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PREPARATION OF CHITIN NANOWHISKERS USING *Pleurotus ostreatus* AND ITS APPLICATION IN DYE REMOVAL

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I.ABSTRACT:

Chitin, a biopolymer abundantly found in the exoskeletons of crustaceans and fungal cell walls, has gained attention due to its biocompatibility and sustainability. The study outlines a chemical method for extracting chitin from *Pleurotus ostreatus* include dimeralization, deproteination, decolourization and subsequently converting it into nanowhiskers through acid hydrolysis. Characterization techniques including SEM, FTIR, and XRD confirm the successful formation of chitin and chitin nanowhiskers with desirable properties such as high aspect ratio, uniform morphology, and crystalline structure. The dye adsorption experiment was performed by using crystal violet dye. The prepared chitin nanowhiskers was added into dye solution. The dye was adsorbed and measured by using UV spectrometer. In conclusion, this study presents a sustainable approach for the preparation of chitin nanowhiskers from *Pleurotus ostreatus* and underscores their promising application in dye removal from industrial effluents.

Key words: chitin, biocompatibility, chitin nanowhiskers, crystal violet dye.

II. INTRODUCTION:

Mushroom is being widely used as food and food supplements from ancient times. Over 14,000 different types of mushrooms can be found in the world. Among all varieties of mushrooms, oyster mushrooms are classified into more than 200 categories. Approximately 600 of the 14,000 species that are known have important pharmacological qualities, and 2000 of them are safe. The presence of dietary fiber, specifically chitin and beta glucans, is primarily responsible for these functional properties of mushrooms. The oyster mushroom is one of the most extensively farmed varieties of mushrooms among all others.

2.1 DESCRIPTION OF OYSTER MUSHROOM

Pleurotus species are often called "oyster mushrooms". *Pleurotus ostreatus* is one of the most widely grown types of mushrooms worldwide. It is the second largest commercially produced mushroom in the world, after *Agaricus Bisporus*. The genus Pleurotus has approximately thirty-eight species from various parts of the world.

2.2 CHITIN NANOWHISKERS

A type of rod-shaped crystal substance is called chitin nanowhiskers (CNW). Owing to its unique nanosize and high modulus, CNW is currently widely utilized as a reinforcing filler in a variety of polymer composites, including polyacrylate, natural rubber, polyvinyl alcohol, chitosan, and maize starch. The only difference between CNW and cellulose nanowhiskers is the presence of an acetamide group in the chitin structure. It can withstand chemical reagents better. In ordinary solvent, the CNW is poorly soluble (Seyyed Salar Meshkat *et al*.,2019).

High-performing nanomaterials known as chitin nanowhiskers (CNWs) can be isolated from chitin, one of the most accessible bioresources. An acid can be used to hydrolyze native chitin to produce this nanomaterial. Chitin nano-whiskers (CNWs) are made hydrolytically with boiling HCl and vigorous stirring from several chitin sources, including crab and shrimp shells. With so many benefits, including low density, nontoxicity, biodegradability, biocompatibility, and ease of surface modification and functionalization, the chitin nanowhiskers (CNWs) are expected to find widespread use in a variety of fields, such as tissue engineering, dye removal, food industry, drug delivery, cosmetics, and reinforcing nanocomposites.

These crystallites are rod-shaped, nanoscale particles that range in length from 150 to 600 nm and in diameter from 1 to 60 nm. Chitin nano-whiskers, like nanofibers, have very large and active surface areas, high stiffness and strength. The benefits of nanomaterials and chitin's adsorption ability are combined in chitin nano-whiskers.

Several adsorbents, including cellulose, CM, ChNWM, and ChNW, were used in the crystal violet absorption experiment. Compared to all other adsorbents, chitin nanowhiskers (ChNW) demonstrated improved adsorption and removal efficiency. The electrostatic force between the positively charged crystal violet molecules and the negatively charged ChNW molecules could be the cause (Sreerag Gopi *et al*.,2016).

III. MATERIALS AND METHODS:

3.1 SAMPLE COLLECTION AND PREPARATION:

The oyster mushroom (*Pleurotus ostreatus*) was collected from the local market, Coimbatore. Oyster mushrooms are an excellent option for chitin extraction because of their high chitin content, rapid development, easy of cultivation, biodegradability, and capacity for large-scale production. The oyster mushroom was cleaned, cut into small pieces, and dried for three days at 50°C in a hot air oven. Samples were put in a mixer and ground into powder. They were stored for chitin extraction.

3.2 EXTRACTION OF CHITIN FROM OYSTER MUSHROOM(*Pleurotus ostreatus*):

Twenty gram milled mushroom sample was weighted for the chitin extraction. Chitin was extracted by using chemical method which consists of the following steps: demineralization by acidic treatment, deproteinization by alkali treatment, and decolorization for the removal of pigments and colours.

