ANTI-MICROBIAL ACTIVITY OF CELLULOSE NANO CRYSTAL DERIVATIVES PRODUCED FROM WASTE TISSUE PAPER

BY

YAFET ALULA WOLDEGIORGISE

A THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED BIOLOGY

SCHOOL OF APPLIED NATURAL SCIENCES

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER'S IN BIOTECHNOLOGY

OFFICE OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

NOVEMBER, 2021

ADAMA, ETHIOPIA

Anti-microbial Activity of Cellulose Nano Crystal Derivatives Produced from Waste Tissue Paper

By: Yafet Alula Woldegiorgise

Advisor: Seid Mohammed (Ph.D.)

Co-advisor: Zerihun Belay (Ph.D.)

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Sciences

In Partial Fulfillment of the Requirement for the Degree of Master's in Biotechnology

Office of Graduate Studies

Adama Science and Technology University

November, 2021

Adama, Ethiopia

DECLARATION

I hereby declare that this MSc thesis entitled “Anti-microbial Activity of Cellulose Nano Crystal Derivatives Produced from Waste Tissue Paper” is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

_____	_____	_____
Name of the student	Signature	Date

RECOMMENDATIONS

We, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled “Anti-microbial Activity of Cellulose Nano Crystal Derivatives Produced from Waste Tissue Paper” prepared under my/our guidance by Yafet Alula submitted in partial fulfillment of the requirements for the degree of Mater’s of Science in Biotechnology.

Therefore, I/we recommend the submission of the revised version of the thesis to the department following the applicable procedures.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date

APPROVAL PAGE OF M.SC. THESIS

We, the advisors of the thesis entitled “Anti-microbial Activity of Cellulose Nano Crystal Derivatives Produced from Waste Tissue Paper” and developed by Yafet Alula, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

_____	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Yafet Alula have read and evaluated the thesis entitled “Anti-microbial Activity of Cellulose Nano Crystal Derivatives Produced from Waste Tissue Paper” and examined the candidate during an open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biotechnology.

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

_____	_____	_____
Department Head	Signature	Date
_____	_____	_____
School Dean	Signature	Date
_____	_____	_____
Office of Postgraduate Studies, Dean	Signature	Date

ACKNOWLEDGEMENT

I would like to extend my foremost and sincere gratitude to my advisor Dr. Seid Mohammed and my co-adviser Dr. Zerihun Belay for their continuing advices, willingness, enthusiasm, and assistance throughout the research. I would like to extend my gratitude to Adama Science and Technology University and my teachers and staffs of Applied Biology for proving me the most pleasurable and challenging educational environment the I had the privilege to enjoy, learn and grow as student.

I would like thanks Dr. Ir. Sreenivas Reddy Bathula for his willingness to guide and support in this research paper.

I would like to extend my gratitude for material engineering school (ASTU) for their collaboration on the XRD, chemical department of Addis Ababa University for allowing me to use FTIR and finally department of applied biology for providing with SEM and working space for my research.

Finally, I would give my deepest gratitude for my family and friends who have been by my side along this wonderful educational journey.

TABLE OF CONTENTS

Contents **Page**

LIST OF FIGURES

LIST OF TABLES

I. LIST OF ABBREVIATIONS AND ACRONYMS

ACNC: Acetyl Cellulose Nano Crystals

AgCNC: Silver cellulose NanoCrystal

BWTP: Bleached Waste Tissue Paper

BNC: Bacterial Nano Cellulose

CNC: Cellulose Nano Crystals

CrI: Crystallinity index

FAO: Food and Agriculture Organization of the United Nation

FT-IR: Fourier-transform infrared spectroscopy

HWTP: Hydrolyzed waste Tissue paper

ICNC: Iodine Cellulose Nano Crystals

MCNC: Methyl Cellulose Nano Crystals

NCCLS: National Committee for Clinical Laboratory Standards

NFC: Nano fibrillated cellulose

SEM: Scanning electron microscope

WTP: Waste Tissue Papers

XRD: X-ray diffraction

II. ABSTRACT

Paper wastes can only be recycled for a limited number of cycles, each recycles decrease the pulp fiber decreasing the usability of the material. New pulps production and accumulation of waste paper have led to deforestation and waste accumulation. Most of the wastes end up as landfills or trash scattered in the environment which is unsafe for the environment. Cellulose is the most abundant polymer with a vast application as a nanocomposite and its derivative. In recent years, biodegradable materials with antimicrobial for wound covering, is in demand. Cellulose nanocomposite gave an alternative method of paper recycling. Therefore, the present study was designed to assess anti-microbial activity of cellulose Nano crystal derivatives produced from the waste Paper. Cellulose Nano crystal (CNC) was prepared in 45% sulfuric acid (H_2SO_4) and four derivatives of CNC were prepared Acetyl Cellulose Nano Crystal (ACNC) and Methyl Cellulose Nano crystals (MCNC) with esterification process, Iodine Cellulose Nano crystal (ICNC) with oxidation process, and Silver Cellulose Nano Crystal (AgCNC). The analytical technique such as Scanning Electron Microscope (SEM) were used for characterization of CNC and AgCNC, X-ray diffraction (XRD) were used for characterization of CNC and Fourier transformed infrared spectroscope (FT-IR) were used for characterization of CNC and it derivative: ACNC, ICNC, MCNC, and AgCNC. Anti-microbial properties of Cellulose Nano Crystal Derivatives were evaluated against Gram positive Staphylococcus aureus ATCC 25923, Gram negative Escherichia coli ATCC 25922, and Candida albicans ATCC 10231. The XRD result shows, CNC prepared at 45% concentration of H_2SO_4 has the highest peak at 2ϴ 22.54, full width at half maximum (FWHM) of 0.03 radian crystallinity index of 81.8%, and crystallinity size of 15.39nm. According to the FT-IR result, the comparative analyses of band change and shift between CNC with its derivatives have indicated successful derivation of CNC, with acetyl, iodine, methyl, and silver. The antimicrobial effects of ACNC, ICNC, and AgCNC have shown better results with increase of concentration. In conclusion, AgCNC is the most effective when compared with others. MCNC is the least effective of all the derivatives.

Key words*:* Acid hydrolysis; Cellulose; Cellulose Nano crystal; Surface modification; Paper waste.

1. INTRODUCTION

1.1. Background of the Study

Cellulose is a carbohydrate homopolymer consisting of β-D-glucopyranose units joint together by β-1,4-glycosidic linkage (French, 2017). Cellulose represents a very appealing material being renewable resources and recyclable material. It is the main constituent of plant cell walls in wood, cotton, hemp and other plant-based materials and plays an essential role in maintaining the plant structural phase. Anselme Payen is a French chemist who discovered in 1838, fibrous composition of plant, isolating cellulose from plant based material and established the chemical structure. Cellulose is the amplest polymer on Earth, which makes it also the most common organic compound. Annual cellulose synthesis by plants is close to 1.841e+12 tons. This equates to approximately 108 tons of pulp produced annually, from this, only 4 million tons are used for further chemical processing annually (Hermanutz *et al*., 2006).

Cellulose Nano Crystals are needle-shaped highly crystalline (~90%) cellulose moieties. The average diameter of the CNC was found to be 3–35 nm and a length of 100-1000 nm. CNC was firstly prepared by Rånby in 1949 by using an acid hydrolysis process and the idea of this hydrolysis process for CNC extraction was firstly introduced by Nickerson and Habrle in 1947. Although, this innovative work done by Rånby was dumped that time but again recognized in the 1970s after the work done by Derek Gray in McGill University (Canada) (Osong *et al*., 2016). There are three fundamental methods of extraction of nano cellulose from natural polymer, chemical, enzymatic and mechanical methods, each method will produce unique nano cellulose pertaining to different characteristics in morphology and polymerization degree (Deepa *et al*., 2011).

Cellulose have been used in the traditional packing and writing material but through nano technology and surface modification, a completely novel way of utilizing cellulose is being discovered. Various applications in different industries such as Composites Plastics, Paper and Packaging, medical, barrier properties and electronics/Metallic particles (Foster *et al*., 2018).

Currently, the use of low-cost and renewable materials has attracted more and more interest globally. Since cellulose is of natural origin, biocompatibility and bio-degradability are other features scientists are looking for in this amazing material also their unique and excellent properties including high young modulus, specific surface area, transparency and low thermal expansion coefficient and biocompatibility. For these positive properties, cellulose was found to have application in different research fields such as the biomedical, energy, environmental and water related fields (Mondal, 2017).

The use of CNC for controlling the proliferation of pathogenic bacteria and fungi, is proven effective in experimental research, but the use of CNC in medical application is at infancy expect in drug delivery. CNC coated with zinc oxide, titanium dioxide and silver oxide have shown to be very effective as antimicrobial agent in CNC hybrid form of film and Nano composites derived using Evaporation-induced self-assembly in aqueous solution and In situ solution casting technique respectively, have shown to be very effective agents *E. coli* and *S. aureus*. Therefore, the current study aimed to show the new recycling direction of cellulose from waste paper to produce CNC and improve its applicability with the aim of anti-microbial property by surface modification.

1.2. Statement of the Problem

The dramatic rise of the population is becoming the reason for excess production and utilization of raw materials to meet the demand and sustain life. In doing so the utilization of raw material for production is exceeding to the extent that we are depleting the resource. Environmental stress imposed by excess utilization row material, byproduct and manufacturing. Eco-unfriendly recycling and lack of efficient waste management techniques resulted in the continuous deposition of toxic and non-biodegradable materials, damaging habitat in turn decrease biodiversity. The issue of waste disposal and ineffective remediation systems are drawing researcher's attention towards harmless and easily biodegradable materials (Qiao *et al*., 2019).

Paper is made from one of the most abundant biomass (cellulose), having the capacity to use in different structures with a variety of functionality. The demand for a paper gives rise to several million tons of papers production which in turn results in the accumulation of waste paper. Even having sufficient recycling of waste paper continues to constitute considerably to the municipal and industrial waste. The maximum ratio of paper-to-paper recycling is reported to be 65%. Despite having good recycling occurrence waste paper

can only be recycled for a given amount of cycle, with every cycle the potential of the pulps is reduced (Bloemhof *et al.*, 1996). The fiber length which is affected by the recycling mechanism will decrease in size consequentially will produce paper with poor quality than virgin pulps. The destiny of these worn-out pulps is either combustion or packed in landfill, which are not the best option regarding the current global warming issue. So, these ever-accumulating wastes have the potential to be used as a form of CNC. This alternative waste paper recycling will provide a safer, environmentally friendly method of recycling and address the issue of byproducts arising from paper to paper recycling (Hamer, 2003).

The formation of biofilm is a stifling reason for slowed and regressed wound healing. Wound cover having the ability to kill microorganism, is a desired trait of wound cover, improving the initial use of wound cover as the name indicates. Lack of antimicrobial drug variation, low-income countries are facing antimicrobial resistance with overuse and continue usage of limited variation of antibiotic drugs. The rise of antibiotic resistance of bacteria and other microorganisms is a serious public health concern and a major cause of morbidity and mortality worldwide (Li and Webster, 2018). At present, it is an urgent necessity to prevent the spread the antimicrobial resistance and to limit the unnecessary use of antibiotics by providing an alternative material that has antimicrobial properties to cover wounds.

