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Synthesis, Characterization and Antimicrobial Activities of Cellulose Nano Crystals Isolated from *Borassus flabellifer*

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Abstract

Cellulose nanocrystals (CNCs) are emerging as a highly promising class of nanomaterials due to their unique combination of mechanical strength, biodegradability, and sustainability. This study focuses on the synthesis and comprehensive characterization of CNCs derived from agricultural by-products aiming to explore their potential applications in fields such as nanocomposites, biomedical devices, and environmentally friendly packaging materials. CNCswere synthesized from *Borassus flabellifer*through acid hydrolysis a well-established method that selectively removes the amorphous regions of cellulose, leaving behind highly crystalline nanostructures. The hydrolysis process was optimized by varying parameters such as temperature, reaction time, and acid concentration to achieve maximum yield and crystallinity. The synthesised cellulose nanocrystals were characterized using FT-IR, XRD, SEM and TEM. Zeta potential. analysis and anti-microbial studies were carried out to find out thestability and sensitive of CNCs towards bacteria and fungi.

Keywords: Cellulose, Nanocrystals, Acid hydrolysis, Nano composites, Bio medical, Ecofriendly.

1. INTRODUCTION

The severe environmental problems, including the increasing difficulties of waste disposal and the deepening threat of global warming caused by the non-biodegradability of a number of polymers used in packing and agriculture field have raised concerns all over the world. To solve the problems caused by plastic waste, many efforts have been done to obtain an environmentally friendly material.The production of novel biodegradable polymers is a major emergent research field both in industry and academic due to the environmental concerns [1-3].Research on polymers made from renewable resources such as cellulose, starch, and gelatin has been on the rise recently.

Cellulose is the most abundant natural bio polymer and is readily available from renewable resources. It is an excellent fibre. Wood, cotton and hemp rope are all made of fibrous cellulose. Cellulose is made of repeat units of the monomer glucose. Cellulose is considered as a nearly inexhaustible raw material with fascinating structures and properties for the remarkable demand for environment friendly and biocompatible products. The goal of chemically functionalizing cellulose is to modify its characteristics for various uses, especially as a chemical feedstock for the synthesis of cellulose derivatives for a range of applications [4,5].Cellulose, which is present in many different types of organisms such as plants, algae, bacteria and tunicates, is one of the most common polymers in nature. Cellulose, which may be made from wood and cotton resources, is accessible in India and around the world. Cellulose is being thoroughly studied for a number of uses, including food, chemicals, textiles, biomaterials, electronics, electrochemical devices, and pharmaceuticals, among others, because of its superior surface and structural qualities [6].

In recent years, one of the emergent applications of cellulose is in the field of nanotechnology [7].It has beenfurther classified into Cellulose microcrystals (CMCs), Cellulose nanofibers (CNFs) and Cellulose nanocrystals (CNCs) according to its morphology and structural characteristics. With dimensions of 50–350 nm for length, 5–20 nm for breadth, and 5–30 nm for aspect ratios, CNCs have a spindle-like appearance [8]. The cellulose-based products could be recycled or degraded where in Sono chemical degradation and enzymatic degradation are attractive approaches. Various cellulose-rich materials have been used as raw materials in the process of CNCs synthesis [9]. Several methods have been employed in the past to prepare CNCs. The cellulose nanoparticles' size, shape, morphology, and structural characteristics are established using these techniques. One popular technique for separating CNCs from amorphous cellulose is acid hydrolysis. However, the use of acid causes potential degradation of cellulose and increases the susceptibility to corrosion due to residual acid [10,11]. It has been found that the ultrasound-assisted method is an attractive option for removing Cellulose nanocrystals from the entire cellulose framework. CNCs have also been used as a filler to produce new nanocomposites due to its low density, abundance, good mechanical properties, and full biodegradability [12].Nanocellulose is used to make emulsions, biomedical devices, packaging and rheology modifiers like hydrogels used in the biomedicine, bio composite films, supercapacitors and xero-gels [13-19].Nano composites containing CNCs are used for making toughened paper, water repellents, wound healing patches, hydrogels, scaffolds, biosensors, bio probes, textiles, batteries, electrical devices, food packaging materials, etc. [20].

In this work, the acid hydrolysis has been performed on mesocarp fibre from *Borassus flabellifer* in the presence of ultrasound sonication to isolate CNCs. Acid hydrolysis treatment is carried out to remove the amorphous region of CNCS. Further, the CNCs was characterized to study the functional groups, crystallinity nature, surface morphology, stability and anti-microbial activity.