3.2.1 Demineralization:

The demineralization process removes impurities and minerals. It is primarily carried out by acid treatment with sulphuric acid, hydrochloric acid, nitric acid, acetic acid, and formic acid, but hydrochloric acid is the most commonly used acid. For 20 g of dried mushroom, 250 ml of 2M HCL was used. The demineralization step took 15 h at 60°C. After this time, the samples were filtered through a filter paper with pore size of 2 μm by rinsing with distilled water to remove excess acid in it.

3.2.2 Deproteination:

Deproteination includes the disruption of chemical bonds between the chitin and protein. NaOH is employed in the process of deproteinization. 250 ml of 2M NaOH were used to treat the sample of demineralized mushrooms. In order to remove any remaining protein residues from the chitin structure, the deproteination step was carried out at 85°C. The sample was filtered and washed with distilled water until it reached a neutral pH after being treated with NaOH for 24hours.The sample was washed to remove excess chemicals present in the sample.

3.2.3 Decolorization:

The decolorization is the next step followed by deproteination to remove color and pigments present in the sample. Chloroform, methanol, and distilled water were combined in a 1:2:4 ratio to accomplish decolorization. It took an hour to bleach the samples. The sample was rinsed with distilled water. Kept in an oven at 50°C until drying. The chitin thus extracted was used for nano-whiskers preparation.

3.3 CALCULATION OF CHITIN CONTENT:

The chitin content (%) was calculated using the following formula:

$$\text{Chitin content (\%)} = \frac{\text{Dried chitin extracted (g)} \times 100}{\text{Raw material (g)}}$$

3.4 PREPARATION OF CHITIN NANOWHISKERS.

Chitin nanowhiskers were prepared using chitin as precursor material, according to the methodology proposed by Pereira *et al*. (2014), with some modifications. Hydrochloric acid (3 mol/L) was used to hydrolyze chitin for 90 minutes at boiling temperatures while stirring. A 30 mL acid solution was used for every 1 g of chitin. The resultant dispersion was diluted with deionized water after being cooled to room temperature. Phase separation was achieved by centrifuging the suspension for 20 minutes at 4000 rpm. Until the precipitate's pH reached neutral, it underwent many centrifugations and washings. Then freeze-dried for 24 h to obtain chitin nanowhiskers. The prepared chitin nanowhiskers was stored and used for dye removal.

3.5 DYE ADSORPTION EXPERIMENT:

To conduct an experiment on dye removal, 250 mg/L of crystal violet was prepared. Crystal violet was dissolved in distilled water to create dye solutions. Around 40 ml of crystal violet solutions was treated with 2 g of powdered chitin nanowhiskers and continuously stirred at 600 rpm for about 1 hour. After incubation, the UV-vis spectrophotometer was used to measure the concentration of crystal violet in aqueous solutions at a wavelength of 584 nm.

The percentage of crystal violet dye removal from aqueous solutions was calculated by the following equation.

$$\% R = \frac{Ci - C0}{Ci} X100$$

Where

Ci (mg/L) is the initial concentration of solution

C0 (mg/L) is the final concentration of solution

3.6 CHARACTERIZATION OF CHITIN AND CHITIN NANOWHISKERS

3.6.1 FTIR analysis

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bond/functional groups. Fourier Transform Infrared Spectrophotometer (FT-IR) spectra of samples were recorded in the range 4000-400 cm to study the molecular structure of the sample. Collection and analysis of IR images were done using spectrum V6.0 software. The wavelength of light absorbed is the salient feature of chemical bonds seen in the annotated spectrum. It is used to determine the presence of specific functional groups like carbon-carbon multiple bonds, aromatic rings, carbonyl, or hydroxyl groups in a molecule. To prepare the samples for FTIR analysis, the dry ground powders are blended with powdered KBr (usually at a ratio of 1:5), and the resulting mixture is compressed into discs.

FT-IR spectra of Ch and ChNW, we can confirm that these spectra are very similar and present the same bands. The infrared band at approximately 2900 cm–1 is caused by the stretching vibrations of CH2 and CH3, and amide I and II, were shown to be correlated with the IR bands at 1650 and 1550 cm–1. The O–H and N–H stretching vibrations were seen in both spectra as a broad band centered at 3400 cm–1. At 1450 cm–1, the distinctive CHx deformation band is visible. The bands that can be seen fall between 1250 and 820 cm^1. They are caused by C–O and C–O–C stretching (Dotto *et al*., 2015).

3.6.2 XRD analysis

X-ray diffraction (XRD) can be used to investigate the crystalline structure. For a complete and consistent X-ray exposure, the powdered components were leveled and positioned inside the sample container. With an X-ray diffractometer and CuK radiation (= 1.5406Å), the X-ray equatorial diffraction patterns of chitin and ChNW were obtained at an operating voltage and current of 40 kV and 40 mA, respectively (Sreerag Gopi *et al.,* 2016).

