination of food. The application of nanoscale particles is motivated by their mechanical barrier property, durability, flexibility, biodegradability, and transparency from a packaging standpoint. The food packaging industry is eager to create biodegradable, lightweight materials, and nanoparticles fill this need. Food quality can be maintained with this type of material in terms of taste and freshness. Additionally, food will have a longer shelf life, which is significant for both consumers and businesses. Nanocellulose can be used as a food thickener, flavour carrier, and suspension stabilizer. It also replaces low-calorie carbohydrate additives[22].

1.5.2 BIO-MEDICAL APPLICATIONS

Growing interest in the use of nanomaterials in biomedical applications has been sparked by their unique features and diverse structures. For biomedical applications, nano celluloses in particular hold great promise as an affordable advanced material due to their low cytotoxicity, biocompatibility, and biodegradability. Furthermore, they are easily modifiable to produce useful products due to their chemical functionality. Among bacterial nanocellulose is one of the many varieties of nanocellulose materials that has gained popularity recently for a variety of biomedical applications. It has proven effective in wound healing, tissue engineering, drug delivery, cartilage replacements, medical implants, and other applications[25].

1.5.3 PHARMACEUTICAL

When combined with other pharmaceutical excipients, cellulose has excellent compaction properties that form dense matrices that facilitate the easy administration of therapeutic drugs. Potential benefits of using nanocellulose as an excipient in drug release are presented. Higher concentrations of therapeutic drugs may have been added to the material's surface due to its large surface area and negative charge, which suggests the possibility of a significant charge and the best possible control over detoxification. The use of nanocellulose for comparable applications is supported by cellulose's demonstrated biocompatibility. The hydroxide groups on the surface provide a site for surface modification, employing various techniques, to a wide range of chemical groups. Drugs that are hydrophobic and non- ionized, for example, can have their charge and release adjusted using surface modification, which is not typically associated with nano cellulose.

1.5.4 PAPER INDUSTRY

With its ability to strengthen fiber-to-fiber bonds and consequently the paper's strength, CNC has potential uses in the paper and paperboard industries. Additionally, CNCs can be added as a wet-end additive to improve retention and dry and wet strength in commodity types of paper and board products, as well as a barrier in greaseproof types of papers[22].

1.5.5 REINFORCING FILLER FOR POLYMERS

When compared to the corresponding matrix materials, polymer nanocomposites' mechanical properties are significantly improved by the addition of CNCs, as evidenced by the notable increases in stiffness and strength. CNCs have a high specific modulus (modulus/density) and an endless supply, which makes them appealing as reinforcing fillers[26].

1.5.6 HEALABLE POLYMERIC MATERIAL

Researchers in academia and industry have been increasingly interested in self-healing materials because of their exceptional mechanical performance, increased functionality, lifetime, and dependability. A variety of stimuli-responsive characteristics, including heat, light, cracks, etc., can effectively reassemble the mechanically damaged network.

1.5.7 WATER TREATMENT USING NANOCELLULOSE

Researchers are currently looking into low-cost, environmentally safe methods of treating wastewater that contains harmful contaminants like dyes and organic pollutants. Based on the wastewater treatment solution, cellulose-based nanocomposite material can be served as a potential agent. It can act as a valuable resource to meet the industrial scalability and low carbon footprint profile because of its bio-renewable environment-friendly, and inexpensive solutions.

1.5.8 COMPOSITES

Nano fillers can be added to polymers to create composites with high mechanical performance. For the preparation of composites, nanocellulose is the best material to use instead of non-biodegradable nanofillers like carbon nanotubes, nano clays, etc. Tensile strength and elasticity are both increased and decreased upon the introduction of CNCs into the polymeric matrix. However, poor dispersion of CNC upon drying and low compatibility with hydrophobic groups make its production extremely challenging. This can be resolved by grafting and surface-modifying hydrophobic groups to strengthen them. Numerous scientific fields and industries, including packaging, aeronautics, adhesives, hydrogels, nano barriers, inks and paints, fire retardants, etc., can benefit from the use of nanocomposites[22][17].

1.6 CHITOSAN

Chitosan, a linear polysaccharide composed of N-acetyl-D-glucosamine and β-(1→4)-linked D-glucosamine, is derived from the chitin shells of crustaceans through the application of alkaline chemicals such as sodium hydroxide. This versatile compound finds numerous commercial and potential biological applications. In agriculture, chitosan acts as a biopesticide and seed treatment, aiding plants in resisting fungal infections. In winemaking, it serves as a fining agent, preventing spoilage of the product. In the industrial setting, it can be incorporated into self-healing polyurethane paint coatings. Additionally, chitosan exhibits antibacterial properties and can be utilized in bandages to reduce bleeding. Furthermore, in the field of medicine, it facilitates the transdermal delivery of medications.