1.3. Significant of the Study

This research will help illuminate. The potential of waste paper for the production of CNC and the potential of solid waste recycling, which will increase efficiency in utilization of waste and produce material with natural composition for it is environmentally friendly. Show the benefit of CNC and increased potential through surface modification. Show the potential of surface modified CNC as an alternative material with antimicrobial properties to cover wounds. prevention of bacterial proliferation on a surface and mitigate problems like multidrug resistance bacteria by CNC and its derivatives. It will also help as an input for further biological or industrial studies related to the topic.

2. OBJECTIVE OF THE STUDY

2.1. General Objective

- To perform anti-microbial activity using cellulose Nano crystal derivatives derived from the Waste Tissue Paper.

2.2. Specific Objectives

- Cellulose was extracted from waste paper.
- The extracted cellulose was characterized by color and viscosity.
- Cellulose nano crystal was prepared of from extracted cellulose.
- The prepared cellulose nano crystal was characterized using SEM and XRD.
- Surface modification was performed on the prepared cellulose nano crystal.
- The modified CNCS were Characterized using FTIR.
- Antimicrobial activity of the CNC derivatives was evaluated against selected infectious strains

3. LITERATURE REVIEW

3.1. Waste Paper

The use of cellulose for production of usable material dates back many years. The most noted is paper, dating back almost 2000 years by cailun in Chain, with the progression of years alongside innovation and technology up rise, different type of material has been developed with cellulose being the base material, used in different sectors such as building material, fibers, packing, consumables, pharmaceuticals and so on (Czernik & Bridgwater, 2004).

The major of varieties of waste papers are comprised of cardboard packaging containers, newspapers, magazines and office waste papers. Packing containers have the highest utilization participation of 93.9%, followed by new paper at 92.8%. Office paper has the lowest utilization rate at 12.8% but makes up 30% of the total collected waste paper (Kumar *et al*., 2020). The principal sources of waste paper generation are industries and business houses which contribute around 52% in the collection or recovery of total waste paper in the form of paper shavings and cuttings. The household participation in the production of waste paper comes at second position with the contribution of 38% and unsold newsprints and magazines are at the third position at 10% in the generation of waste paper (Rodriguez *et al*., 2017).

Waste paper is one of the most recyclable cellulosic sources throughout the world. The recycling rate of waste paper at the global level is around 57.9% with (85%) in Australia, 72% in Europe, 70% in Canada, 64% the United States and 61% in South Africa (FAO, 2018, State of The Global Paper Industry 2018 - Report). Waste paper is one of the most recyclable cellulosic sources and is usually recycled 3–4 times (theoretically, 6–7 times) on average for papermaking (Joshi *et al*., 2017). With every cycle of waste paper reuse decreases the fiber strength due to negative changes in fiber morphology (shorter length and dimension), chemical composition and surface conditions of the fiber, fiber bonding (inter and intra), the polymerization degree of cellulose and water retention value. After a certain number of recycling most of the pulp fibers become too short and unfit to be used. Thus, it produces have a negative impact on paper sheets and is usually rejected as waste. This recycled paper sludge or deinking sludge ends up as in incineration or landfill, are unfriendly to the environment as these create greenhouse gases and harmful leachate (Bloemhof *et al*., 1996).

3.2. Solid Waste Management in Ethiopia

Urbanization, economic development and population growth of Ethiopia have led to an increase in waste production. The total waste production per year is estimated from 0.6 to 1.8 million tons in rural areas and 2.2–7 million tons in urban areas (EPA, 2004). The waste generation rate in Ethiopia is among the lowest; regardless the solid waste management system is very poor in Ethiopia. The infamous Koshe located in the southeastern part of Addis Ababa, with a surface area of 25 hectares is an illustration of a problematic method of waste disposal in Ethiopia. The most common practices are collecting waste and unloading on an open dumpsite without the necessary step such as sorting and pretreatment (Figure 1). The overall waste collection is below 50% and only 5% of waste is recycled (Teshome, 2020).

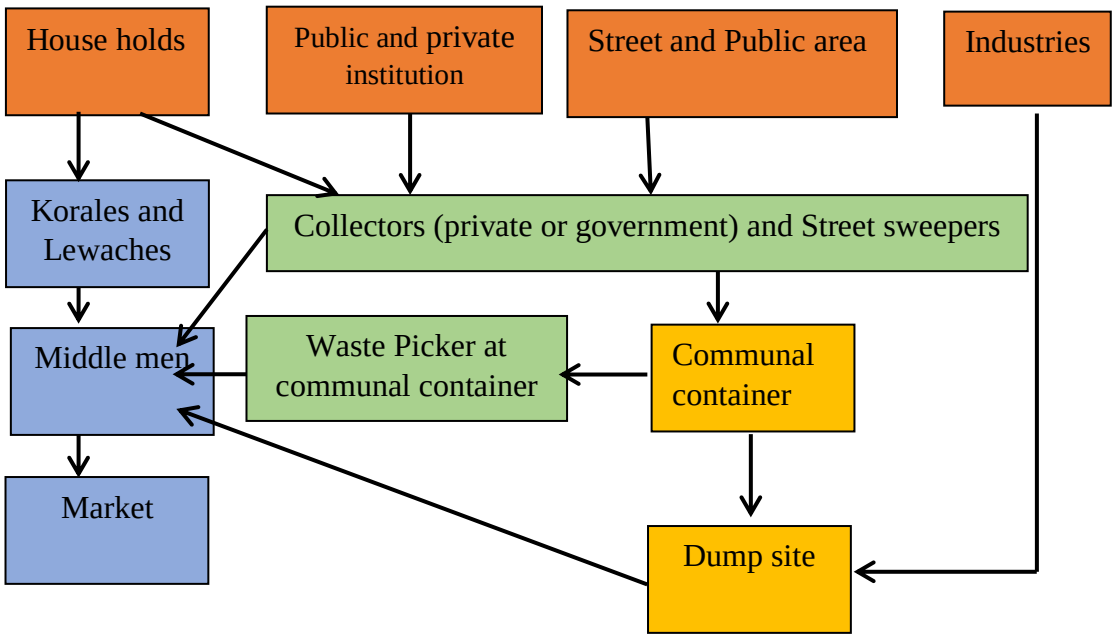

Figure 1. Diagrammatic description of the current waste management system in Ethiopia (Teshome, 2020).

3.3. Chemical and Structural Composition of Cellulose Fibers

Cellulose is a high molecular weight organic compound comprised of a homopolymer, of anhydro-d-glucose units, linked β-(1→4) glycosidic bond. The pyranoses sugar units are linked by removing the H from hemiacetal and –OH group from alcohol to from acetal with the process of condensation. Hence glucose units in the cellulose polymer are referred to as anhydroglucose units. Acetal linkages joined by single oxygen atoms between the pyranoses occur between the C-1 of one pyranose ring and the C-4 of an inverted pyranose, the next ring. Linking two of these sugars produces a disaccharide called cellobiose (Figure 2) (French, 2017).

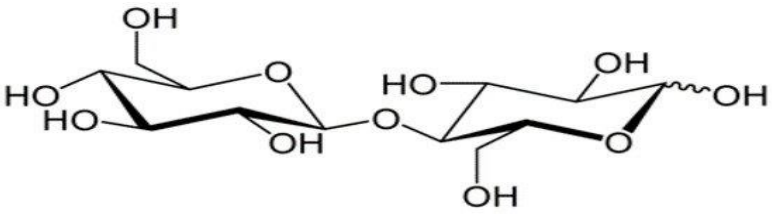

Figure 2. Cellobiose unit (French, 2017).

The cellulose molecule contains three different from of anhydroglucose units, the reducing end with a free hemiacetal at C-1, the nonreducing end with a free hydroxyl at C-4 and the internal rings joined at C-1 and C-4 (Figure 3) (Habibi *et al*., 2010).

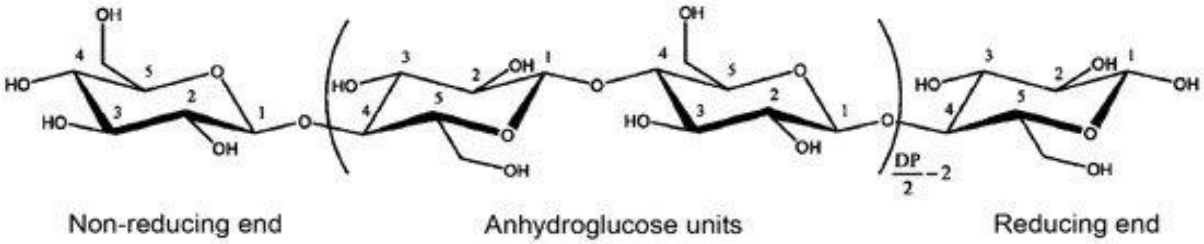

Figure 3. Chemical structure of cellulose (Habibi *et al*. 2010).

Other than acetal linkage, hydrogen bonds help maintains the structural integrity of cellulose. OH, group on cellulose has two possible interactions route. The intramolecular hydrogen bond between a sequences of cellulose bonded with C2-OH and C6-OH groups and C3-OH with endocyclic oxygen. The other intermolecular interaction occurs between the vertically parallel cellulose, between C-3 and C6-OH, and is responsible for the uniform packing nature of cellulose (Habibi *et al*. 2010).

Cellulose has two distinct regions as illustrated in (Figure 4a) crystal regions, where the polymers are packed tightly together in crystallites, which are stabilized by a strong and very complex intra- and intermolecular hydrogen-bond network. The amorphous regions are loosely packed as chain dislocations on segments along the elementary fibril where the microfibrils are distorted by internal strain in the fiber and proceed to tilt and twist (Hearle *et al.*, 1958).