The Bragg's law is a fundamental equation in crystallography that relates the wavelength of incident X-rays (or other electromagnetic radiation) to the diffraction angle and the lattice spacing in a crystalline sample. It is expressed as:

$2d \sin\theta = n\lambda$

Where:

d is the interplanar spacing (distance between parallel planes of atoms)

θ is the angle of incidence (or the Bragg angle)

n is an integer representing the order of diffraction

λ is the wavelength of the incident X-ray beam.

3.6.3 SEM analysis

The morphology and physical status of the chitin surface can be visually confirmed through the use of scanning electron microscopy. Without pores, the surface of Ch is homogenous, smooth, and extremely compressed. Samples were examined at tensions between 5 and 15 kV in low vacuum mode on a field emission gun SEM. Working distance ranged from 1 to 100 mm using a large field detector (LFD).

SEM was used to examine the morphology of chitin and chitin nanowhiskers. An SEM image of native chitin reveals a densely packed structure containing minerals, proteins, and hydrogen bonds between and within molecules. The amorphous and crystalline areas of native chitin are incorporated in the protein and mineral matrix, resulting in reduced dispersibility in aqueous solution. The rough surface of chitin nanowhiskers is caused by incomplete dissolution of the amorphous region, which may result in moderate dispersibility in a water medium. Lastly, in the case of ChNW, a rod-like morphology can be observed, which improves ChNW's dispersibility in comparison to other forms of chitin. Additionally, this shape of ChNW aids in the adsorption process by making it easier for the dye molecules to occupy the adsorption sites, which can be challenging in raw chitin due to its lower area-volume relation (Sreerag Gopi *et al.,*2016).

IV. RESULTS AND DISCUSSION:

4.1 Collection of mushroom and preparation of mushroom sample:

In this study, the oyster mushroom (*Pleurotus ostreatus*) was chosen for chitin extraction. The oyster mushroom (*Pleurotus ostreatus*) was collected from the local market, Coimbatore. The quantity of one packet of mushroom was about 200g. For 200g of mushroom,20 g of dried mushroom was obtained. The dried mushroom was ground into fine powder for chitin extraction. The percentage of dried mushroom obtained was about 10%.

4.2 Chitin extraction:

To extract chitin in its pure form from mushrooms, the mineral components must be eliminated, most notably calcium carbonate (CaCO3) and calcium phosphates, which are removed with a strong acid. Following the mineral extraction process, a higher concentration of sodium hydroxide (NaOH) ranging from 0.125 to 5.0 M was used to remove the chitin-associated proteins. In addition to causing deproteination, NaOH also causes the biopolymer to hydrolyze. Using a combination of solvents, an additional step was carried out to remove color and pigments related to the chitin structure. Pigments such as carotenoids were removed using acetone or a mixture of organic solvents. Decolorization was occasionally carried out. At last, chitin was extracted from *Pleurotus ostreatus*.

For 12 hours, 50.0 g of *Hericium erinaceus* was submerged in 500 mL of 4% HCl solution at room temperature. The acid-insoluble residue was then collected using a 400-mesh filter cloth and cleaned with distilled water until it reached a neutral . After that, 400 mL of a specific concentration of NaOH was added to the resulting residue, and it was heated for a predetermined amount of time at a specific temperature. Following the reaction, a 400-mesh filter cloth was used to filter the mixture, and it was then cleaned until the pH was neutral. The 300 mL NaClO solution (7.5%, pH 4.0) was then combined with the alkali insoluble residue at 75°C for two hours. Additionally, the residue underwent filtering and a neutralizing wash in distilled water. After being freeze-dried, the obtained chitin was pulverized and passed through a 100-mesh sieve(Jing Liao *et al*.,2020).

4.3 Drying of chitin and calculation of chitin content.

Decolourized sample content was dried to obtain chitin at 50°C in a hot air oven. The extracted chitin was stored for nanowhisker extraction. For 20 g of dried mushroom, 0.85 g of chitin was extracted. The chitin content of oyster mushroom (*Pleurotus ostreatus*) was about 3.5 to 4.2%. Chitin concentration of *P. ostreatus* fruit bodies are: 3.78% DM (±0.97) and 2.8% DM (±0.75) for pileus and for stipes. Yield of chitin from *Pleurotus ostreatus* (whole fruit body) was 4.77–4.95 %(Janos Vetter *et al*.,2007). In our current study same amount of chitin content was extracted.