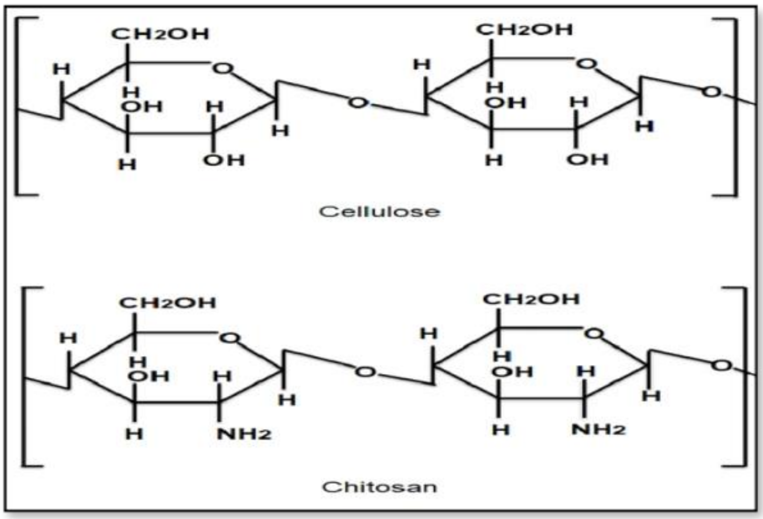

Fig.8 Structure of Cellulose and Chitosan

1.7 PROPERTIES OF CHITOSAN

The molecular weight and level of acetylation of chitosan are its primary properties. These determine the functional characteristics of chitosan, ranging from its solubility and ability to form materials to its biodegradability and variety of bioactive features. Elevated DDA chitosan films demonstrated increased tensile strength, elastic modulus, and crystallinity.

1.8 APPLICATIONS OF CHITOSAN

Chitosan is employed in a wide range of extremely diverse goods and applications, from water treatment and plant protection to pharmaceutical and cosmetic products, because of its physical and chemical characteristics. Different chitosan properties are needed for different applications. These characteristics vary depending on the molecular weight and degree of acetylation, for example. Many biopharmaceutical research areas, including mucoadhesion, permeation enhancement, vaccine technology, gene therapy, and wound healing, have proposed the use of chitosan. Chitosan has recently found use in the following areas: gastrointestinal, colon-specific, vaginal, nasal, sublingual, buccal, transdermal drug delivery, mucosal vaccination, and gene carrier. Additionally, it can be utilized in the pharmaceutical sector to directly compress tablets, function as a disintegrant for tablets, create controlled-release solid dosage forms, or enhance drug dissolution. Derivatives of chitosan were created to enhance biological activity as well as water-soluble chitosan films can be made. In our work, we are creating a CNC-Chitosan thin film that may find application in packaging by taking advantage of the antibacterial properties of chitosan.

1.9 CHARACTERISATION TECHNIQUES

1.9.1 XRD

X-ray diffraction (XRD) is a widely used technique in the characterization of nanoparticles. XRD is commonly used to determine the phase, crystalline grain size, crystalline structure, and lattice parameters. When n 2dsin), where n is an integer, is met, Bragg's Law is satisfied. When an X-ray beam of a corresponding wavelength is incident upon a crystal with an inter planar spacing of d (the crystal lattice constant), the X-ray diffraction or constructive interference between elastically dispersed X-ray beams can be observed at specific angles 20. The powder diffraction pattern of the nanomaterial reveals the amount of crystallinity or amorphous content, phases present, phase concentrations, structure, and crystallite size and strain[27].

1.9.2 FTIR

Since Fourier Transform infrared spectroscopy (FTIR) reliably predicts the chemical changes that follow treatment using a chemical approach[28], it has been widely used in research involving polysaccharides like cellulose. The technique is based on measuring the absorption of electromagnetic radiation at mid-infrared wavelengths (4000–400 cm–1). The positions of bands linked to the kind and strength of bonds as well as specific functional groups are displayed in a recorded spectrum, providing details regarding the composition and interactions of molecules. Attenuated total reflection (ATR) sampling, when used in conjunction with traditional infrared spectroscopy, enables materials to be directly viewed in either a solid or liquid condition without the need for additional preparation. The changes that an internally reflected infrared beam undergoes as it comes into contact with the chosen sample are measured by an accessory for attenuated total reflection. The selected materials must come into reasonable contact with the surface of the crystal. In the parts of the spectrum where the sample absorbs energy, the wave will shift or become weaker[29].

1.9.3 SEM

One of the most popular methods for characterizing nanomaterials and nanostructures is the scanning electron microscope (SEM). The signals that result from electron-sample interactions provide details about the sample, such as its chemical makeup and surface morphology (texture). In a SEM, electrons that have been accelerated possess a substantial amount of kinetic energy. The energy is released when the incident electrons in a solid sample slow down, resulting in a variety of signals from the electron-sample interaction. SEM micrographs have a large depth of field because of the extremely narrow electron beams, producing a distinctive three-dimensional appearance helpful in comprehending the sample's surface structure. The specimen's atoms absorb the energy of an electron beam striking them and release a secondary electron of their own. The secondary electron, which has a positive charge of roughly 300 V, is detected by a detector. Secondary electrons (SE) can create an extremely high-resolution image of the sample surface, revealing details smaller than 1 nm. SE are emitted from very close to the specimen surface. An image of the surface is typically created by combining the position of the electron beam with the detected signal, which is typically scanned in a raster pattern. Sample preparation for SEM takes more time. Since only a small number of particles are visible in the viewing field at once, the information obtained is typically descriptive and visual rather than quantitative. SEM can, however, offer useful extra information on particle texture when combined with other methods, such as laser diffraction, which may help to explain agglomeration or flow issues[30].