2. EXPERIMENTAL PROCEDURE

2.1 MATERIALS

Mesocarp fibre from*Borassus flabellifer* collected from local market, India. Concentrated sulphuric

acid (H_2SO_4, 98%)was purchased from sigma Aldrich. sodium hypochlorite solution (NaOCl), sodium hydroxide pellets were acquired from shiv chemical laboratory, India.Deionized water and ethanol were used.

2.2 PREPARATION OF CELLULOSE NANOCRYSTALS

The mesocarp fibre from*Borassus flabellifer*as shown in fig 1 was grounded to powder form. 5g of powdered mesocarp fibre was washed with distilled water to remove the impurities. The dried sample was bleached using 15% of NaOCl and heated for about 4 hours. The bleaching treatment was done to purify the sample and also for the whitening of the sample. The obtained cellulose as shown in fig 2 was washed with deionized water and dried at room temperature.

The dried sample was treated with concentrated H_2SO_4. 35ml of 98% concentrated H_2SO_4 is added to the dried sample. It was kept undisturbed for 2 hours. The mixture was kept in a cold ice bath to terminate the reaction. The pH was adjusted to 6 by adding excess of NaOH and sonicated for about 90 minutes. The resultant sample was washed with ethanol followed by deionised water, filtered and dried in a hot air oven at 60ºC for 48 hours. Fig 3 shows the extracted CNCs obtained from *Borassus flabellifer.*

2.3 CHARACTERIZATION

The FTIR of CNCs was recorded using a Thermo Nicolet iS50. The samples were mixed with KBr powder and then pressed into thin pellets. The sample's wavelength range was measured between 4000 and 400 cm^{-1}. With the aid of a Bruker D4 X-ray diffractometer, the resulting CNCscrystallinity index was examined. The measurement was done with Cu Kα radiation at 40 kV. With an acceleration voltage range of 0.5 to 30 kV, the JEOL 6390LA/OXFORD XMX N apparatus was used to take the scanning electron microscopy (SEM) images. A detector of secondary electrons (SE) was used to take the pictures. Using a JEOL 1011 TEM operating at 100 kV, transmission electron microscopy was used to examine the morphology of CNCs. The IS Morada 4K CCD camera system was used to take the images. TEM pictures were captured using a 200 kV LaB6 electron cannon with a 0.23 nm point resolution and a 0.14 nm lattice resolution.Kirby-Bauer test is widely used to determine the sensitivity or resistance of bacteria and fungi to various antimicrobial compounds, and it uses the Mueller Hinton agar. Mueller-Hinton agar is a non-selective, non-differential medium capable of growing a wide range of non-fastidious organisms. Kirby-Bauer test is also known as disk diffusion method.