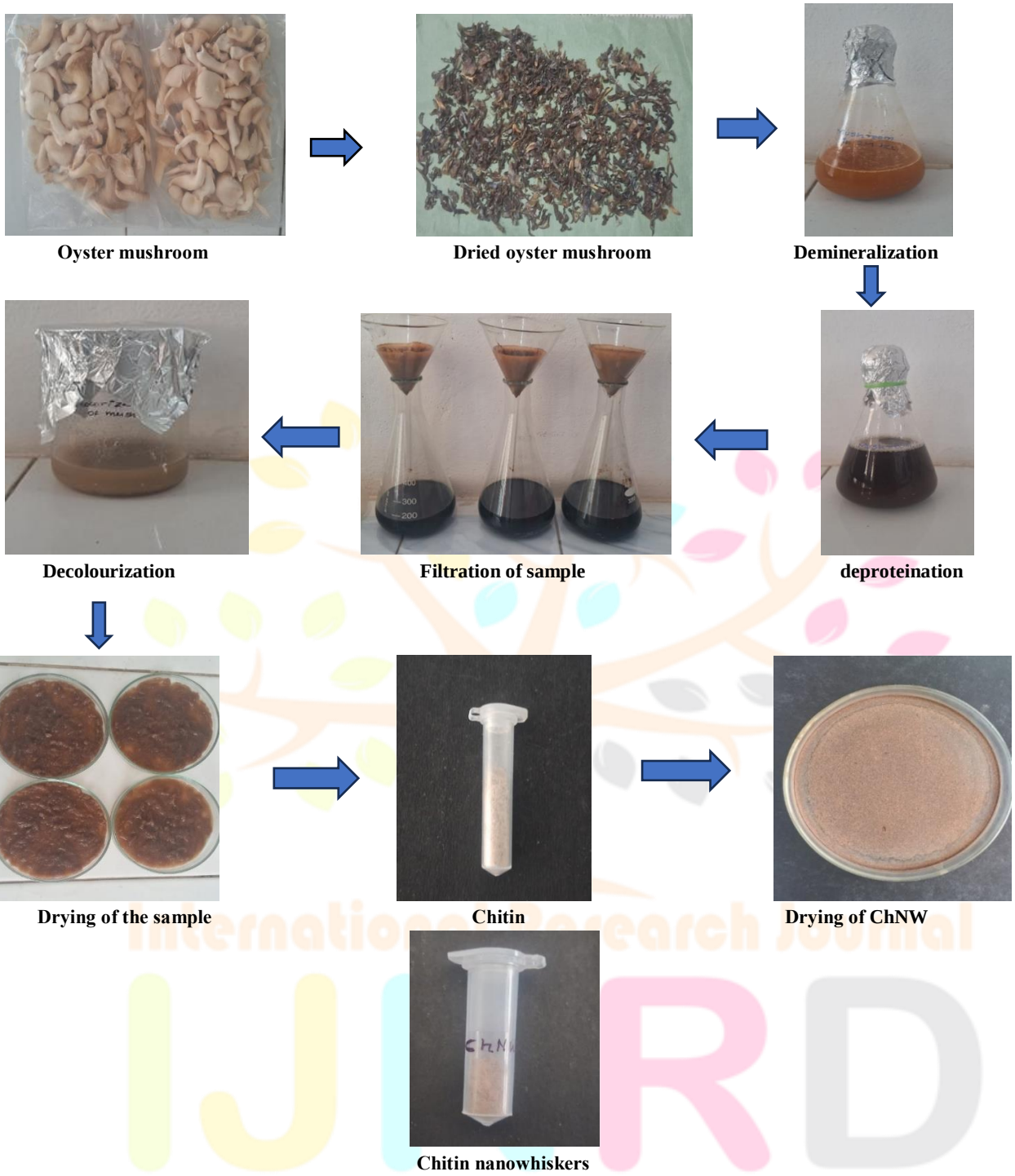
4.4 Chitin nanowhiskers

Chitin was converted into chitin nanowhiskers by acid hydrolysis of native chitin. The chitin was broken into chitin nanowhiskers by treating it with hydrochloric acid. The amount of nanowhiskers from 0.35g of chitin was about 0.119g. The percentage of chitin nanowhiskers from *Pleurotus ostreatus* was 34%. Chitin was disrupted to obtain chitin nanowhiskers. The obtained chitin nanowhiskers was used for dye removal in contaminated water.

The chitin was bleached for two hours at 80°C using a sodium hypochlorite solution (17 g of sodium hypochlorite in 1 L of 0.3 M sodium acetate buffer at pH 4.0).Subsequently, the washed residue was stored in 5% of potassium hydroxide for 72 h to remove the residual proteins. After adding hydrochloric acid to the resultant dispersion, centrifuging, and repeating a series of washings, the ChNW suspensions were moved to a dialysis bag and dialyzed until a pH of 6 was obtained. After supersonically dispersing the ChNW, isopropyl alcohol was

used to chill the mixture to 36°C. A fine powder that was utilized as an adsorbent was produced by lyophilizing and freeze-drying frozen samples (Sreerag Gopi *et al*.,2016).