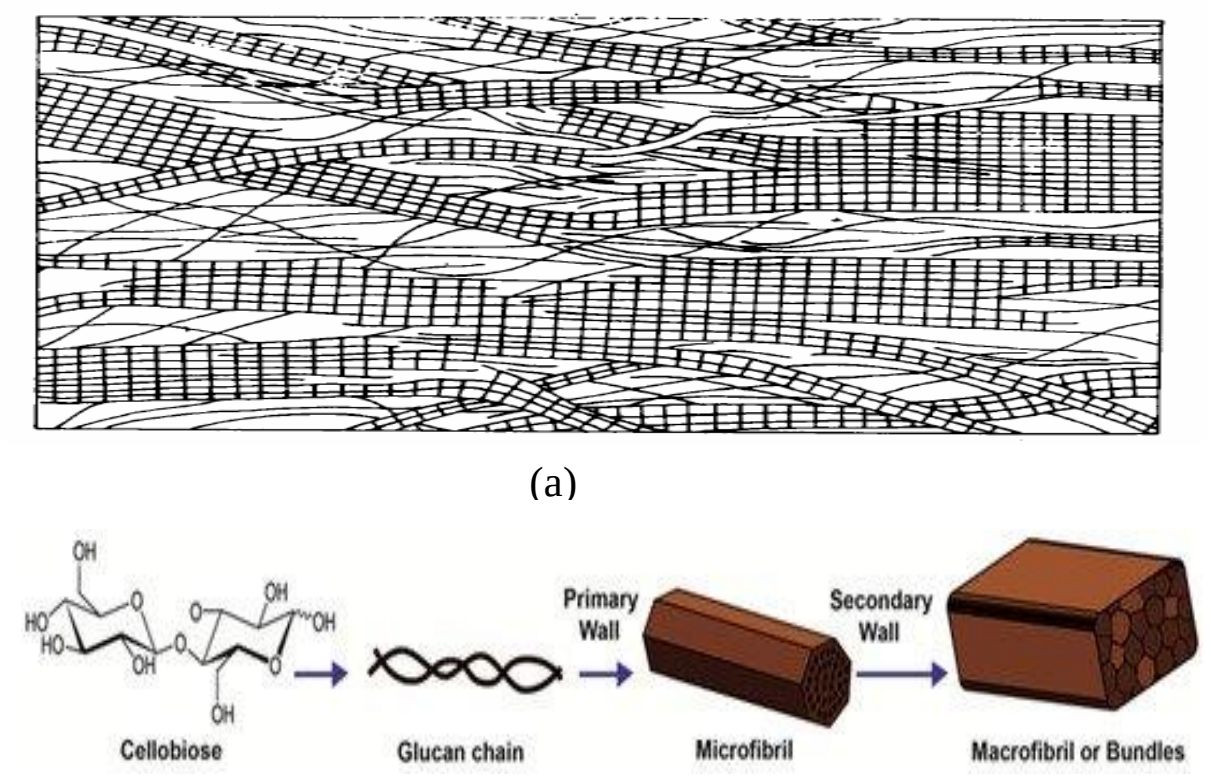

Figure 4. (a) Fringed fibril model of the super molecular structure of cellulose, regions: low ordered (amorphous) and highly ordered (crystalline) (Hearle, 1958). (b) Glucose residues polymerized from an individual chain and further assemble into microfibrils and macro fibrils or bundles (Festucci *et al.*, 2007).

Cellulose synthesis has separate initiation and elongation process. Then spun to from bundle in hierarchical order at the site of biosynthesis. In the primary cell wall of plants, pyranoses are polymerized into glucan chain. Later, 36 chains of glucan are assembled together to form a microfibril with a diameter of 28nm. During the synthesis of the plant secondary wall, microfibrils often associate further to form bundles of macro fiber (Figure 4b). However, the chain length may vary ranging from as low as 2000 up to 20,000 glucose residues (Festucci *et al.*, 2007).

3.4. Natural Source of Cellulose

Cellulose can be found from various sources. The traditional and primary source is the cotton and wood of higher plants. Cellulose also origins from agricultural residues such as palm trunk and empty fruit bunch, corncobs, wheat straw, sugarcane bagasse, corn stover, coconut husks, wheat rice, empty fruit bunches and *Eragrostis teff* Straw (Ebise & Hundessa, 2021). Softwoods are preferred over hardwoods for nano cellulose production due to long fibers and the requirement of less amount of energy for better fibrillation (An *et al*., 2016).

In addition to its plant origins, bacteria belonging to the genera *Acetobacter, Agrobacterium, Alcaligenes, Pseudomonas, Rhizobium*, or *Sarcina* are able to synthesize cellulose extracellularly having α configuration, which is different from a plant with β configuration. It is also synthesized by marine animals such as tunicates, algae, fungi (Iwamoto *et al*., 2007).

3.5. Nano Cellulose

Nano cellulose is a nanomaterial that is extracted from natural fiber cellulose with a size ranging from 1nm to 100nm in diameter and several micrometers in length. Its unique property such as being lightweight, low density (around 1.6 g/cm3), and outstanding strength, have given it a wide range of applications. Biodegradability of this natural fiber is also a plus in the utilization, recycling, and easy disposal. The nano cellulose is pseudo-plastic, a gel exhibiting viscosity property, under ambient conditions. These properties are thixotropy, change under conditions like agitation, shake and when pressurized, becoming thin, losing its viscosity. Then normalize into a viscous gel reverting to its original state (Khalil *et al*., 2012).

3.5.1. Extraction of Nano Cellulose

There are three fundamental methods of extraction of nano cellulose from natural polymer, chemical, enzymatic and mechanical methods. The preparation method and source of the cellulose greatly influence the nature of the nano cellulose, diameter and length (Deepa *et al*., 2011). The chemical method uses chemicals to hydrolysis the hydrogen bond, which is the amorphous part, that holds the polymers, sulfuric acid and hydrochloric acid are the most used in a laboratory and industrial level, yielding CNC with high crystallinity (Figure 5). The crystallinity is directly proportional to the concentration of the chemical. The most

recent ionic liquids are being used are solvent and catalyst for the direct derivation of the CNC. The enzymatic method uses a mixture of enzymes to degrade cellulose, cellobiohydrolases ablate on crystalline cellulose and endoglucanases ablate the amorphous cellulose, working in a coactive manner. This extracted cellulose can be processed by acid hydrolysis to yield the nanocellulose. (Henriksson *et al*., 2007).

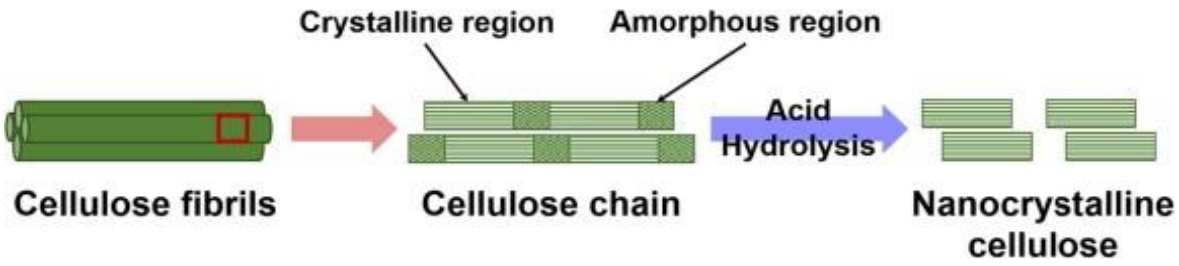

Figure 5. Schematic of CNC after extraction by acid hydrolysis, separating the amorphous region from the crystalline region (Sharma *et al*. 2019).

Mechanical methods use force and pressure to reduce the size of the cellulose, induce delamination and fibrillation of the fibers and separation of nano fibrillated cellulose. The main mechanical treatment methods are homogenization, grinding, refining, extrusion, blending, ultrasonication, cryo crushing, steam explosion, ball milling and aqueous counter collision. Among these, homogenization, grinding, refining and ultra-sonication are the most popular methods and are being used by many researchers for the preparation of nano fibrillated cellulose, the mechanical process is more eco-friendly (Kumar *et al*., 2020).

3.5.2. Type of Nano Cellulose

Nanocellulose can be categorized into three main types (Figure 6); nano fibrillated cellulose, the long up to several micrometers, flexible and web-like intertwined cellulose fibers, having a diameter in nanoscale dimensions 10–100 nm. Bacterial nanocellulose produced and secreted by some aerobic bacteria by using low molecular weight sugars are twisted ribbon-like fibers with average diameters of 20–100 nm, which is accumulated assembly of nanofibers with 2-4 nm in diameter and few lengths in micrometers (Jonoobi *et al*., 2015). These are the fibers with high crystallinity (80–90%) and degree of polymerization (3000–9000). *Agrobacterium, Acetobacter, Alcaligenes, Rhizobium* or *Sarcina*, and Pseudomonas are bacterial species that can produce cellulose, a gram-negative bacterium, *Gluconacetobacter xylinus*, and *Acetobacter xylinum* are the most efficient producer of BNC (Huang *et al*., 2016).

Figure 6. Electron micrographs of (a) NFC (b) CNC (c) BNC (Huang *et al*. 2016).

3.6. Cellulose Nano Crystals

Cellulose Nano Crystals are needle-shaped highly crystalline (~90%) cellulose moieties. The average diameter of the CNC was found to be 3–35 nm and a length of 100-1000 nm. Extracted from materials containing cellulose through a chemical or enzymatic method. The characteristics and morphology of CNC differ from extraction method, type of the hydrolyzing chemicals (Figure 7), condition of extraction (concentration, temperature, and time), and source of the cellulose (Maiti, 2013).

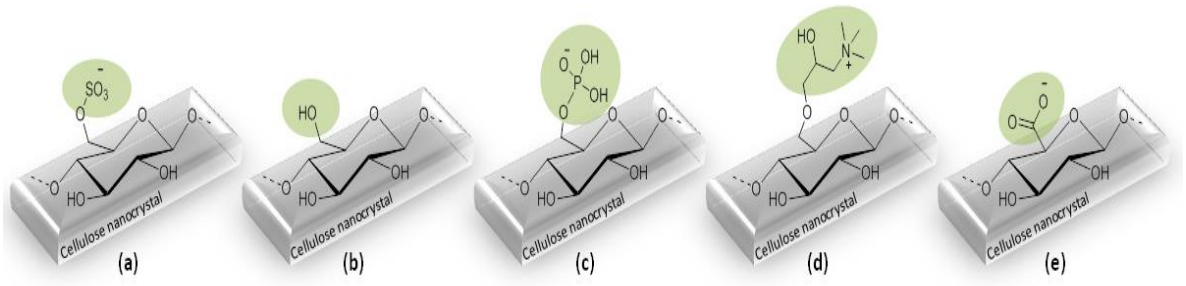

Figure 7. Functional groups attached to the surface CNC where (a) shows the CNC after H2SO4 hydrolysis, (b) after HCl or HBr hydrolysis, (c) after H3PO4 hydrolysis, (d) after H2SO4 hydrolysis followed by a surface cationization, and (e) after an HCl/HBr hydrolysis followed by a TEMPO-oxidation (Börjesson & Westman. 2015).

Moreover, the natural attribute of CNC is compelling, since they are environmentally friendly with recyclable, renewable, sustainable with minimum carbon footprint upon extraction and utilization and can be used in different sectors due to their low toxicity. Although there is a uniformity in the chemical composition of the crystalline region, there is variation in morphology, particle size, crystallinity, and different functional group of the surface of the CNC due to the source of material and method of hydrolysis. This property makes it difficult to initialize a standard procedure to produce a CNC with constant quality. Making it difficult for the industry to produce CNC with standard quality (Lavoine *et al*., 2012).

3.7. Properties of Cellulose Nanocrystal

3.7.1. Viscosity

CMC has a storage modulus value that is particularly high (104 Pa at 3% concentration). It has been found that the storage and loss modulus were independent of the angular frequency at all nanocellulose concentrations between 0.125% to 5.9%. CNC has high viscosity at low nanocellulose concentrations makes CNC very interesting as a non-calorie stabilizer and gallant in food application. CNC gels are highly sheared thinning, decrease viscosity under pressure. The shear-thinning behavior is specifically useful in a range of different coating applications (Zakani & Grecov, 2020).