Fig 1: Mesocarp fibre from *Borassus flabellifer*

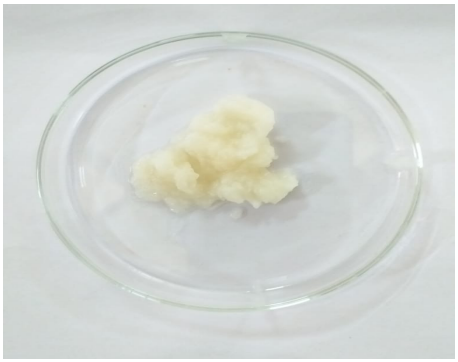


Fig 2: Cellulose

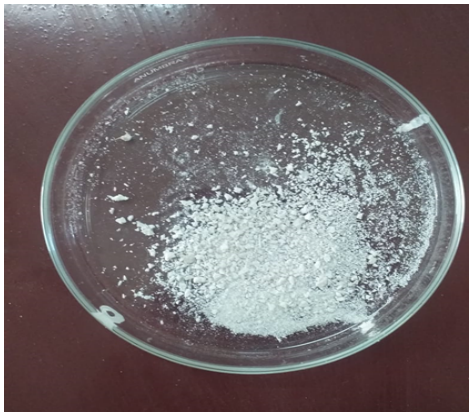


Fig 3: Cellulose nanocrystals

3. RESULTS AND DISCUSSION

3.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Analytical methods such as FTIR are highly effective in determining the molecular structure of cellulose nanocrystals. FTIR spectrum of isolated CNCs is shown in fig 4. The presence of broad band at 3376 cm^{-1} shows the presence of OH stretching vibrationswhich confirm the presence of alcoholic group. The characteristic C-H stretching at 2902.64cm^{-1}confirms the presence of alkane group. The appearance of strong group at 1635.07 cm^{-1} indicatesthe presence of aromatic ring. The appearance of weak peak at 1370.08 cm^{-1} indicates thepresence of CH_2 bending vibrations in the molecule. The peak at 1034 cm^{-1} shows the presence ofC-O-C pyranose ring skeletal vibration. The appearance of peak at 897.57cm^{-1} confirms thepresence of C-O-C stretching at the b-(1-4) glycosidic linkage [21].

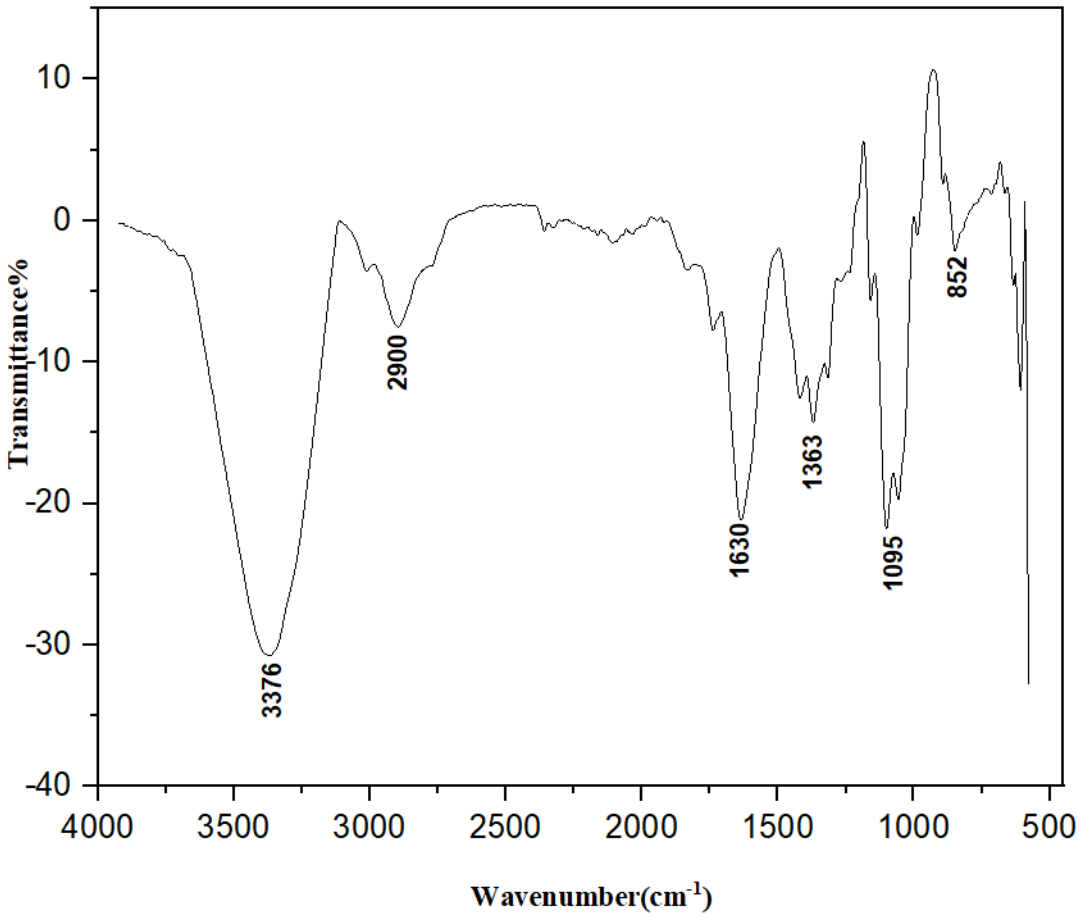


Fig 4: FTIR spectrum of CNCs

3.2 X-RAYDIFFRACTION ANALYSIS (XRD)

The crystalline structure of the material can be determined by the CNCs X-ray diffraction (XRD) pattern, which is shown in fig 5. The graph shows a prominent peak at about 22°, which is indicative of the crystalline areas of cellulose and corresponds to cellulose (200) plane. This peak reveal that the CNCs have a high level of crystallinity. The sharp and intense peaks observed at 2θ = 31.56º,45.19º,22.14º [22]. The existence of tiny peaks at higher 2θ values (ranging from 30° to 80°) and a progressive reduction in intensity indicate the presence of amorphous regions within the cellulose structure. The amorphous fractions in the CNCs sample could be the cause of the peaks' broad nature in the 30°–50° range. Overall, the diffraction pattern shows that the sample is mostly crystalline with a little amount of amorphous content, which is characteristic of cellulose nanocrystals that are obtained from natural sources. The amorphous nature of cellulose is removed during the acid hydrolysis treatment. The particle size of CNCs was calculated using the Scherrer formula.