1.9.4 PARTICLE SIZE ANALYSIS

Particle size analysis is employed to determine the range of particle sizes in a given sample. Particle size analysis is a crucial test utilized in various industries to ensure quality control. It plays a significant role in determining the efficiency of manufacturing processes and the performance of the final product in industries where milling or grinding is involved. The importance of particle sizing is evident in industries such as pharmaceuticals, building materials, paints and coatings, food and beverages, and aerosols.

Among the different methods employed for particle size analysis, laser diffraction has emerged as one of the most widely used techniques, particularly for particles ranging from 0.5 to 1000 microns. This method operates on the principle that when a beam of light, specifically a laser, encounters a group of particles, the angle of light scattering is inversely proportional to the size of the particles. In other words, smaller particles result in a larger angle of light scattering. Laser diffraction has gained popularity due to its versatility, as it can be applied to various sample types, including dry powders, suspensions, emulsions, and aerosols. Additionally, it is a rapid, reliable, and reproducible technique that can measure particle sizes across a wide range[31].

1.10 ANTIBACTERIAL ACTIVITY

Bacteria and fungi are ubiquitous in every corner of the world, posing a significant global challenge to human health[32]. The excessive and inappropriate use of antibiotics over an extended period of time has resulted in the emergence of multiple drug-resistant (MDR) bacteria, further complicating the treatment of infectious diseases. Additionally, both fungi and bacteria can cause widespread plant diseases. To combat these plant diseases, pesticides are extensively employed, but their excessive and continuous use often leads to severe environmental issues. In terms of bactericidal effects, non-functional cellulose exhibits a slightly superior performance against Gram-negative bacteria compared to Gram-positive bacteria. Polysaccharides are commonly used as biomaterials for various applications. In the case of chitosan, it may possess inherent antimicrobial activity. However, to achieve specific functionalities, bioactive compounds need to be immobilized or incorporated into the polymer matrix, similar to the case of cellulose[33].

1.11 OBJECTIVES OF OUR PROJECT

The utilization of pineapple crown leaves for the production of a value-added product, namely cellulose nanocrystals (CNC), involves several key steps. Initially, fibre is extracted from the pineapple crown leaves, followed by bleaching process. Subsequently, CNC is prepared through acid hydrolysis. The raw material and the resulting cellulose nanocrystals were characterized using X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR). Furthermore, a green composite is prepared by combining CNC with chitosan. Finally, the antibacterial properties of the chitosan-CNC composite were also studied.

Chapter 2

Materials and Methods

This section covers the methods and materials used to analyze and create raw material bleached samples, as well as Cellulose Nano Crystals (CNCs).

2.1 SYNTHESIS OF CELLULOSE NANOCRYSTALS USING PINEAPPLE CROWN LEAVES

2.1.1 CHEMICALS REQUIRED

1. Sodium hydroxide (NaOH)
2. Hydrogen peroxide (H_2O_2)
3. Conc. Sulphuric acid (H_2SO_4)

2.1.2 MATERIALS REQUIRED

1. Pineapple crown leaves

2.1.3 APPARATUS REQUIRED

1. Magnetic stirrer
2. Centrifuge
3. Sonicator

2.1.4 EXPERIMENTAL METHODS

2.1.4.1 EXTRACTION OF FIBER FROM PINEAPPLE LEAVES

Pineapple crown leaves collected from the nearby shops are properly washed and are soaked in water for about 2 weeks for easier fiber extraction. The fiber is then being extracted manually. The extracted fiber is washed, dried and crushed.

2.1.4.2 ALKALINE PRETREATMENT

For mercerization-Every 5 g of fiber is made to react with 100 ml of 5% sodium hydroxide and is kept in the magnetic stirrer at 70°C for an hour. The pH of the sample obtained after 1 hour stirring is made neutral by washing it in excess distilled water. It is then filtered and dried. It is then subjected to undergo bleaching. Every 5 g of sample obtained is then made to react with 100 ml of 24% hydrogen peroxide and 4% of sodium hydroxide taken in 1:1 ratio. It is then kept in the magnetic stirrer for about 2 hours at 50°C.The pH of the bleached fiber is made neutral by washing it with excess distilled water.

2.1.4.3 ACID HYDROLYSIS

For every 5 g of bleached fiber, 100 ml of 30% concentrated sulphuric acid is being added and is placed it in the magnetic stirrer at 50°C for about 2 hours under vigorous stirring. The reaction is being terminated by the addition of excess distilled water in 1:5 (v/v) ratio of solution and distilled water. The sample is now being centrifuged at 1100 rpm, where each cycle stands for 10 minutes. At the end of each cycle, the acid supernant is discarded and excess distilled water is being added, the pH of the hydrolysed sample is thereby made neutral. The hydrolysed sample is further subjected to ultra sonification for 15 minutes.