4.5 SCHEMATIC REPRESENTATION OF EXTRACTION OF CHITIN AND CHITIN NANOWHISKERS



4.6 Dye removal using chitin nanowhiskers

The prepared nanowhiskers was used for the removal of dye in the contaminated water. In this study, Crystal violet dye was used as an example for the study of dye removal by chitin nanowhiskers. The concentration of crystal violet in aqueous solutions was determined by UV–vis spectrophotometer at the wavelength of 584 nm. The percentage of removal of crystal violet from aqueous solutions was 95% . The obtained chitin nanowhiskers was the best naturally available dye adsorbent. It was considered as a potent source for dye removal.

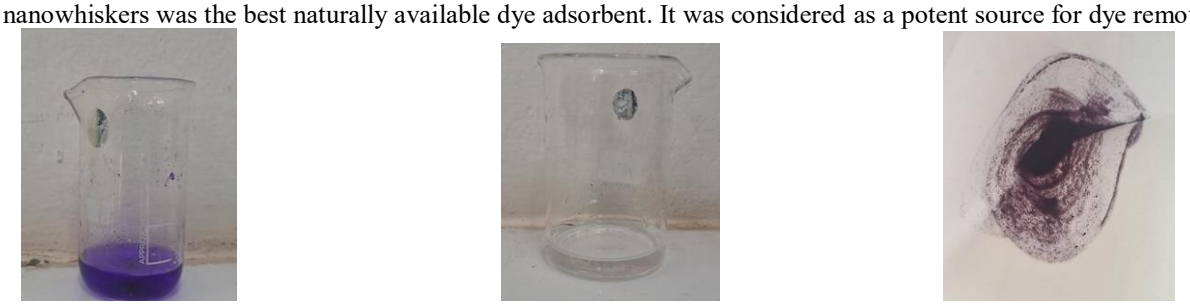


Figure:1 Dye adsorbed by chitin nanowhiskers

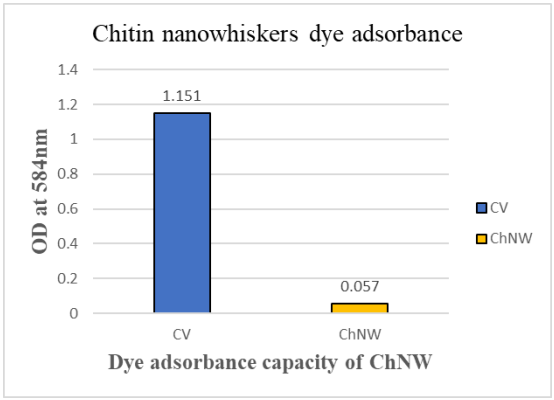


Figure:2 Dye adsorbance capacity of chitin nanowhiskers

4.7 CHARACTERIZATION OF CHITIN AND CHITIN NANOWHISKERS

4.7.1 FTIR ANALYSIS:

CHITIN

Figure 3 showed the FTIR spectra of chitin .In that the peak at 3741.90 cm^{-1} showed the presence of hydroxyl (OH) groups. The peak at 1527.62 cm^{-1} showed the presence of amide II vibrations arise from the bending motion of N-H bonds and the stretching vibration of C-N bonds within the amide groups of the polymer backbone. The peak at 578.64 cm^{-1} in chitin spectrum typically represented the presence of vibrations associated with saccharide ring deformation. The vibrations at around 578.64 cm^{-1} are characteristic of the ring breathing or bending motions, contributing to the overall fingerprint of chitin's FTIR spectrum. The peak at 516.92 cm^{-1} - 470.63 cm^{-1} typically represented vibrations associated with C-O-C linkage in the glycosidic bonds that connect the N- acetylglucosamine units in chitin's polymer chain. The peak at 493.78 cm^{-1} typically represented bending vibrations of the C-C bonds in the pyranose ring of the N-acetylglucosamine units that make up chitin's polymer chain. The peak 432.05 cm^{-1} typically represented vibrations associated with the stretching of C-O-C linkage with the glycosidic bonds that connect the N-acetylglucosamine units in chitin's polymer chain.