$$D=K\lambda/\beta\cos\theta$$

where, D is the crystalline size, K is Ḃraggs constant, β is Full width at half maximum, θ is Bragg

angle, and λ is X-ray wavelength. From Scherrer formula the particle size is calculated as 12nm. The crystallinity index (C.I) of the isolated CNCs was calculated by Segal Empirical Equation.

$$CI=\frac{(I_c-I_{am})}{Ic} \times 100$$

where I_c is the maximum intensity of crystalline peak at 2θ = 22º and $I_{am}$is the intensity of amorphous peak at 2θ = 35º. The calculated crystallinity index of CNCs was 64.51%.

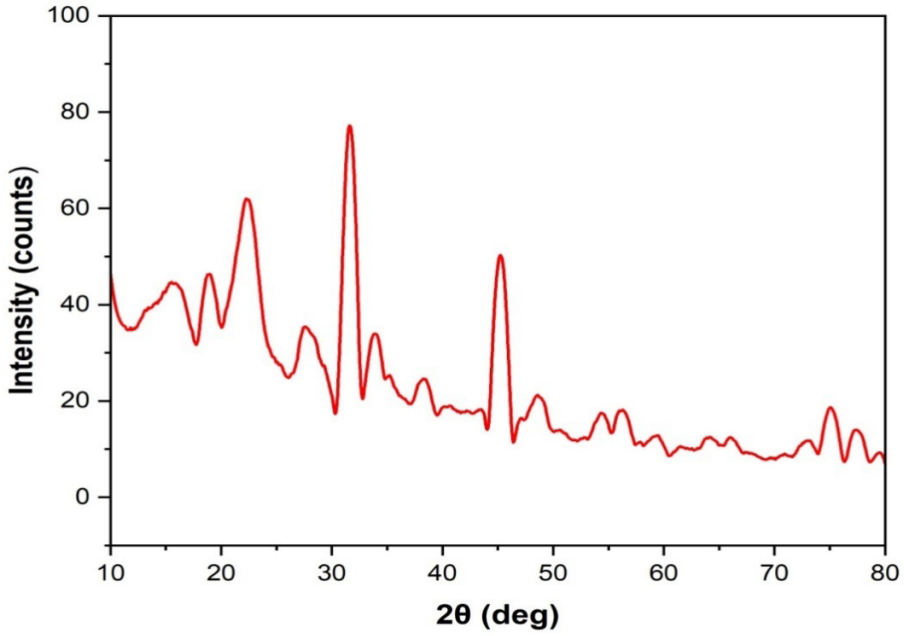
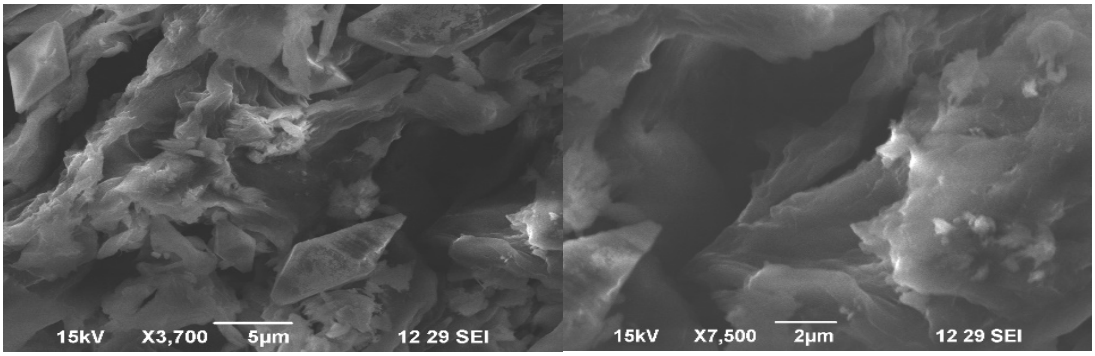