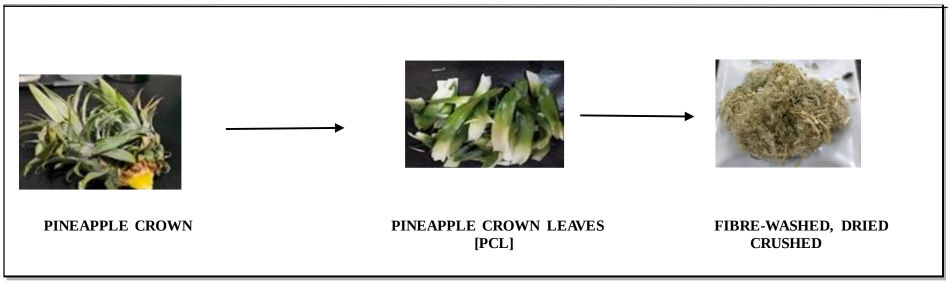

Fig.9 FLOW CHART SHOWING THE EXTRACTION OF PINEAPPLE CROWN FIBRE [PCF] FROM PINEAPPLE CROWN LEAVES

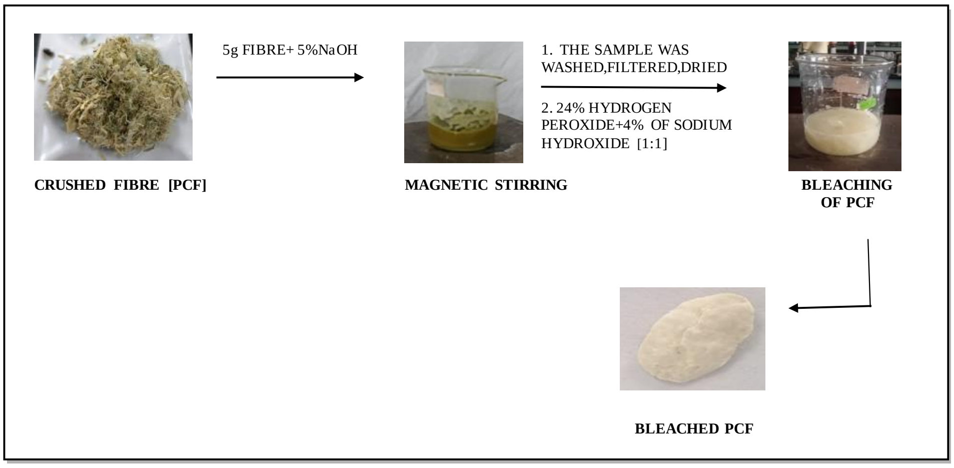

Fig10 FLOW CHART SHOWING THE ALKALI PRE-TREATMENT PROCEDURE

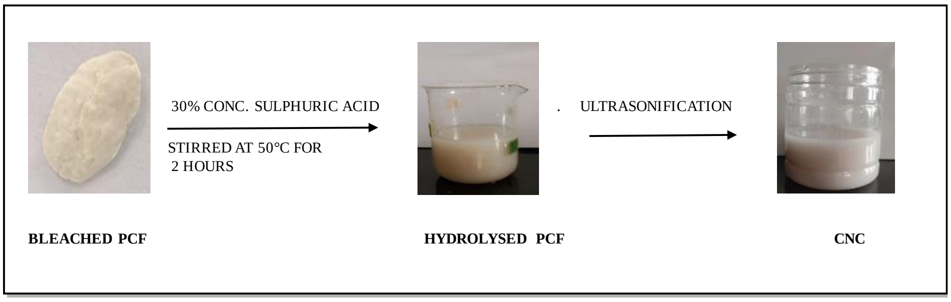

Fig.11 FLOW CHART SHOWING THE ACID HYDROLYSIS PROCEDURE

2.2 CHARACTERIZATION TECHNIQUES

2.2.1 X-RAY DIFFRACTION (XRD)

The Shimadzu XRD-700 X-RAY Diffractometer was utilized to analyse the crystallinity index of the material both before and after undergoing chemical modification. Steel sample holders were used to position the raw materials and CNCs in the form of milled powder, ensuring a consistent and complete exposure to X-rays. The analysis took place at a temperature of 25°C, employing a monochromatic Cuk radiation source with a wavelength of - 0.1539 nm and an angle range of 10° to 60°.

2.2.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The identification of functional groups through chemical modification allowed for the manipulation of structural changes in the samples. The investigation of the changes in functional groups of the materials, namely the Raw material and CNCs, was conducted using FTIR spectroscopy with the Nicolet 50 FTIR spectrophotometer (Thermo Nicolet, USA). The FTIR spectra of the samples were recorded in the transmittance mode within the range of 400-4000 cm^3.

2.2.3 PARTICLE SIZE ANALYSIS (PSA)

The particle size distribution after 60 min ultrasonication was tested with a Malvern Zetasizer Nano ZS instrument. Samples of nanocellulose suspended in water were diluted and analyzed with a particle size analyzer using dynamic light scattering (DLS). Non-Invasive Back Scattering technique was used with a 173◦ detector angle using a HeNe 4 mW laser (633 nm). Measurements were repeated 3 times for the sample[3].

2.3 SYNTHESIS OF ANTIBACTERIAL COMPOSITE FROM CNC AND CHITOSAN

1g of chitosan was added to 100ml 1% acetic acid and dissolved.

(i) Composite of 1:9 (CS: CNC)

1ml of 1% chitosan [CS] solution was mixed with 9ml of previously prepared CNC solution and stirred it for 2 hours without heating.

(ii) Composite of 9:1 (CS: CNC)

9ml of 1% chitosan solution was mixed with 1ml of CNC solution and stirred it for 2 hours without heating.