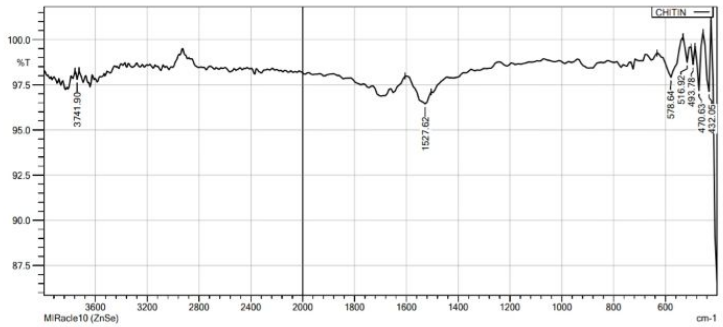


Figure:3 FTIR SPECTRA OF CHITIN

CHITIN NANOWHISKERS

Figure 4 showed the FTIR spectra of chitin nanowhiskers. The peak at 3348.42 cm^{-1} showed the presence of hydroxyl (OH) groups. The peak at 1481.33 cm^{-1} showed the presence CH2 bending vibrations. This peak indicates the bending vibration of CH2 groups. In chitin nanowhiskers, this peak reflects the molecular structure of chitin, which consists of repeating units of N-acetylglucosamine (GlcNAc) linked by β-(1→4) glycosidic bonds. The peak at 1373.32 cm^{-1} showed the presence of bending vibration of CH3 groups. The peak at 1049.28 cm^{-1} showed the presence of C-O stretching vibrations, which are commonly found in polysaccharides. The peak at 725.23 cm^{-1} showed the presence of C-H bending vibrations in the polysaccharide structure. The peak at 648.08 cm^{-1} showed the presence of stretching vibration of the C-N bond in chitin nanowhiskers. This peak provides valuable information about the presence of nitrogen-containing functional groups. The peak at 601.79 cm^{-1} showed the presence of bending vibration of the N-H bond. The peak at 563.21 cm^{-1} showed the presence of stretching vibration of the C-O-C glycosidic linkage. The peak at 532.35 cm^{-1} showed the presence of the stretching vibration of C-O bonds in the glycosidic linkage. The peak at 493.78 cm^{-1} showed the presence of stretching vibration of the C-O-C glycosidic linkage. The presence and intensity of this peak can provide information about the crystallinity and structural organization of the chitin nanowhiskers. The peak at 462.92 cm^{-1} showed the presence of corresponds to the vibration of the C-N bond in the amide II region. The peak at 408.91 cm^{-1} showed the presence of crystalline structure of chitin nanowhiskers. The appearance of this peak suggests the presence of crystalline regions in the chitin nanowhiskers, likely arising from the ordered arrangement of the polysaccharide chains. One peak range differentiating between chitin and chitin nanowhiskers in FTIR spectroscopy is the range associated with the glycosidic linkage vibrations, typically around 1150-950 cm^{-1} . FTIR peak of chitin and chitin nanowhiskers differs based on aliphatic C-H stretching vibrations.

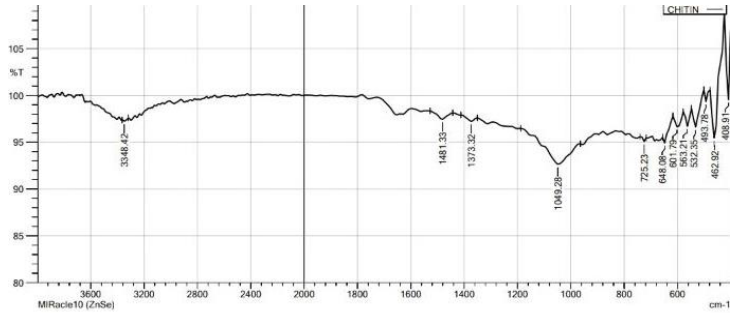


Figure:4 FTIR SPECTRA OF CHITIN NANOWHISKERS

Broad band at 3,430 cm^{-1}, which corresponds to the vibrational stretching of the hydroxyl group (Sandra Patricia Ospina Álvarez *et al*.,2014). The band at 2,922 cm^{-1} represents the C–H a symmetric stretching (Teng *et al*., 2001) reported that bands near to 2,900 cm^{-1} are representative bands for chitin. The band at1,657 cm^{-1} corresponds to the amide I stretching of C=O, while the band at 1,564 cm^{-1} corresponds to the stretching or N–H deformation of amide II, and bands at 1,380 cm^{-1} and 894 cm^{-1} correspond to the symmetrical deformation of amide III.(Sandra Patricia Ospina Álvarez *et al*., 2014).

4.7.2 XRD ANALYSIS:

CHITIN

The XRD (X-Ray Diffraction) data table and image provide valuable information about the imperfect crystalline lattice structure of the analyzed material. The Bragg's law is a fundamental equation in crystallography that relates the wavelength of incident X-rays (or other electromagnetic radiation) to the diffraction angle and the lattice spacing in a crystalline sample. It is expressed as:

$$2d \sin\theta = n\lambda$$

Assuming the X-ray wavelength (λ) used in the experiment is known (e.g., 1.54 Å for Cu Kα radiation), we can rearrange Bragg's equation to calculate the interplanar spacing (d) for each diffraction peak:

$$d = n\lambda / (2 \sin\theta)$$

From the Tabulated Value

For the peak at 2θ = 23.5506°:

$$d = (1 \times 1.54056\ Å) / (2 \times \sin(11.7753°))$$

$$d = 3.77772\ Å$$ (matching the value in Table 1)

For the peak at 2θ = 26.6369°:

$$d = (1 \times 1.54056\ Å) / (2 \times \sin(13.3185°))$$

$$d = 3.34662\ Å$$ (matching the value in Table 1)