Fig 5: X-ray diffraction analysis of CNCs

3.3 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) is a powerful technique used to examine the surface morphology and topography of materials at high resolution. The surface morphology of CNCs is typically characterized as needle-like shape. SEM images of isolated CNCs is shown in fig 6. This morphology arises due to the crystalline regions of cellulose, which remain intact after the amorphous regions are removed through processes such as acid hydrolysis. By knowing the surface morphology of CNCs, it can be optimized in variety of applications, such as reinforcement in films, coatings, nanocomposites, and biomedical materials, where their special qualities such as high strength, low weight, and biodegradability make them very appealing.

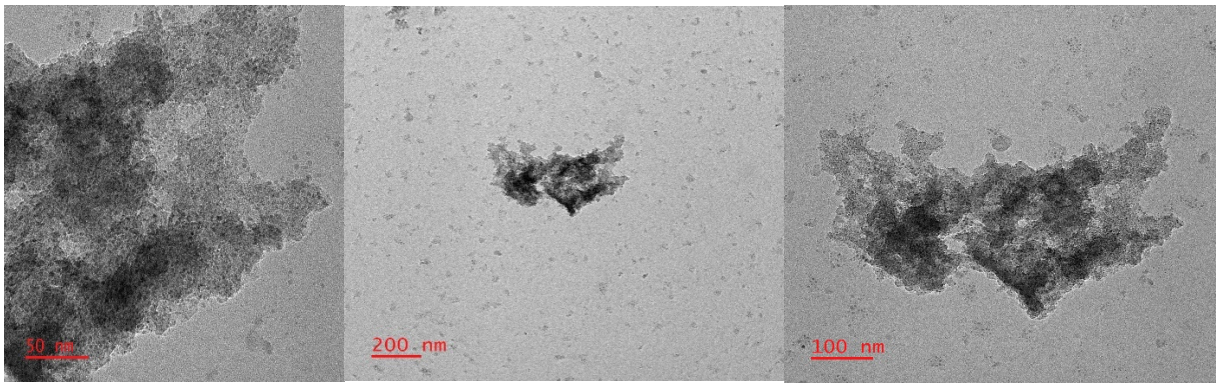


(a) (b)

Fig 6: SEM images of CNCs

3.4 TRANSMISSION ELECTRON MICROSCOPY

Transmission electron microscopy (TEM) is a potent method for seeing a material's ultrastructure at an atomic level of complexity and resolution. TEM is a useful tool for gaining deep insights into the morphology, size, and structure of CNCs Due to CNCs extremely crystalline structure, their edges are frequently distinct and sharp.Due to their large surface area and tendency to form hydrogen bonds, CNCs appear as small clusters or bundles in TEM images, especially if not well dispersed. TEM images of CNCs is shown in fig 7. The cluster or aggregated shape observed in TEM is crucial in understanding how CNCs interact with other materials, as their high aspect ratio enhances their reinforcing potential when embedded in matrices, like polymers, or applied in thin films. From TEM image the particle size is found to be 18.5nm which is represented in fig 8.TEM combined with techniques like electron diffraction can provide further insights into the crystal structure and orientation of CNCs, offering a comprehensive understanding of these biologically derived nanomaterials.mportant details describing the dimensions, surface structure, and aggregation behavior of CNCs can be found within the TEM pictures. The mechanical, optical, and barrier properties of the accomplished composite are all directly determined by the shape and size distribution of CNCs, which makes them particularly significant in applications where they are utilized as reinforcing agents or as nanofillers in polymers.



(a)(b) (c)