(iii) Composite of 5:5 (CS: CNC)

5ml of 1% chitosan solution was mixed with 5ml of prepared CNC solution and stirred it for 2 hours without heating.

2.4 ANTIBACTERIAL STUDIES OF CHITOSAN-CNC COMPOSITES

2.4.1 METHODOLOGY

2.4.1.1 PREPARATION OF NUTRIENT MEDIA

To create nutrient broth, 1.3 grams of nutrient broth were dissolved in 100 millilitres of distilled water. Then, 5 millilitres of the nutrient broth were added to test tubes and sterilized using an autoclave. For the nutrient agar media, 1.3 grams of nutrient broth and 2 grams of agar agar were mixed in 100 millilitres of distilled water. The media was autoclaved and 20 millilitres were poured into sterile petri plates under aseptic conditions.

2.4.1.2 PREPARATION OF MICROBIAL CULTURES

The test organisms, namely E. coli and S. aureus, were introduced into 5 ml of sterilized nutrient broth and incubated overnight at a temperature of 37°C.

2.4.1.3 WELL DIFFUSION METHOD

A culture of each bacterium was prepared on a lawn using sterilized cotton swabs. The swab, which had been sterilized, was dipped into the bacterial suspension and moved from side to side, covering the entire plate. The plate was then rotated to 90 degrees and the same procedure was repeated to ensure complete coverage with bacteria. Once the lawn was prepared, wells with a diameter of 6 mm were cut into agar plates using a sterile well cutter. The wells were labelled and 20µL of different samples (CNC, Chitosan [CS], 1:9 [CS:CNC], 9:1 [CS:CNC], 5:5 [CS:CNC]) were loaded into the corresponding wells. The antibacterial activity of these samples was compared with standard antibiotics. The plate was then incubated at 37°C for 24 hours. The radius of each zone of inhibition, which indicates the effectiveness of the compound, was measured using a standard ruler in centimetres. If the compound is effective against bacteria at a certain concentration, no colonies will grow. After the experiment, the plates were autoclaved for 20 minutes to kill and dispose of the bacteria. All the glassware used in the experiment was also autoclaved to remove any bacteria that may have been present[34].

Chapter 3

Results and discussion

3.1 CHARACTERIZATION OF CELLULOSE NANOCRYSTALS

3.1.1 PHYSICAL APPEARANCE

The visual characteristics of the unprocessed substance derived from the leaves of pineapple crowns, chemically refined cellulose, and nanocrystals produced through hydrolysis techniques are presented in the following manner.

Fig.12 RAW MATERIAL
.

Fig.13 CHEMICALLY PURIFIED CELLULOSE

Fig.14 CNC

The application of NaOH and H_2O_2 to the raw material resulted in alterations to both its color and texture. The emergence of a white hue signifies the elimination of impurities and the attainment of a high cellulose content. By subjecting the extracted cellulose to hydrolysis using H_2SO_4, a stable colloidal suspension of Cellulose Nanocrystal was obtained.

3.1.2 XRD

Cellulose, a vital constituent of PCL, possesses a crystalline structure that arises from the arrangement of its chains through acid hydrolysis. The diffraction patterns acquired for the Raw Material and CNC are depicted in Figure 15 and Figure 16, respectively. In both cases, the peaks observed at 2θ = 22.737 and 35.044 for the raw material, and 2θ = 15.351 and 22.805 for CNC, correspond to the cellulose I region across all samples. Furthermore, Table 1 provides a comprehensive summary of the crystallinity percentage and amorphous percentage.