3.7.2. Mechanical Properties

CNC is a rod-like or whisker shape particle, with a width of 3-20nm and a length of 50-2000nm. CNC has many physical attributes that make it ideal for different applications: high axial stiffness at ~150 GPa similar to Kevlar, the strongest material used for super-strong bulletproof material. Films made from CNC have been shown to have a high tensile strength at ~7.5 GPa, low coefficient of thermal expansion at ~1 ppm/K, withstand thermal up to ~300°C, have a high aspect ratio (length to width) of 10–100, low density at ~1.6 g/cm_3, have lyotropic liquid crystalline behavior (can self-organize into a chiral nematic ordering) (Liu *et al*., 2014).

3.7.3. Surface Modification

The hydroxyl groups on the surface of the CNC are the reason that make the surface modification of CNC possible. When the amorphous regions of cellulose are removed, it exposes the hydroxyl group on C-2, C-3, and C-6. C-2 and C-6 are the most reactive (Eichhorn *et al*., 2010). Using a different type of reaction such as oxidation, esterification, amidation, carbamation, etherification, and nucleophilic substitution. Different functional groups can be added to the CNC. This property gives CNC to acquire property, that was not present on CNC. Due to this reason, the CNC is being used in different industries (Table 1) (Thomas, 2018).

3.8. Mechanism Surface Modification of cellulose Nanocrystal

The different techniques of surface modification are shown in (Figure 8), surface modifications concerned with this research paper are discussed.

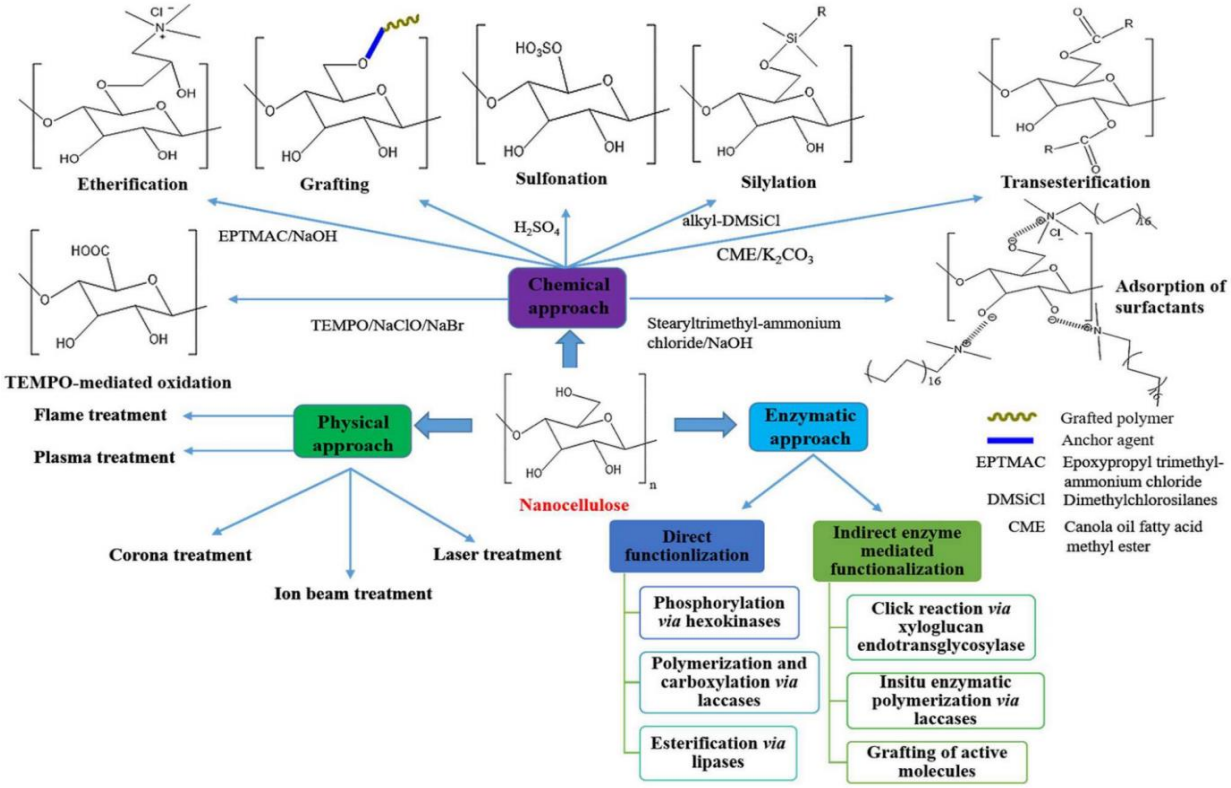

Figure 8. Schematic representation of the most commonly used surface modification routes of nano cellulose (Trache *et al*. 2020).

3.8.1. Oxidation

Oxidation reactions are used to add functional groups such as carboxylic acid the most common reaction is nitroxyl based oxidation to functionalities carboxylic acids selectively at primary alcohols or aldehyde on the surface of the cellulose, by periodate oxidation functionalities aldehydes from vicinal diols. The use of Tetramethylpiperidinyloxyl radical (TEMPO) oxidation for surface modification of cellulose is the most recent for surface functionalities cellulose in large quantify. The oxidation of this reaction is done with a small amount of TEMPO, which is recycled using secondary oxidants such as sodium hypochlorite or sodium chlorite. By Oxidation of CNC with TEMPO, water resistant films have been prepared, applicable for a fluorescent sensor for nitroaromatics detection (Golmohammadi *et al*.,2017).

3.8.2. Esterification

Sulfation and phosphorylation are the most common and simple esterification. Esterification reaction happens during the hydrolysis process of the poly chain of cellulose. Acetylation reaction is also categorized under heterogeneous Esterification. use in acetic anhydride, the use of anhydride in surface modification of CNC is uncommon. surface acetylation can be carried out in pyridine anhydrous since it is a low reactive polar solvent it makes ideal as a catalyst and solvent to disperse the CNC. 4-dimethylaminopyridine can also be used as a catalyst and is more effective. Acetylation has the tendency to reduce the diameter of the crystal, with minimum change on the length of the crystal. Transesterification is also known as grafting is used in surface modification CNC using poly(3-caprolactone) or poly(lactide) by ring-opening polymerization. Tin (II) 2-ethylhexanoate is the conventional catalyst, acid catalysts are not so common (Boufi & Belgacem, 2006).

3.9. Methods of Cellulose Nanocrystal Characterization

Several techniques have been developed to characterize the physical structure and chemical composition of cellulose and it nano composite. The most commonly used compound characterization techniques are Fourier Transform Infra-Red (FTIR) Spectroscopy. It used to identify functional groups of certain compounds. Nuclear Magnetic Resonance (NMR) is also used to determine molecular identity and structure. It is commonly known that Scanning electron microscope (SEM) able to employ for characterization of particle morphology. X-ray diffraction (XRD) commonly used for determination or characterization of Nanocrystal of certain substance such as that cellulose (Chandra *et al*., 2016).

3.9.1. Fourier Transformed Infra-Red (FTIR) Spectroscopy

FTIR stands for Fourier transform infrared, the preferred method of infrared spectroscopy. When IR radiation is allowed to pass through a sample, depending on the sample, some radiation is absorbed, and others transmitted. The vibrational motion of atoms in a molecule is influenced by the masses of atoms, their geometrical arrangement, and the strength of their chemical bonds. The resulting signal at the detector is a spectrum representing a molecular ‘fingerprint’ of the sample, measuring bond vibrational motions of atoms in molecules. It is a powerful technique for chemical characterization,

identification of functional groups and used to achieve information regarding the attachment of the capping agents (Kruer *et al*., 2018).

3.9.2. Scanning Electron Microscope

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm) (Sutton *et al.*, 2007).

3.9.3. X-ray Diffractometer (XRD)

The X-ray diffraction (XRD) technique is applied to characterize the crystalline nature of a material. XRD works by treating materials with non-destructive radiation with incident X-rays and measure the incident and scattering angles of the X-rays. These diffracted X-rays are then detected, processed and counted. This generate a unique diffraction pattern that specific crystallin structure. These X-rays are generated in cathode ray tube, by accelerating electrons within a potential difference and colliding them onto a target metal (usually Cu or Mo). The collision of electron with an atom of target metal will remove electron. When the created space, is filled with another electron, this will generate X-ray. The X-ray are filtered with thin sheet of metal to produce monochromatic radiation, passed through a small gap to concentrate, and guide the beams to the sample. By scanning the sample through a range of 2θangles and the random orientation of powdered material, the attainment of all possible diffraction directions of the lattice will be possible. A key component of all diffraction is the angle between the incident and diffracted rays (Takagi, 1969).

3.10. Major application of cellulose Nanocrystal and/or Nanocomposites

Property such as lightweight, low density and outstanding strength make CNC a desirable material. The OH group on the surface can be modified to enhance or change, self-assembly, dispersion for a wide range of suspensions, matrix polymers and to control interfacial properties in composites the property of the CNC. The new character is heavily influenced by the function group added to the CNC, changing the property of cellulose, the modification CNC can exhibit improved mechanical properties, high surface area to volume ratio, low defects, and engineered surface chemistries (Sinko *et al*., 2015).

Table 1. Applications of CNC in different industries (Sharma *et al*., 2019.)

No	Applications field	Product features
1	Composites and Plastics	Shelf life extension; Dimensional stability;
2	Paper & Packaging	Intelligent packaging, Ultra-violet screening packaging; Anti-microbial packaging;
3	Medical	Drug delivery and controlled release; Scaffold in tissue engineering; Implants;
4	Barrier properties	Shelf-life extension; Down-gauging of films; Oxygen scavenger;
5	Electronics/Metallic particles	Time-temperature integrator; Freshness indicator; Gas and Leakage detector;
6	Aerogel	Self-healing material

Cellulose based nanocomposites are being adopted in different sectors of industry, but a large portion of its use is dominated by the paper and packaging industry, despite the potential application. Due to their properties, the demand for cellulose nanocomposites have risen in several industries such as construction, automotive, electronics, pharmacy and cosmetics. The most recent application of nano cellulose composites as membranes for

ultrafiltration, ion exchange, fuel cells and etc (Sharma *et al*., 2019). The nanocellulose having a wide range of applications has been extensively reviewed in the last decade as summarized in (Table 1).

3.11. Skin Pathogen and Wound Healing

Skin is the protective organ that separates the inner body from the outside environment. This makes it prone to damage and injury. Major Skin injury (such as abrasion Laceration Avulsion and Puncture) caused open wounds and present a critical threat to victims, especially those with large areas of skin damage. This is the primary reason for dehydration, systemic infection and other complications suffered by victims. One key factor for the effective treatment of this type of skin injuries is to close the wound as soon as possible (Zannis *et al*., 2009).

The traditional wound covering bandage made from cotton or wool, only function as a wound dressing to maintain temperature and protection from the external environment to minimize additional injury. For this reason, the likely hood of bandage contamination is high. Due to this reason, an advanced wound dressing with an extremely intricate system of wound healing, that employing incorporated active ingredients, dressings can be functionalized with many classes of antibiotics (such as quinolones, tetracyclines, aminoglycosides, and cephalosporins, etc.) (Boateng and Catanzano, 2015).