Fig 7: TEM images of CNCs

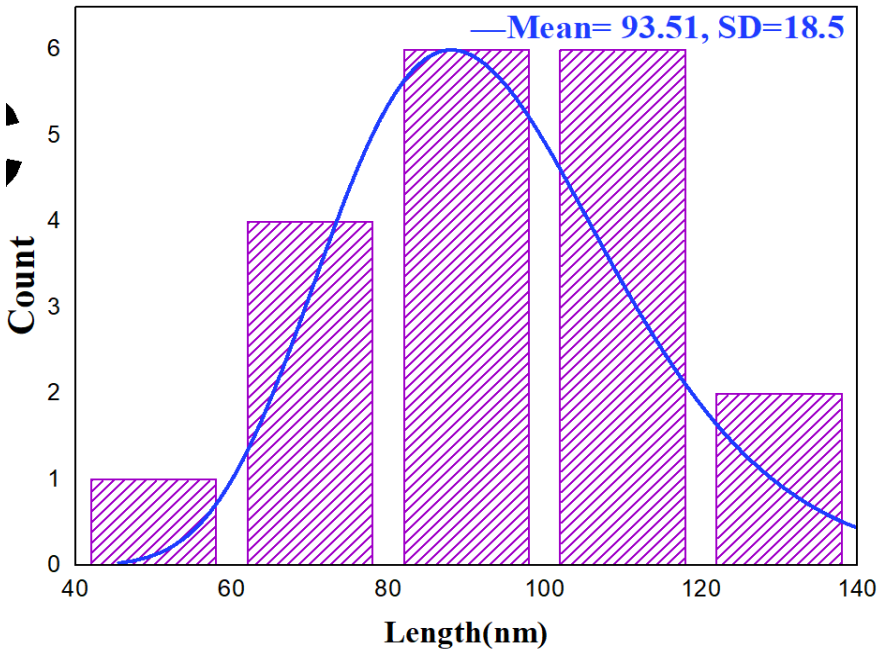


Fig 8: TEM graph of CNCs

3.5ZETA POTENTIAL

Zeta potential is the electrical potential difference between the dispersion medium and the stationary layer of fluid attached to a dispersed particle.Zeta potential is directly related to the stability of colloidal dispersions as it measures the magnitude of the electrostatic repulsion or attraction between particles. High zeta potential (positive or negative) indicates strong electrostatic repulsion between particles, which prevents aggregation and maintains stability. Low zeta potential can lead to particle aggregation and instability.If the values are greater than ±30 mV indicate a stable suspension and if the values are between ±10 mV and ±20 mV suggest low stability. The zeta potential value for the synthesised CNCs

is found to be -0.0335 which shows highly stable colloidal system. The negative value indicates that the particles have absorbed more anions which is shown in fig 9.