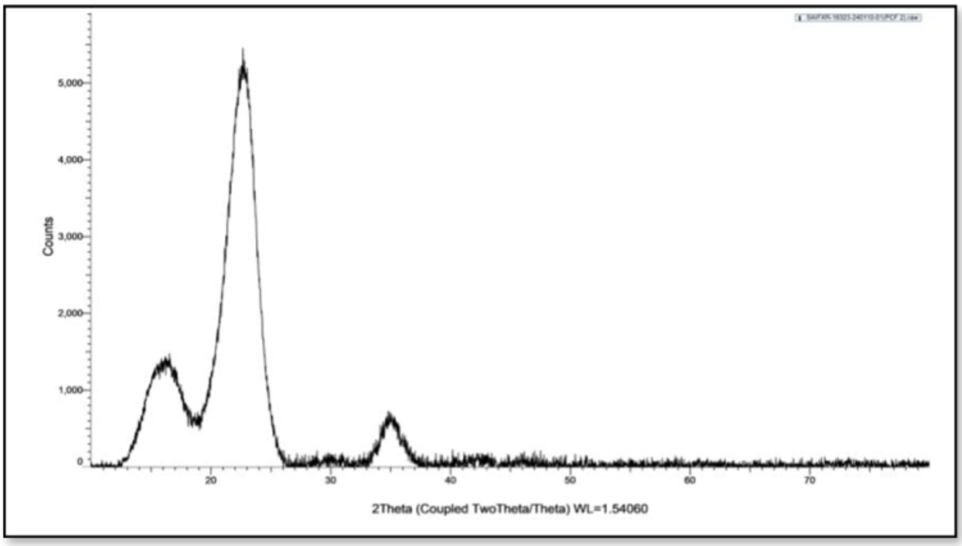

Fig.15 XRD Graph of Raw Material

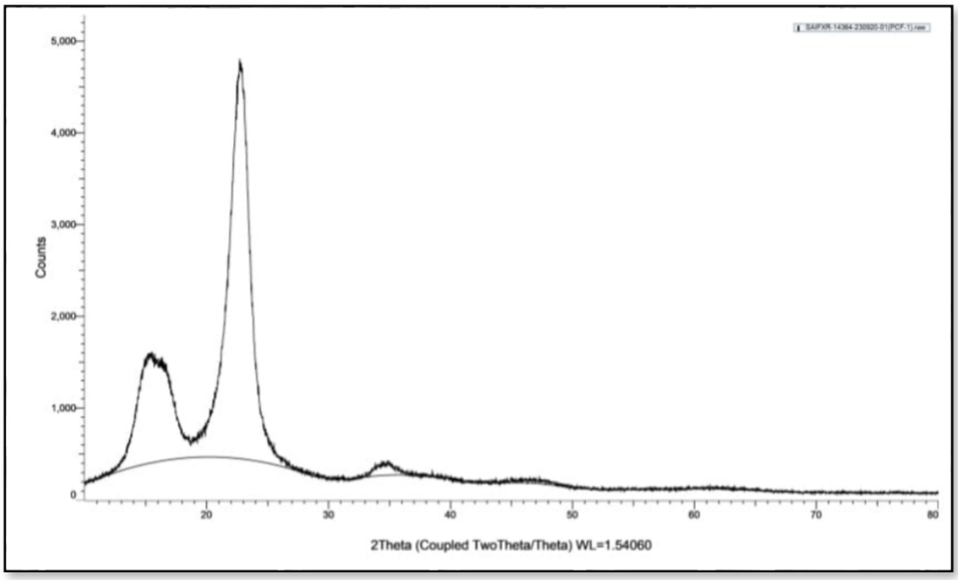

Fig.16 XRD Graph of Cellulose Nanocrystals

The samples exhibit crystallinity values of 22.8 and 75.1, respectively. Moreover, the crystallinity values of CNC are greater in comparison to those of the raw materials. This disparity can be attributed to the elimination of amorphous regions of cellulose through acid hydrolysis. This process prompts the hydrolytic cleavage of glycosidic bonds, resulting in the release of a higher number of individual crystals.

PROPERTY	RAW MATERIAL (%)	CNC (%)
CRYSTALLINITY	22.8	75.1
AMORPHOUS	77.2	24.9

Table 1: Crystallinity and Amorphous Percentage

Furthermore, in the course of the acid hydrolysis procedure, the amorphous component dissolves, liberating individual crystals that have the potential to enhance the crystallinity of cellulose. The augmentation in crystallinity is directly linked to the reinforcement of the cellulose structure's rigidity, thereby resulting in heightened tensile strength of the fibers. Consequently, this elevation is anticipated to bolster the mechanical characteristics of composites. It is worth noting that the crystal size of CNC measures 48.54 nm.

3.1.3 SEM

The Figure illustrates the appearance and morphology of CNC subjected to acid hydrolysis. The analysis revealed that the shape of CNC resembled that of a rod**.**

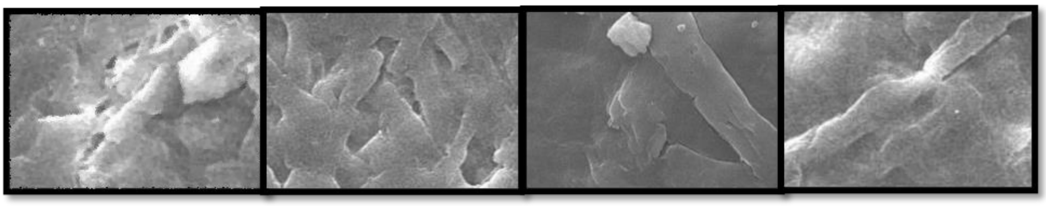

Fig.17 SEM images of Cellulose Nanocrystals

The scanning electron microscopy (SEM) images presented in Figure depict the morphological surface of PCL following various treatments. Upon undergoing bleaching treatment, the bonding between lignin and hemicellulose is disrupted as a result of the removal of the amorphous content, leading to the formation of microfibril bundles. This observation is further supported by the X-ray diffraction (XRD) crystallinity index data. Furthermore, the SEM image of PCL after acid hydrolysis treatment reveals a decrease in Fiber size. The PCL cellulose exhibits microfibril bundles with diameters ranging from approximately 1 to 5 µm. Additionally, Figure displays the SEM image illustrating the morphological surface of PCL subsequent to homogenizing treatment.

3.1.4 PARTICLE SIZE ANALYSIS (PSA)

PSA was employed to determine the crystal size following 60 minutes of ultrasonication. The suspension, consisting of 1 wt. % nanocrystals, underwent dynamic light scattering analysis, as depicted in Fig. The average diameter of these crystals was then computed. These findings provide additional support for the effective synthesis of Nano sized cellulose, demonstrating uniformity in both length and width. Moreover, the particle size distribution reveals that the nanocrystals obtained from PCL through mechanical treatment exhibit a high level of homogeneity.