The antimicrobial resistance problem is challenging in low-income countries due to the high prevalence of infections, irrational uses of antimicrobials, overuse availability of drugs, and lack of clinical microbiology laboratories for antimicrobial susceptibility testing. According to (Tadesse *et al*., 2017) majority of bacteria were reported, 26.7% *Streptococcus pneumoniae* were resistant to Penicillin, 34.0% *Haemophilus influenzaa* were resistant to amoxicillin and 88.1%, 80.7%, and 29.8% *E. coli* were resistant to amoxicillin, trimethoprim, and gentamicin respectively, all data recorded on median resistance. Infections caused by resistant bacteria will proliferate with limited hindering factors, this will affect the outcome of the treatment drastically, increase cost, illnesses duration and reduce other optional treatments like chemotherapy (Abera *et al*., 2014).

These infections are particularly difficult to treat because the bacterium has become resistant to many commonly used antibiotics as mentioned in the above paragraph and others such as methicillin-resistant *S. aureus*, vancomycin-resistant *enterococci* and macrolide-resistant *Streptococcus*, while examples of antibiotic-modifying microbes such as *Pseudomonas aeruginosa* and aminoglycoside-resistant *Acinetobacter baumannii* (Rice, 2006). Infections, either by bacteria or fungi, can lead to deterioration of the wound healing process and severe systemic complications.

Medical Nanotechnology represents a developing field that manipulates materials in the nanometer size range or molecular/atomic scale and enhances potency or gives novel properties. Widening the application of the material or diverging it from the original use for medical applications such as regenerative medicine and preventing various diseases. The hydroxyl group on the surface of cellulose Nanocrystal gives it a vast opportunity for modification to give new propriety and enhances the potentials applicability of cellulose as a nanoparticle (Liu *et al*., 2004).

4. MATERIAL AND METHODS

4.1. Description of Study Area

Waste tissue papers were collected from available surrounding in Adama town and Adama Science and Technology University. Adama is located at 08°32′29″N 39°16′08″E in East shoa zone, Oromia Regional State, 99 km south east of Addis Ababa, the capital city of Ethiopia. The town is found in area of average altitude about 1712 m above sea level and has annual rain fall of 7600 mm and mean annual monthly temperature of 21°C. The climate of the area is hot or arid climatic type. The city is divided into 14 sub cities and and 99 woredas (kebeles).

4.2. Sample Collection and Preparation

Thirty grams of Waste tissue papers (WTP) were collected from available surroundings with low contaminants such as dirt, oil, and other wastes. The experiment for reuse of waste Paper for production cellulose Nano Crystals, which included extraction of CNC from waste tissue paper, derivation of the extracted CNC, SEM morphological structure, and antimicrobial property of the derivative against pathogen bacteria and fungi were performed in the bimolecular laboratory and XRD characterization of CNC was conducted in material science laboratory Adama science and technology university, Ethiopia. FT-IR characterization of the derivative of CNC was carried out in Addis Ababa University, chemistry laboratory.

Twenty grams of collected waste tissue paper (WTP) was submerged in 2 liters of distilled water (distilled water) (w/v) for 24 hrs and occasionally was stirred, to enhance dispersion and remove any water-soluble substance. After the formation of slurry, excess water was removed and air dried for 48 hrs, then milled to a fine powder. The obtained fine powder of WTP was treated by 2% of NaClO in 500 ml of (distilled water) (v/v) at 70^{o}c for 2 hrs. NaClO was used as a bleaching substance to remove dirt and grease. The bleached waste tissue paper (BWTP) was filtered and washed until neutral pH is achieved. Finally, the bleached sample was air dried and collected for further analysis for preparation of cellulose Nanocrystal (Abdel, 2012).

4.3. Preparation of Cellulose Nano crystal

Three grams of BWTP were measured and added into four beakers containing different concentrations of H_2SO_4. The concentration employed were 65%, 55%, 45%, and 35% of which the total volume (w/v) was being 30ml in a 50ml borosilicate beaker. After optimization was performed using color change and thickness, a 45% concentration of H_2SO_4 (/v/v) was employed for cellulose Nano crystal s preparation. Briefly, a10 grams of BWTP was milled and hydrolyzed with 45 % H_2SO_4 (v/v) in 100ml of distilled water at 45^oC with constant string for 1 hour, the cellulose to acid ratio was 1/10 (g/ml). The mixture was diluted to 15 % H_2SO_4 with 600ml of distilled water (v/v) and 5N of KOH was added and stirred gently to neutralize the acidity. The hydrolyzed waste tissue paper (HWTP) was centrifuged at 950 rpm for 30 minutes to remove excess water and salt. This HWTP was then rinsed with 2000ml of distilled water and dried in an oven at 45^o C. The cellulose Nano crystal extraction was performed according to Dong *et al*., (2016). Conformation of the formation of CNC was checked using SEM and XRD. The total yield of the cellulose Nano crystal was calculated following the methods of Meyer & Mark (1928).

Total yield % = W/W_0 *100 Where W_0 is the initial dry weight of the BWTP and W is weight of dried sediment.

4.4. Preparation of Cellulose Nano crystal Derivatives

4.4.1. Acetylation Cellulose Nano crystal

Acetylation was performed under a laminar hood in a 250 ml round bottom flask. A one gram of CNC was added into 20 mL anhydrous pyridine and dispersed with ultrasonic (JEJOTECH UC-20) at 28^oC for 15 minutes. The solution containing CNC and anhydrous pyridine was stirred and centrifuged at 400 rpm and heated at 80^oC. Then 5ml acetic anhydride was added drop wise to the reaction and the mixture was kept for 5 hrs at 85^oC. The product was precipitated with 500ml of distilled water and washed with 500ml of the same distilled water (w/v). Finally, the acetylated cellulose Nano crystals collected and named as acetyl cellulose Nano crystal (ACNC). This Nano crystals were oven-dried under vacuum at 38◦C for further analysis (Lin *et al*., 2011). Conformation of the formation of ACNC was checked using FTIR.

4.4.2. Iodination of Cellulose Nano crystals

Iodination was performed in a 250 ml glass beaker. A one gram of CNC was added into 20ml of 85 % phosphoric acid (H_3PO_4) (w/v) and dissolved at room temperature. Stirring was performed at 180 rpm for 3hrs until a viscous solution was obtained. A 4.35g potassium iodide and 6.40g potassium iodate (KIO_3; KI) were mixed and grinded to obtain fine powder and were added in a mixture of CNC and H_3PO_4 within in a fume hood. After 24 hrs, the mixture was precipitated by adding 200 ml of cold EtOH (− 20 °C) followed by stirring at high speed. The precipitated compound was filtered and washed several times with 96% EtOH (v/v). Finally, the iodine Cellulose Nano crystal collected and named as Iodine cellulose Nano crystal (ICNC) (Pagliaro, 1999). Conformation of the formation of ICNC was checked using FTIR.

4.4.3. Methylation of Cellulose Nanocrystal

One gram of CNC was added to 3.5N NaOH in 30 ml distilled water and kept in a hot water bath for 30 minutes at 70°C. Before, a solution of 95% methanol (v/v) and 34% HCl (v/v) was prepared in 3:2 ratio and kept in the refrigerator for 24hrs. After 30 minutes the cooled solution was added to NaOH and HWTP mixture. After 30 minutes 500ml of distilled water was added to stop the reaction and filtered. Finally, the methyl cellulose Nano crystals collected and named as methyl cellulose Nano crystal (MCNC).Then was slowly dried in an oven at 35^{o}C. The Methylation of cellulose was conducted under a modified procedure adopted by (Ichihara and Fukubayashi, 2010). Conformation of the formation of MCNC was checked using FTIR.

4.4.4. Preparation of Silver Cellulose Nano crystal

One gram of CNC was added in 10ml of 0.02M of silver nitrate ($AgNO_3$) then the solution was added to a 250ml beaker containing 90ml of distilled water and dispersed with a magnetic stirrer rigorously to obtain a uniform suspension. After 8 minutes 0.05M of trisodium citrate dihydrate in 1 ml of distilled water was added. After 2 minutes the solution was heated in a water bath for 10 minutes at 80^{o}C and the solution was filtered and washed with 500ml of distilled water. These silver cellulose Nano crystals were collected and named as silver cellulose Nano crystal (AgCNC). Finally, it was dried at 40^{o}C and preserved morphological and structural characterization using Scanning electron microscope (SEM), Fourier Transform Infrared (FT-IR) Spectroscopic and X-ray

Diffraction (Xu *et al*., 2016). Conformation of the formation of AgCNC was checked using FTIR.

4.5. Characterization of Cellulose Nano crystal and its derivatives

4.5.1. Morphological characterization of Cellulose Nano crystal using Scanning electron microscope (SEM)

Observation of microstructure and surface morphology of the CNC was performed using Scanning electron microscope (SEM) that found at the Department of Applied Biology Laboratory, ASTU. The observation was performed for dry powders of CNC and AgCNC that were fixated on to a sticky carbon fiber tape. The SEM (JMC-6000Plus) was performed with accelerating voltage of 15 Kv and examined at 5, 10, 50, 100, and 200 µm.

4.5.2. X-Ray Diffraction

The crystallinity of CNC was characterized by an X-ray diffractometer (XRD). Measurements were performed on dry powders of CNC and CMC, at ambient temperature on XRD-7000 X-RAY Diffractometer quipped with nickel filtered Cu-Kα radiation (λ = 0.1506 A^0 =0.15406 nm) orating at 40KV and 30mA in a range of 2ɵ= 5–80◦ using a fixed time mode with a step interval of 0.02, at Adama Science and Technology University (Agarwal *et al.*, 2017). Mathematical tool: The crystallinity index (CrI) was calculated according to Segal equation (Segal *et al*, 1959).

CrI= (I_{002}−I_{am}) /I200X 100 where I_{002} is the diffraction intensity at 2θ = 22-23˚; and I_{am} is the minimum diffraction intensity at 2θ = 18-20˚. The crystallite size was calculated as per the Scherrer equation (Kale *et al*., 2018). Crystallite size = 0.94×λ/β×cosθ where λ is X-ray's wavelength; β is the full width at half maximum in radians; and θ is the Bragg angle.

4.5.3. Fourier Transform Infrared (FT-IR) Spectroscopic

The crystals of cellulose (CNC) and all the derivatives were collected and characterized using FT-IR. These crystals were placed on a polytetrafluoroethylene plate to form thin layer film which was used to the transmission IR spectra measurement. The transmission IR spectra were measured on a Fourier transform infrared spectroscopy (FTIR) FT-IR-8300 spectrophotometer (spectrum 65 FTIR PerkinElme) scanning from 400 to 4000 cm^{-1}

using KBr pellets. The data was collected and processed using the origin Pro 8.0 software (Arcos-Hernandez, *et.al*. 2010).

4.6. Antimicrobial Property Detection

Antimicrobial activities were performed for AgCNC, ACNC, ICNC, and MCNC, on testing microorganisms *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *C. albican* ATCC 10231. Agar diffusion method was used to evaluate qualitatively the antibacterial activity of AgCNC, ACNC, ICNC, and MCNC. Comparative analysis of the derivative and effect of concentration on the test organism was evaluated on Muller Hinton Agar (MHA), Acid Hydrolysate of casein (17.50gm), starch (1.50 gm), agar (17.00 gm), and distilled water, (1000 ml) and adjusted at pH 7.3 ± 0.1 and 25ºC.