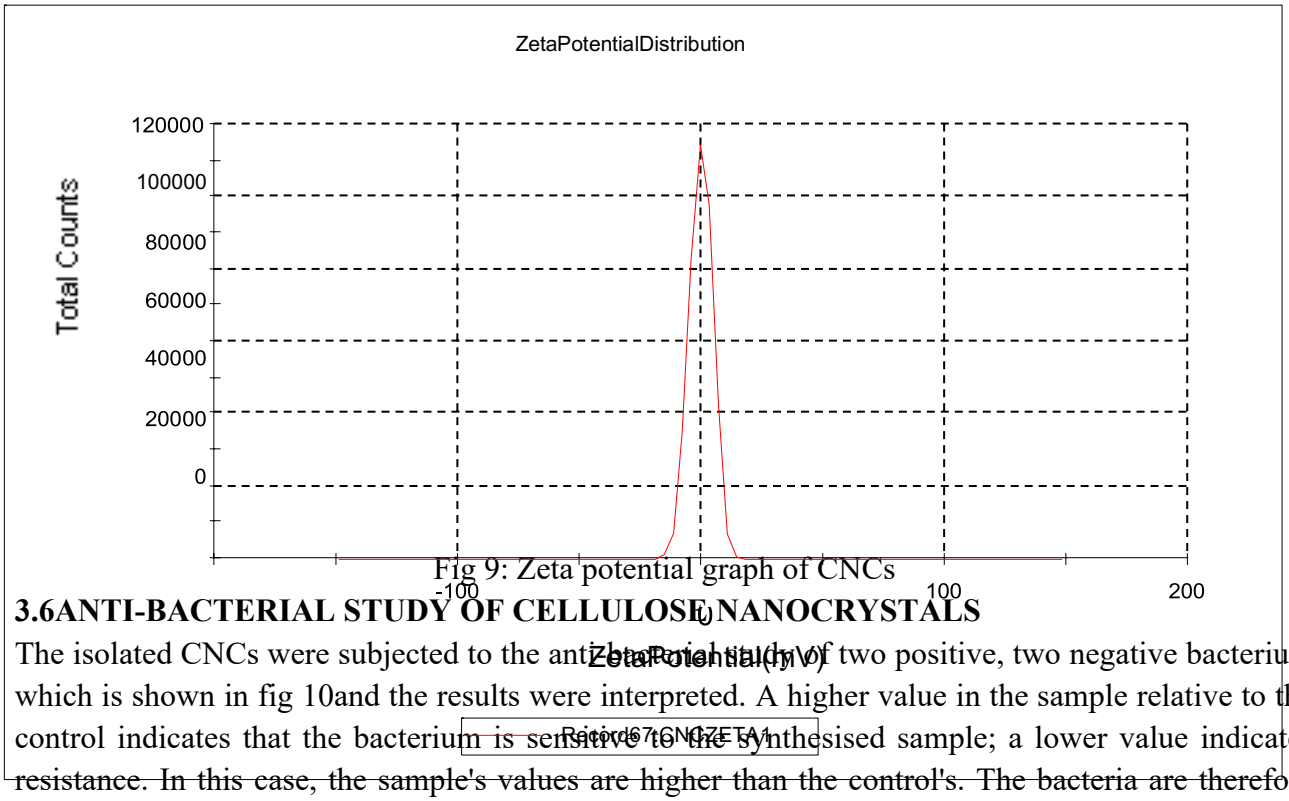
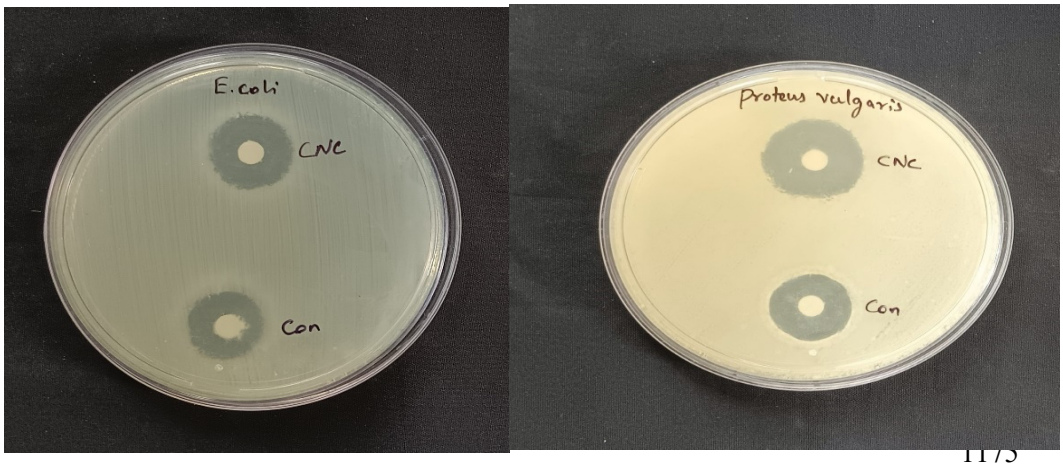