Discussion:

The observed diffraction peaks in the XRD pattern (Fig. 5) confirm the crystalline nature of the sample. The positions of these peaks, represented by the 2θ and d-spacing values, can be explained by Bragg's law, which relates the diffraction angle to the interplanar spacing and the wavelength of the incident X-ray radiation. Table 1, presents the higher intensity peak at 23.5506° suggests that the corresponding set of lattice planes has a higher population of atoms or a higher degree of ordering, contributing to a stronger diffracted beam. In contrast, the lower intensity peak at 26.6369° may indicate a lower atomic density or a higher degree of disorder along those particular lattice planes. It is important to note that the presence of only two prominent diffraction peaks in the XRD pattern may suggest a relatively simple or highly symmetric crystal structure.

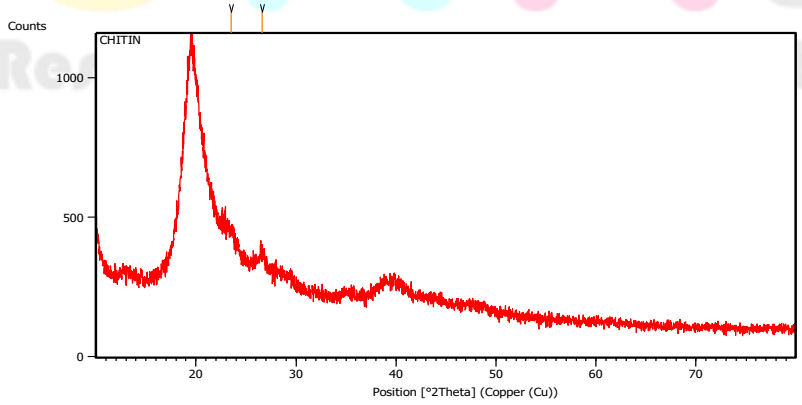


Figure:5 X-ray diffractograms of chitin

Table:1 XRD values of chitin

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
23.5506	31.77	0.8029	3.77772	63.01
26.6369	50.42	0.5353	3.34662	100.00

X-ray diffraction (XRD) can be used to investigate the crystalline structure. XRD findings were interpreted to determine the kind of chitin. Alpha chitin's X-ray diffraction pattern usually exhibits prominent peaks at approximately 2θ values of 9.3°, 19.7°, and 26.6°.The alpha chitin crystal lattice's (020), (110), and (130) planes are represented by the peaks, in that order. For beta chitin, the most noticeable peaks are found at approximately 2θ values of 19.2°, 20.2°, and 9.2°. The beta chitin crystal lattice's (020), (110), and (120) planes are represented by these peaks, in that order.

CHITIN NANOWHISKERS

The XRD pattern of the chitin nanowhiskers is shown in Figure 6. The diffractogram exhibits three prominent diffraction peaks at 2θ values of 22.9758°, 26.5301°and 29.3852°.

Discussion:

The observed diffraction peaks in the XRD pattern (Fig. 6) confirm the crystalline nature of the sample. Table 2, presents the higher intensity peak at 22.9758° suggests that the corresponding set of lattice planes has a higher population of atoms or a higher degree of ordering, contributing to a stronger diffracted beam. In contrast, the lower intensity peak at 29.3852° may indicate a lower atomic density or a higher degree of disorder along those particular lattice planes. It is important to note that the presence of only three prominent diffraction peaks in the XRD pattern may suggest a relatively simple or highly symmetric crystal structure. A peak position at around 29.3852° in an XRD result typically suggests a crystalline structure in the material being analyzed. This value corresponds to the diffraction angle (2θ) at which X-rays are diffracted by the crystal lattice planes of the sample. The difference between the XRD result of chitin and chitin nanowhiskers indicates the crystalline structure of chitin nanowhiskers.

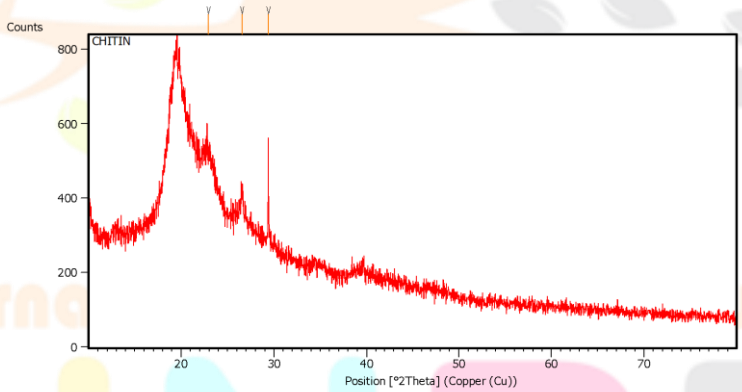


Figure:6 X-ray diffractograms of chitin nanowhiskers

Table:2 XRD values of chitin nanowhiskers

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
22.9758	58.07	0.8029	3.87093	20.19
26.5301	76.31	0.5353	3.35984	26.52
29.3852	287.69	0.0502	3.03957	100.00