Test microorganisms for human pathogenic bacterial and fungi were collected from the Oromia region laboratory. These test microorganisms (strains) were including such as *S. aureus* ATCC 25923 for Gram positive strain, *E. coli* ATCC 25922 for Gram negative strain, and *C. albican* ATCC 10231 for fungi strain. These test microorganisms were activated using sterilized nutrient broth medium consisting of (in g/L): peptone, 5; NaCl, 5 and yeast extract, 1.5 at pH 7). Using aseptic technique Mueller-Hinton agar medium (MHA) was prepared and autoclaved for 15 minutes at 121 ºC and poured on to petri dish (100 x 15mm). These petri dish containing waited until it fully solidified then filliped. Agar surface of each plate was streaked by a sterile cotton swab with the reference bacterial strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *C. albican*).

Antimicrobial activities were performed. Briefly, disk of 6mm diameter was prepared from whatman filter paper using a paper puncher. The dicks were soaked within the solution containing the derivatives, five different concentration of CNC derivatives with 0.0066, 0.013, 0.02, 0.026**7** and 0.0333mg/ml concentration. The CNC derivatives were sonicated in 3ml solution for 5minute. MCNC in distilled water , ACNC in 70% ethanol, ICNC in 5% Dimethyl sulfoxide and Ag 5% Dimethyl sulfoxide. Each disc was placed onto the media and plates were incubated overnight at 37°C, disk diffusion tests were performed according to NCCLS methods. CNC was used as the negative control in the experiment dissolved in 5% of Dimethyl sulfoxide in 3ml distilled water (v/v), Ceftriaxone (0.03µg/ml) and Ketokonazole (0.03µg/ml) as positive control for bacteria and fungi respectively. Finally, zone of inhibition was detected and measured (Kiehlbauch *et al*., 2000).

5. RESULT AND DISCUSSION

5.1. Preparation of Cellulose Nano crystal

As shown in the (Figure 9)**,** four different concentrations of H_2SO_4 were used ranging from 35 to 65% with color shown. These concentrations of H_2SO_4 were able to hydrolysis of β-anhydroglucose linkage in cellulose structure. Depending on the quality and type of paper, the content of cellulose various with district H_2SO_4 treatment for Nanocrystal preparation (Reddy and Yang, 2005). It was reported that Lignin and hemicellulose were removed with concentrated H_2SO_4 (Kim, 2011).

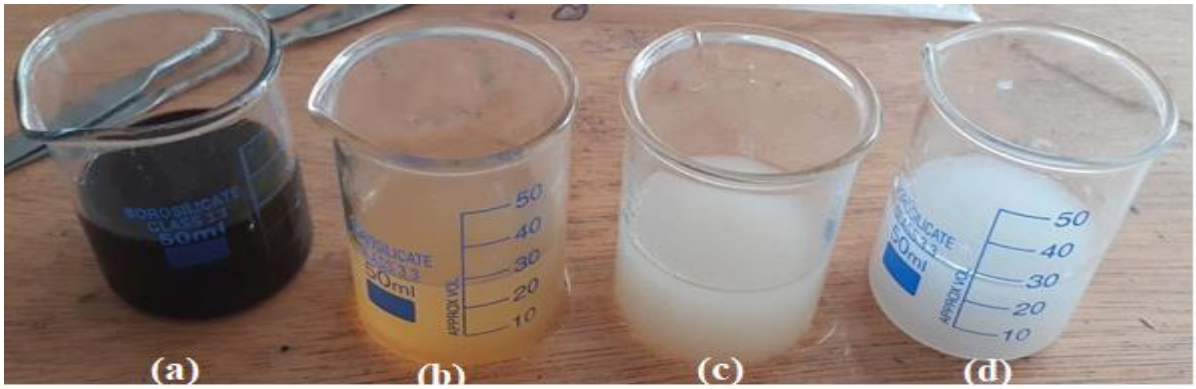

Figure 9. Testing of different concentrations of H_2SO_4 to obtain CNC without damaging the structural integrity of the cellulose. (a) 65%, (b) 55%, (c) 45% and (d), 35% of H_2SO_4.

Table 2. Color change, thickness and thinness of cellulose solution in different contraptions of H_2SO_4

Cellulose	H_2SO_4 Concentrations	Color	Viscosity
A	65%	Dark	low viscosity
B	55%	Golden brown	low viscosity
C	45%	White	low viscosity
D	35%	white	highly viscous

As illustrated in (Figure 9) and (Table 2), after adding the cellulose into a 65% H_2SO_4 (w/v), $_{it}$ immediately turned into the dark cloud which is an indication of carbon release hence denatures. At 55% also showed a significant amount of denaturing hence the color change into golden brown. However, 45% showed a promising result there was no color change and the solution had low viscosity and on 35% of H_2SO_4 the solution in the beaker

had highly viscous and coagulate no significant amount of hydrolysis. The cellulose regenerated from 45% of H_2SO_4 (w/v) had a yield of 90% CNC (w/v). The correlation with viscosity and H_2SO_4 concentration is direct proportional, the viscosity increases as the concentration increase due to negative surface charge formation which occurs when H_2SO_4 hydrolyze the cellulose increasing the phase separation and thinning the viscosity (Abitbol *et al.*, 2018).

5.2. Characterization of CNC

5.2.1. Morphological characterization

SEM was used to observe the morphological structure, the HWTP observed in the range of 20-200µm. As shown in (Figure 10), there is a clear formation of crystalline cellulose. Size, diameter and length were unable to determine since the resolution was clear up to 20µm. Further analysis was done with XRD to investigate the crystalline size and crystalline index.

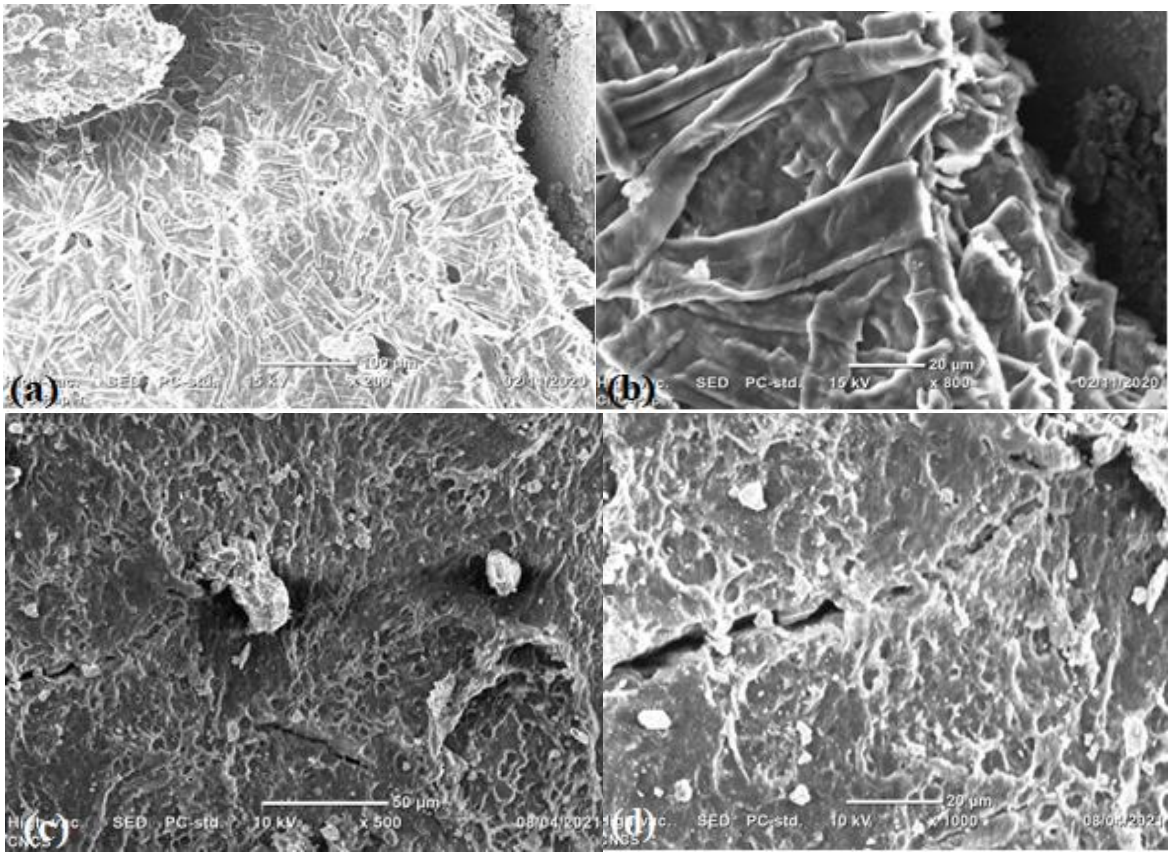

Figure 10. Image obtained using Scanning electron microscopic (SEM) images for CNC and AgCNC. Both Figure 10 a&b indicates SEM image for CNC, (a) CNC at a 100 µm magnification and (b) at a 20µm magnification. Both figure 10c & d indicates SEM image for AgCNC, (c) AgCNC at a 50 µm magnification and (d) 20µm magnification.

AgCNC have shown a surface change due to the reduction of Ag nanoparticle on to the surface of the CNC. The surface morphology derivation of AgCNC from CNC is clearly in (Figure 10) c&d, AgCNC surface is covered with spread dust of silver nanoparticle that adhere to the surface of the CNC. The absence of dust like particle on the CNC is an evident which characterizes the morphological change of CNC upon addition of Ag nanoparticles.

5.3. Characterization of the derivatives cellulose nano crystals using analytical instruments

5.3.1. X-Ray Diffraction (XRD)

X-ray diffraction able to characterize material particle based on its 2ɵ degree. In this study, the crystallinity of the CNC was checked using this X-ray diffraction. As it was displayed in (Figure 11), the profile of cellulose Nano crystal with corresponding reflections of the orthorhombic crystalline lattice were listed at 2θ =15^{o}, 16^{o}, 22^{o}, and 34$^{o.}$ These results are indicating the conformity with (110), (110), (200) and (004) crystal planes, respectively. The XRD spectra were also predicted for CNC that prepared using acid hydrolysis at 45% (w/v) H_2SO_4 for BWTP (Table 2). The sample was recorded in the range of 2ɵ (5 to 80^{o}). Depicting the character of cellulose Iβ structure with these results were also coherent with Xing *et al*. (2020), using 58% H_2SO_4 (w/v) that produced crystallinity size of 15.39 nm at 2θ =15^{o}, 16^{o}, 22^{o}, and 34^{o} having an aliment plane of conformity with (110), (110), (200) and (004) which showing the character feature of cellulose Iβ structure.