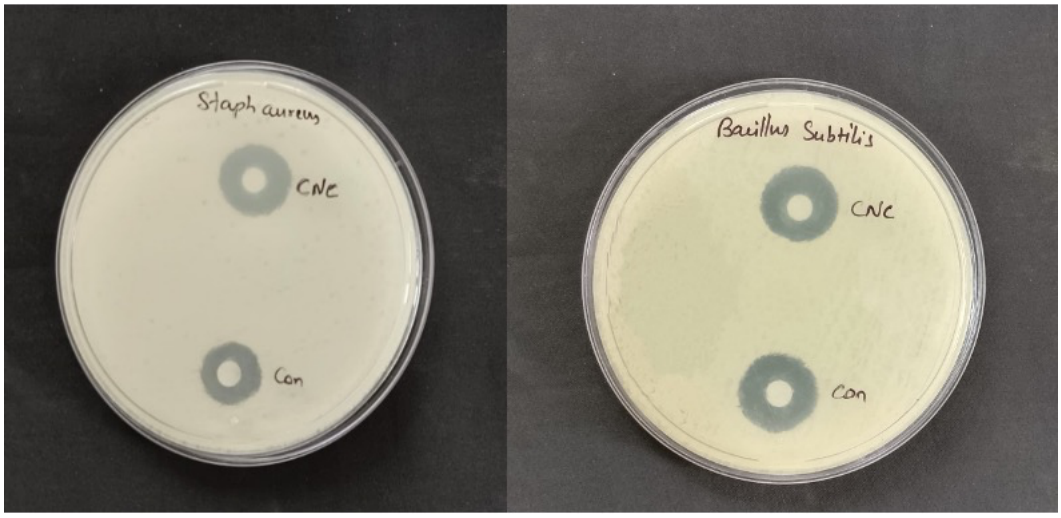
Fig 9: Zeta potential graph of CNCs

3.6ANTI-BACTERIAL STUDY OF CELLULOSE NANOCRYSTALS

The isolated CNCs were subjected to the anti-bacterial study of two positive, two negative bacterium which is shown in fig 10and the results were interpreted. A higher value in the sample relative to the control indicates that the bacterium is sensitive to the synthesised sample; a lower value indicates resistance. In this case, the sample's values are higher than the control's. The bacteria are therefore sensitive to prepared CNCs. This demonstrates that CNCs has strong antibacterial properties, making it suitable for widespread usage in the medical industry. If the prepared sample's qualities were further investigated and improved, it might be utilized in place of strong antiseptics. Therefore, the synthesized CNCs exhibits good resistance to these microorganisms. Table 1 shows the anti-bacterial activity of CNCs.

Negative Bacteria:





Positive Bacteria:

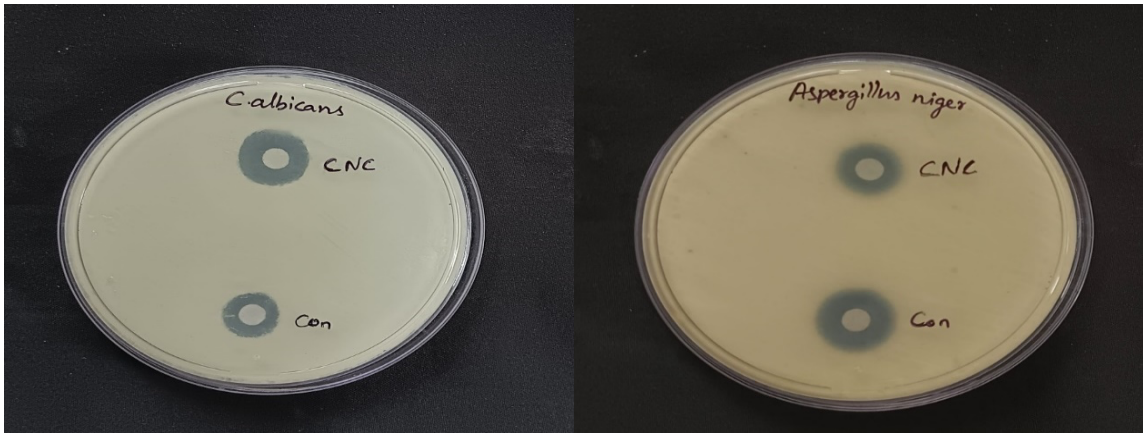
Fig 10: Anti-Bacterial activity of CNCs

Table 1: Activity of positive and negative bacteria

Bacteria	CNC	Control (Amikacin)
Escherichia coli	19.2mm	17mm
Proteus vulgaris	23mm	19mm
Bacillus subtilis	18mm	19mm
Staph aureus	19.2mm	15mm

3.7 ANTIFUNGAL ACTIVITIES OF CELLULOSE NANOCRYSTALS:

The antifungal activity of CNCs was investigated against Candida albicans and Aspergillus niger. Candida albicans exhibited maximum inhibition zone as shown in fig11 indicating stronger antifungal effect. Table 2 shows the anti-fungal activity of CNCs from *Borassus flabellifer.*



Fungus	CNC	Control (Nystatin)
Candida albicans	16mm	13mm
Aspergillus niger	14mm	16mm

4. CONCLUSION

Using a combination of acid hydrolysis and ultrasound treatment, CNCs have been successfully produced. In acid hydrolysis, the amorphous regions of cellulose were completely removed resulting in nanocrystals with high surface area. Characterization techniques such as FTIR, XRD, SEM and TEM were carried out.FTIR confirms the presence of functional groups. The crystallinity index of CNCs was calculated as 64.51% and the average particle size was found to be 12nm. From SEM image the surface morphology of CNCs was found to be needle like structure. The synthesized CNCs' zeta potential value was -0.0335, indicating a highly stable colloidal system.

CNCs showed high anti-bacterial and anti-fungal activity. These nanomaterials can be used as bio composites for reinforcing polymers, creating biodegradable composites, and serving as templates in nanostructured materials. Understanding the synthesis parameters and their impact on the characteristics of CNCs is essential for tailoring their properties for specific applications, ultimately contributing to advancements in sustainable and high-performance materials.

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6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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