4.7.3 SEM ANALYSIS:

SEM (Scanning Electron Microscopy) analysis of chitin and chitin nanowhiskers can reveal important information about their morphology and structure at the micro- and nanoscale levels. The surface morphology of chitin and chitin nanowhiskers can be seen in high-resolution pictures obtained by SEM. Owing to their tiny size, chitin nanowhiskers may have a more homogeneous and sophisticated morphology than chitin, which usually manifests as a fibrous material with a hierarchical structure. Nanowhiskers and chitin particles can be identified by their size and shape using SEM images. A rod-like or needle-like structure with dimensions in the nanometer range is expected for chitin nanowhiskers, while chitin particles may appear larger and more asymmetrical in shape. SEM images can provide insights into the surface properties of chitin and chitin nanowhiskers, including roughness, porosity, and the presence of any surface modifications or coatings.

CHITIN

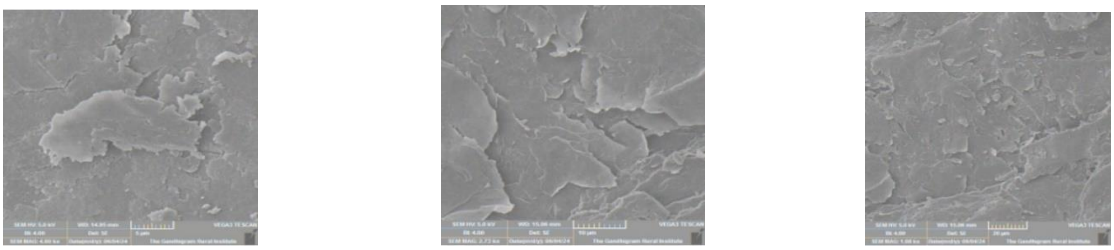


Figure: 7 CHITIN

CHITIN NANOWHISKERS

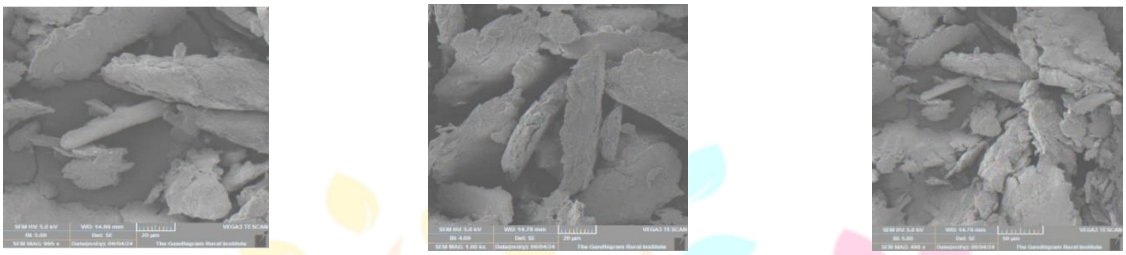


Figure: 8 CHITIN NANOWHISKERS

The SEM analysis of chitin indicates the improper structure of chitin. The formation of rod shape in chitin nanowhiskers synthesized from *Pleurotus ostreatus* which ranges from 20nm to 50nm was confirmed by scanning electron microscopy.

V. SUMMARY AND CONCLUSION:

The study demonstrated the successful preparation of chitin nanowhiskers from *Pleurotus ostreatus* biomass and explored their efficacy in dye removal applications .Chitin, a natural biopolymer found in the cell walls of fungi, mushroom, crustaceans and the exoskeletons of arthropods, possesses unique properties such as biocompatibility, biodegradability, and adsorption capabilities, making it suitable for various applications including environmental remediation. Chemical method was used for chitin extraction. Chemical method include demineralization, deproteination and decolourization. The synthesized chitin and chitin nanowhiskers was characterized using XRD, FTIR and SEM which were used to analyze the morphology, structure, functional group and surface properties of the chitin and chitin nanowhiskers. XRD of chitin nanowhiskers confirms its crystalline nature. SEM analysis of chitin nanowhiskers confirms its rod shape. Chitin nanowhiskers offer potential adsorbents due to their high surface area, porosity, and functional groups capable of interacting with dye molecules. The project's findings contribute to the development of sustainable and eco-friendly materials for wastewater treatment, addressing the growing concern of dye pollution in water bodies. Future research could focus on optimizing the preparation method for chitin nanowhiskers, investigating their adsorption kinetics and mechanisms, exploring potential regeneration and reuse strategies, and evaluating their performance under real-world conditions. Overall, the project highlights the potential of chitin nanowhiskers derived from *Pleurotus ostreatus* in environmental remediation efforts, paving the way for further advancements in this field.

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