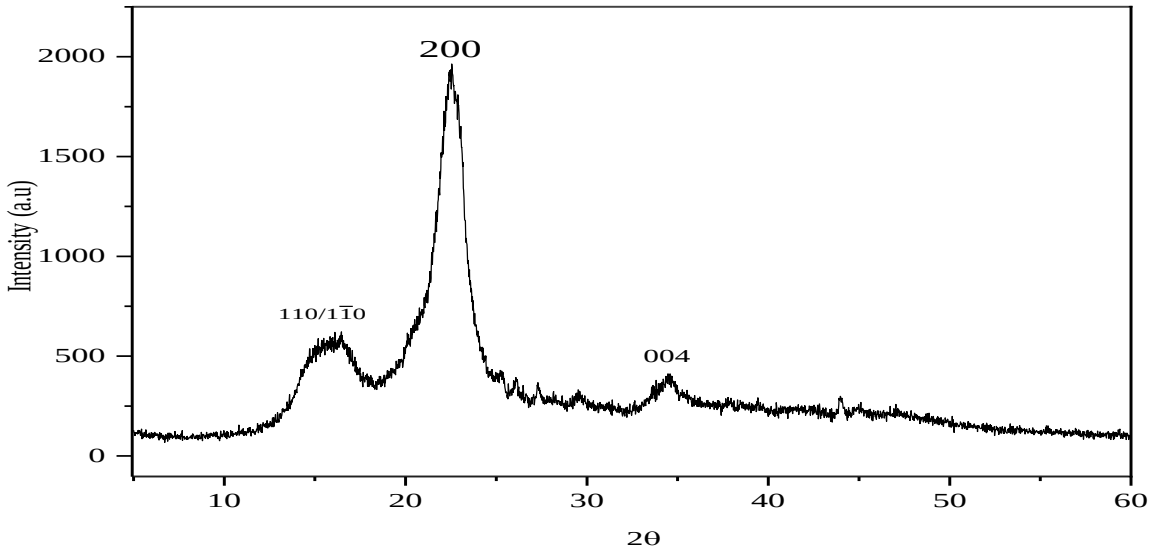

Figure 11. XRD pattern for Cellulose Nano Crystals (CNC) prepared using 45% H2SO4 hydrolysis from WTP and miler index.

The data from the XRD was analyzed using origin 2019b software, as shown in the (Figure 11). The crystallinity index was calculated using Segal equation. The crystallite size was calculated as per the Scherrer equation.

Table 3. X ray diffraction with Pos. [°2Th.], full widths at half maximum (FWHM) in radians, intensity, d-spacing, crystallite size (nm) and Crystallinity for cellulose Nanocrystal.

CNC	2ɵ (degree)	Cosɵ	FWHM (degree)	radian	Intensity	d-spacing	Crystallinity size
1.	15.8526	0.96	3.3734	0.05884	213	5.58596	2.504
2.	16.2517	0.96	2.2134	0.03861	213	5.44967	0.3819
3.	19.2850	0.96	0	0	80	4.59880	0
4.	20.3418	0.93	1.34	0.02337	201	4.36221	6.514
5.	22.5220	0.92	1.7388	0.03033	1063	3.94462	5.073
6.	24.0928	0.91	0	0	177	3.69088	0
7.	24.2724	0.91	0.88	0.01535	135	3.66397	10.140
8.	25.1108	0.9	0.8266	0.01441	69	3.54351	10.923
9.	26.1141	0.89	0.57	0.00994	35	3.40960	16.014
10.	34.4084	0.82	1.42	0.02477	101	2.60432	6.97
11.	35.5574	0.81	1.2	0.02093	39	2.52275	8.351
12.	64.3304	0.43	0.54	0.00942	50	1.44695	34.954
13.	77.4542	0.21	0.55	0.00959	61	1.23127	70.429
	Average particle size						**15.39nm**

Materials found in the range between 1 to 100 nm diameters are categorized as nanoparticles. As indicated from (Table 3), the crystallinity size of the CNC is 15.39nm which is in the range of nanoparticles size. The crystallinity index of the sample analyzed was found to be 81.8% indicating the formation of CNC, this result was in agreement with previous study such as results Listyanda *et al*. (2020), extracted cellulose from ramie fibers using 58% H_2SO_4 (w/v) and produced CNC with Crystallinity size of 6.67nm and Crystallinity index of 90.77%. According to Lacerda *et al*. (2013), the difference in Crystallinity size and index is due to the concentration increase, as the concentration of the hydrolyzing factor increase the Crystallinity of the CNC increases in both size and index. Using sulfuric acid concentration from 5%- 25% the result showed an increased

Crystallinity index from 65% to 75% over all Crystallinity index is low due to the concentration.

5.3.2. FT-IR Spectroscopy

5.3.2.1. Acetylated Cellulose Nano crystalline

CNC was added into anhydrous pyridine, since it is a low reactive polar solvent, it makes is ideal as a catalyst and solvent to disperse CNC alone with sonication, since acetic acid is highly soluble in water and other solvents. After adding acetic anhydride, the solution was stirred for 5 hrs, acetylation of CNC was a slow reaction. A distilled water and acetone were used to remove unreacted acetic anhydride. The obtained ACNC was 0.93 gram from 1gram of CNC.

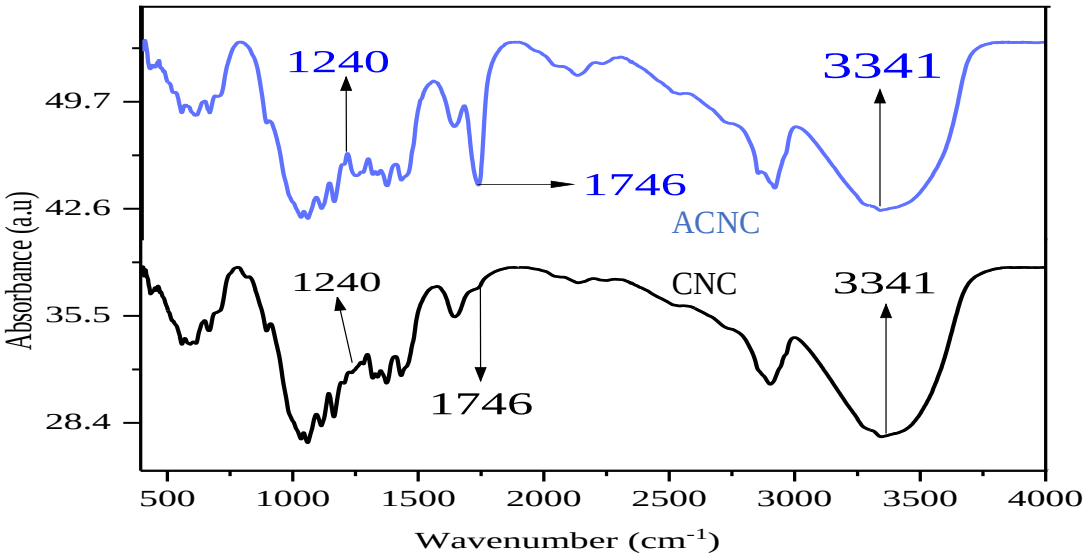

Figure 12. The Fourier Transform Infrared spectroscopy spectra of the CNC and ACNC.

The difference in absorption peaks and formation of the new peaks between CNC and ACNC are the indication of acetylation. As shown in Figure 12, three absorptions located at 1240 cm^{-1}, 1746 -cm^{-1} and 3341 cm^{-1} are evidence affirming the acetylation of CNC with acetyl. The emerging of a new peak in the carbonyl area around 1746 cm^{-1} peak is linked with the formation ester group. The change in absorption at 1240cm^{-1} is an indication of carbonyl C=O stretch vibration (Figure 12). Additionally, the decrease in intensity peaks situated at 3341cm^{-1} is an indication of acetic anhydride chemical modification of assigned O–H stretching of the CNC component which is inconsistence with Lin *et al*., (2011) found that the a lowered intensity in absorption of C=O stretching

band 1240cm^{-1} and the O-H stretching band 3341cm^{-1} is an indication of acetylation of CNC and with the new peak formation at 1746 cm^{-1} points ester group.

5.3.2.2. Iodine Cellulose Nano crystalline

CNC was added in 85% phosphoric acid which turned into thick transparent liquid after 3 hrs of string. Upon addition of potassium iodide and potassium iodate, a color change occurred into black purple due to a release of molecular iodine (Pagliaro, 1999). Little heat was released. After 24 hrs cold EtOH was added and white gray powder was precipitated. EtOH was used to remove unreacted iodine. The final gram of the powder was 4.5 grams from 1 gram of CNC. Iodination of cellulose reaction initiated with an attack of cellulose glucosyl –OH groups on I_3^+followed by iodine removal and the formation of cellulose hypoiodite (Besemer & Vanderkam, 1994).

As shown in Figure 13, wavenumber 530cm^{-1} is an indication of iodination of CNC. In agreement to this study, Pagliaro (1999), stated iodization of CNC with KIO_3 and KI, all the hydrogen hydroxyls were replaced with iodine residues, apart from C, H and O a complete substitution.

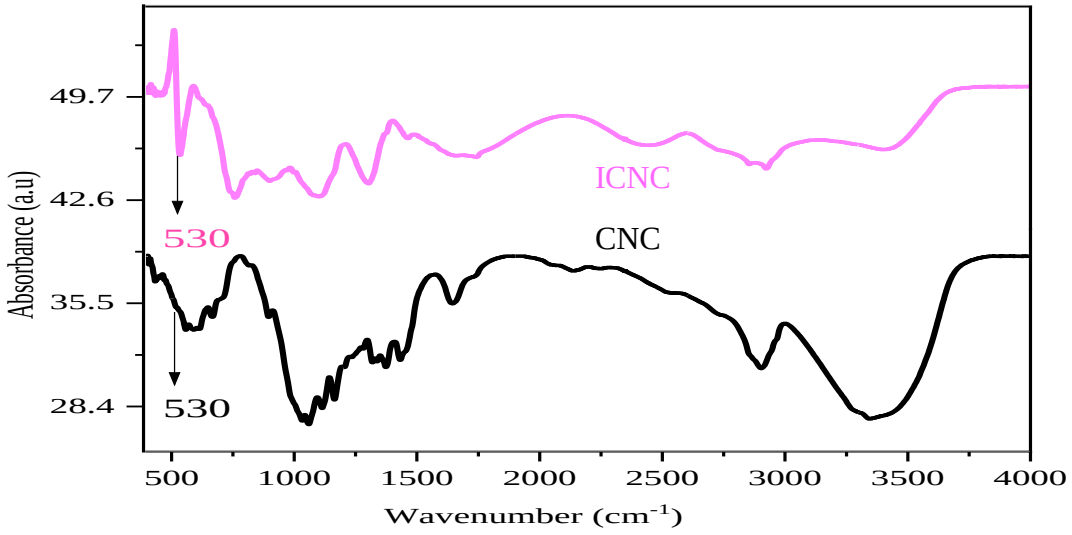

Figure 13. The Fourier Transform Infrared spectroscopy spectra of the CNC and ICNC.

As indicated in Figure 13, a high absorption peak located at 530 cm^{-1} is an indication of the stretching mode of the I–O bond.