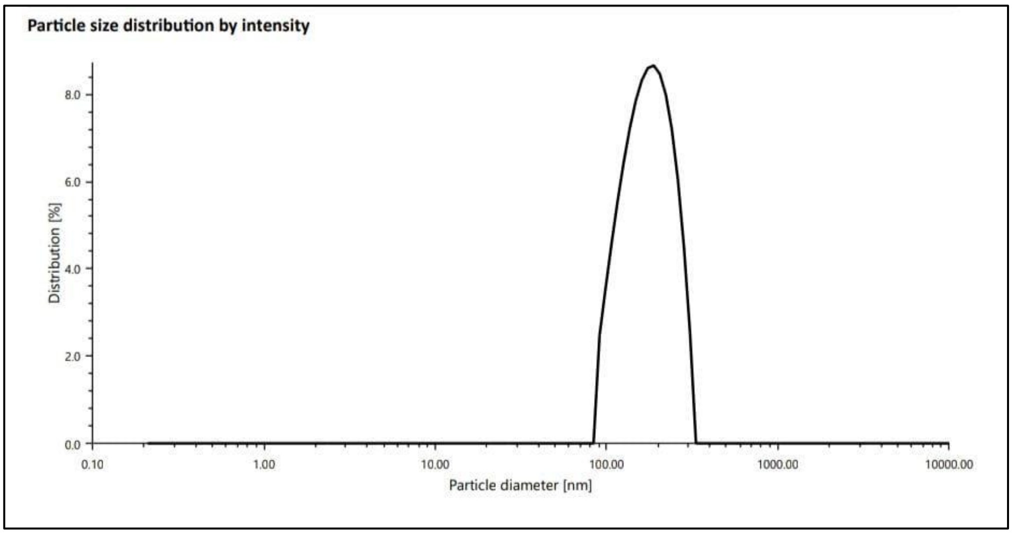

Fig.18 PSA Graph of Cellulose Nanocrystals

3.1.5 FTIR

Infrared spectroscopy operates by analyzing the atomic vibrations within a molecule that is being tested. By examining the FTIR spectra of the sample, we can determine any structural changes that occur before and after chemical treatment.

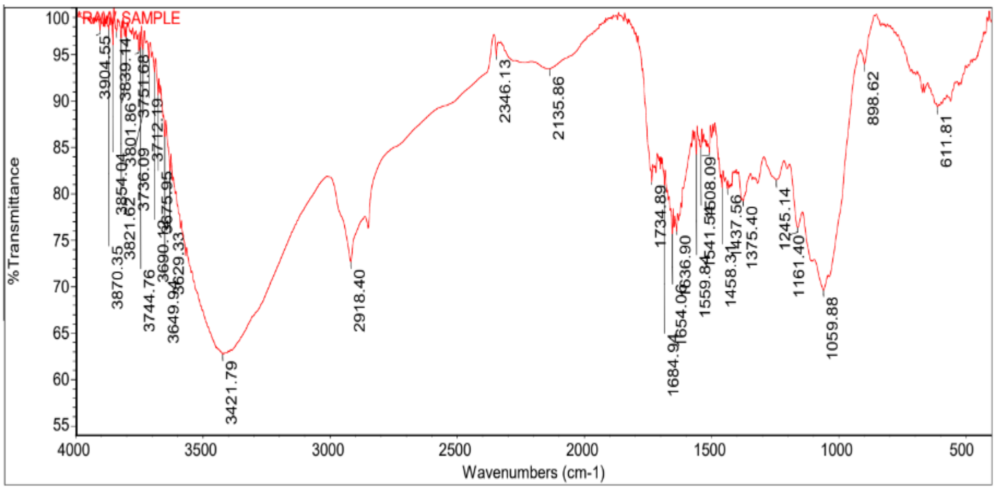

Fig.19 FTIR spectra of Raw Material

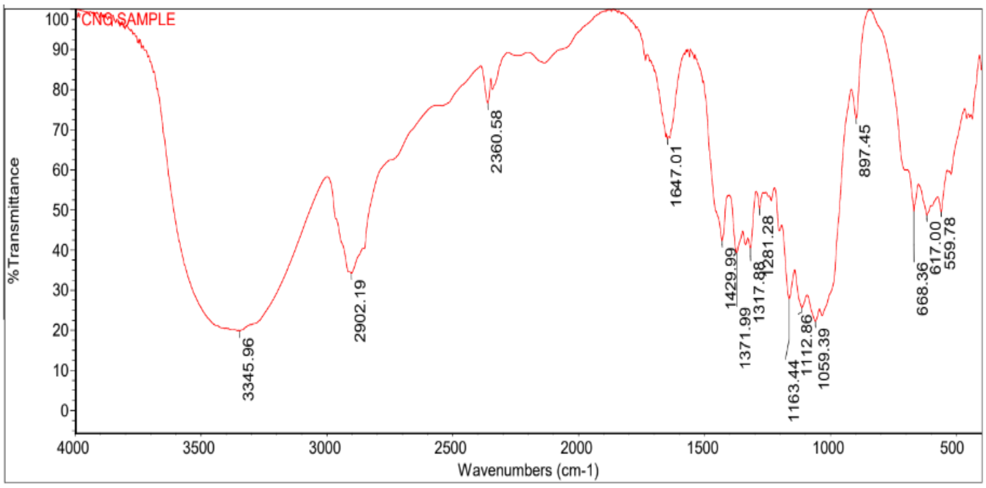

Fig.20 FTIR spectra of Cellulose nanocrystals

When comparing the FTIR analysis graphs of the raw material and CNC (cellulose nanocrystals), we observe a peak in the 1500-1200cm range, which corresponds to the free OH stretching vibration. Additionally, there is a peak in the 2800-2000 cm range, indicating the CH stretching vibration. Another peak in the 1650-1640cm range signifies the OH bonding of absorbed water. These peaks all exhibit an increase in intensity when the raw material is bleached to form cellulose nanocrystals. Notably, there is a crucial peak at 890-900cm, representing the glycosidic linkage of glucose units in cellulose. This peak intensifies from the raw material to CNC, indicating the complete conversion of the raw material into cellulose. At approximately 1734.89cm¹, there is a peak that indicates the presence of hemicellulose in both the raw materials and the bleaching sample. However, this peak is absent in the FTIR of CNC, suggesting the elimination of hemicellulose. Furthermore, a peak at 1245.14 cm represents the ether linkage of lignin, which diminishes as it reaches CNC, confirming the removal of lignin. In conclusion, the analysis reveals an overall increase in the peak representing cellulose and a decrease in the peaks associated with hemicellulose, lignin, and other cellulosic components[35].

3.2 ANTIBACTERIAL ACTIVITY ASSAY

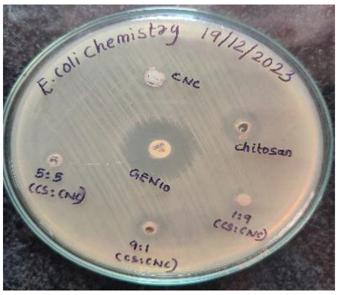

Fig.21 Antibacterial activity of CNC, Chitosan [CS], 1:9 [CS:CNC], 9:1 [CS:CNC], 5:5 [CS:CNC] against E. coli

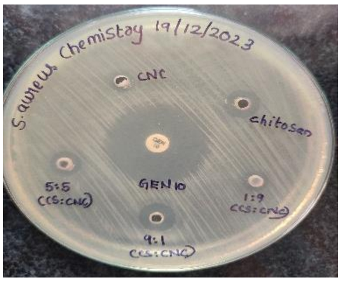

Fig.22 Antibacterial activity of CNC, Chitosan [CS], 1:9. [CS:CNC],9:1 [CS:CNC], 5:5 [CS:CNC] against S.aureus

All five samples were shown to be bactericidal against the gram-negative bacterial stain of E. Coli and gram-positive bacterial stain of Staphylococcus aureus after being incubated for 24 hours. Chitosan demonstrating a 1.0cm diameter zone of inhibition against the E. Coli bacterial strain and 1.1cm diameter zone in S.aureus. The indicated S.aureus zone of inhibition for CNC has a diameter of 0.7cm and no zone of inhibition in E.coli. In the zone of inhibition in S.aureus, the Chitosan-CNC composite of 9:1 ratio exhibited more antibacterial activity than both Chitosan and CNC. And the composites of 1:9 and 5:5 ratios have a zone of inhibition of diameters of 0.7 and 0.9 respectively. The composite of 9:1 ratio of Chitosan-CNC has a larger zone of inhibition in E.coli and other has a smaller zone of inhibition . Therefore it can be concluded that the composite prepared by the addition of Chitosan to CNC will increase the antibacterial activity of CNC. And the prepared composite of Chitosan and CNC from PCF show more antibacterial activity against S.aureus gram-positive bacterial stain.

SAMPLE	ZONE OF INHIBITION [E. coli]	ZONE OF INHIBITION [S. aureus]
CNC	Below 0.5cm	0.7cm
Chitosan [CS]	1.1 cm	1cm
1:9 [CS:CNC]	Below 0.5 cm	0.7cm
9:1 [CS:CNC]	0.9cm	1.1cm
5:5 [CCNC]	Below 0.5 cm	0.9cm

Table 2: Antibacterial activity

Chapter 4

Conclusion

The increasing focus on the utilization of biomass waste for valuable products, such as through recycling and recovery methods, has garnered significant attention due to its potential to reduce environmental impact. This research aims to generate interest in the production of cellulose nanocrystals (CNCs) from renewable sources and agricultural waste. Specifically, CNCs were extracted from pineapple crown using an acid hydrolysis technique, resulting in a stable milky white suspension containing spherical nanocrystalline cellulose. These CNCs possess several desirable properties, like lightweight, strong mechanical strength, biocompatible, biodegradable, high surface area, and having customizable surface chemistry. These characteristics make them suitable for the development of high-performance materials. The stability of the suspension is attributed to the presence of anionic repulsive charges on the crystal surfaces. Analytical techniques such as XRD, FTIR, and SEM were employed to provide evidence for supporting the formation of nanocellulose.

Furthermore, this study has revealed the potential use of pineapple crown as a raw material for the production of CNCs, which also exhibit antibacterial properties when combined with chitosan. This innovative application of waste material not only reduces the burden on landfills by utilizing agricultural waste but also helps mitigate environmental pollution, thereby promoting the sustainable utilization of waste materials for the production of valuable products.

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