5.3.2.3. Methyl Cellulose Nano crystalline

CNC was dispersed and homogenized with NaOH, NaOH also help in swelling (Sun *et al.*, 2015). After adding 3:2 ratio methanol and HCL a white smoke appeared and subsided after 1 minute. The obtained MCNC was 0.9 gram from 1gram of CNC. As indicated in Figure 14, the difference between CNC and MCNC is located at the region from 3600 cm^{-1} to 2700 cm^{-1}. Absorbance located at 2900 cm^{-1}, 2922 cm^{-1} and 3400cm^{-1} are indicators of methylation of CNC. In agreement with the current study, Sekiguchi *et al*., (2003) found that the lowered intensity in absorption of O-H stretching band 3400 cm^{-1} and the C-H stretching band 2900 cm^{-1} is an indication of methylated CNC.

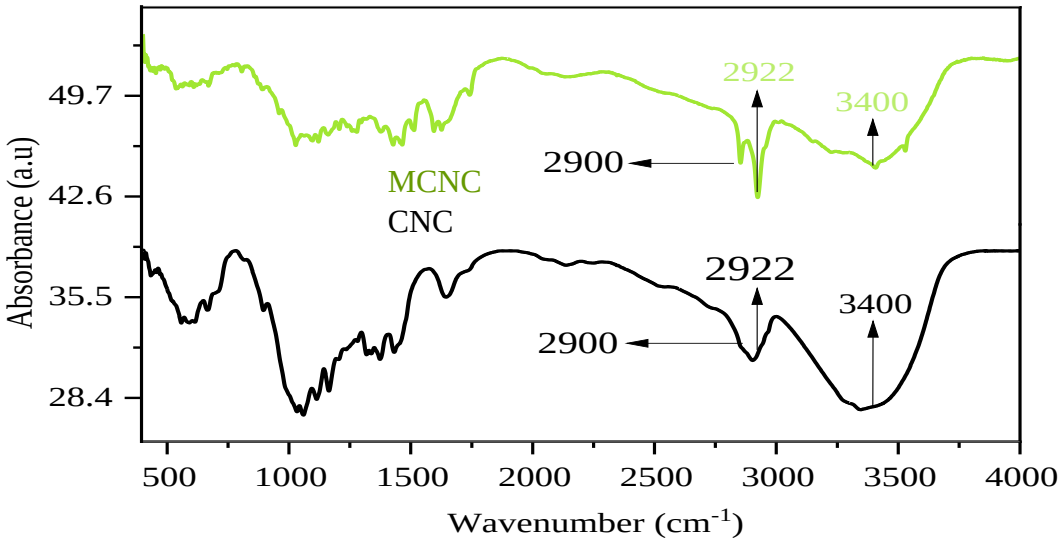

Figure 14. The Fourier Transform Infrared spectroscopy spectra of the CNC and MCNC.

The ratio difference between the intensity of CNC and MCNC, around O-H stretching band 3400 cm-1 and the C-H stretching band 2900 cm-1 are lower in the spectrum of MCNC than CNC. This is an indication of methylation of CNC, when methoxyl groups are in place of the hydroxyl group of glycosidic units, increasing the methyl group in comparative to hydroxyl groups (Nasatto *et a.*, 2015).

The change of shape around the band around 2922 cm-1 and the displacement of this to higher frequencies is an indication of methylation of CNC when compared to CNC. The same displacement also indicates the O-H stretching band of MCNC. The stretching of the C-H band of the CH2 and CH3 groups is an indication of substitution of the hydroxyl group by methoxyl group, increasing the methyl group. According to Sekiguchi *et al*, (2003) alters the pattern of hydrogen bonds on the methylated cellulose, which evidently changes the O-H stretching region.

5.3.2.4. Silver cellulose Nano crystalline (AgCNC)

CNC and $AgNO_3$ were homogenized in a beaker containing distilled water, under heavy stirring. NaOH was added dropwise, the color of the solution started to change from milky white to dark brown as the concentration of NaOH increase. The milky white is due to silver ion, In other study, it has been shown that color change happened due to higher pH value. The rate of reduction of $AgNO_3$ also increase in basic medium. It was also stated that the color change is an indication of surface plasmon resonance excitation and vibration during the reaction (Coseri *et al*., 2015).It was confirmed that $C_6H_9Na_3O_9$ used as a reducing agent to aid silver deposition onto CNC in the preparation of cellulose silver Nanocrystal. The reaction between silver ion and cellulose generates hydrogen ion then cellulose citrate is formed when carboxyl group of $C_6H_9Na_3O_9$ reacts with CNC, which is cellulose ester, increasing reducibility of CNC, enhancing the deposition of silver ion on to the surface of CNC (Xu *et al*., 2016).

As shown in (Figure 15), the spectrum obtained from FT-IT region found between 1250cm^{-1} and 3500 cm^{-1} are the characteristic features of AgCNC and CNC. Band located at 1384cm^{-1}, 1426cm^{-1}, 1730cm^{-1}, 3350cm^{-1} and 3417cm^{-1} are evidence for successful derivation of AgCNC.

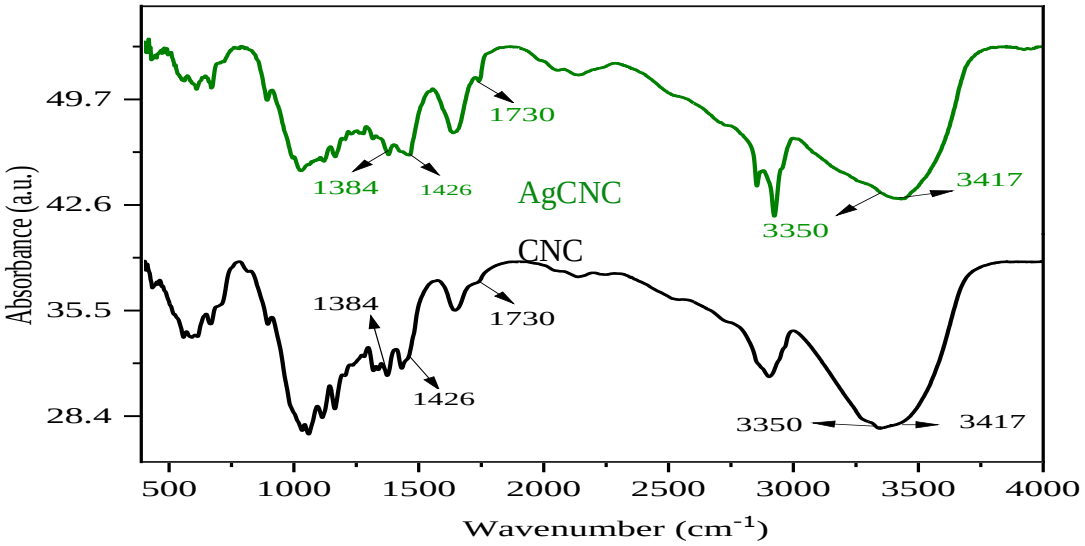

Figure 15. The Fourier Transform Infrared spectroscopy spectra of the CNC and AgCNC.

As shows in (Figure 15), the absence of the band at 1730cm^{-1} is characteristic feature of a reaction between aldehyde groups and Ag^+. The specific peak at 1384cm^{-1} is an indication of the silver nanoparticles. The change of the band shape at 3350cm^{-1} indicates O-H that shifted to 3417 cm^{-1} and the change of shape became narrower which is an evident and

indication of the interaction between AgNPs and hydroxyl. The decrease of the peak located at 1426cm-1 is behavior of symmetric bending vibrations for CH_2 groups in the C_6 of CNC chains after the reaction occurred (Xu *et al*., 2016), a similar result to the current our study (Figure 15), which is1426cm-1. It was found that there is an involvement of some hydroxyl groups in the C_6 of CNC chains in the reaction. All the above result validates the reaction between CNC and Ag nanoparticle which is agreement with Lokanathan *et al*. (2014) finding. In this study, 1730cm^{-1} is an evident for the reaction between aldehyde groups and Ag^+ and 1384cm^{-1} indicate the Ag nanoparticle on CNC as it was also reported by Shin *et al*. (2008).

5.4. Antimicrobial property detection

Antimicrobial activities were performed for AgCNC, ACNC, ICNC, and MCNC, against testing microorganisms such as *S. aureus* ATCC 25923 which is Gram positive strain, *E. coli* ATCC 25922 which is Gram negative strain and *C. albican* ATCC 10231 which is fungi strain. Agar diffusion method was used to evaluate qualitatively the antibacterial activity of AgCNC, ACNC, ICNC, and MCNC. Comparative analysis of the derivative and effect of concentration on the test organism was evaluated on Muller Hinton Agar. Zones of inhibition were detected after measuring using Anti-Biotic Zone reader. These inhibition zones were used as quantitative evaluation for cellulose Nanocrystal derivatives.

Table 4. The Anti-microbial effect of CNC derivatives (mean ± standard deviation, n=3).

Test microorganism	**Staphylococcus aureus ATCC 25923**				**Escherichia coli ATCC 25922**				**Candida albicans ATCC 10231**			
Concentration of CNC (mg/ml)	Iodine	methyl	Acetic	Silver	Iodine	methyl	Acetic	silver	Iodine	methyl	Acetic	silver
0.0066	7±0.5	0	7±0.4	8.2±0.6	7.3±0.4	0	7.2±0.5	8.8±0.9	6.5±0.5	0	0	7.5±0.5
0.013	8.2±0.9	0	7.5±0.5	8.6±0.7	8±0.6	0	7.5±0.4	9.5±0.8	7±0.4	0	7.2±0.4	8.2±0.7
0.02	9.7±0.8	0	8±0.8	9.3±0.9	8.6±0.8	0	8.7±0.8	10.3±1.9	7.2±0.8	7±0.5	8±0.6	9.6±0.9
0.0267	10±0.7	0	9.5±0.5	10.6±1.08	9.4±0.6	6.5±0.5	9±0.6	11.4±1.2	8±1.1	7.5±0.6	8.5±1.2	10±0.7
0.0333	11.2±1.1	7.7±0.3	10±0.8	12±0.8	10±0.9	7.3±0.3	9.7±0.9	13.5±0.8	8.5±0.9	8.7±0.6	9±0.6	11.5±1.1
CNC		0				0					0	
Ceftriaxone 0.013(mg/ml)		18.3±1.3				15.7±0.9					ND	
Ketokonazoe 0.013(mg/ml)		ND				ND					18.5±1.4	

Note: The measurements are in mm, not determined (ND)

The concentration of ICNC has a profound effect on both bacteria and fungi, as the concentration increases the size of the inhibition zone also increases as supported by p value of 0.005 < 0.05 as shown in (Table 4) . Minimum (7±0.5mm) and maximum zone of inhibition (11.2±1.1mm) were detected for *S. aureus* ATCC 25923 at concentration of 0.0066and 0.0333*mg/ml*, respectively. ICNC is more effective on bacteria than fungi especially for *S. aureus* ATCC 25923. *E. coli* ATCC 25922 shown minimum zone of inhibition (7.3±0.4 mm) at a concentration of 0.0066*mg/ml.* For the same strain the maximum zone of inhibition (10±0.9 mm) detected at a concentration of 0.01*mg/ml*. In this study, zone of inhibition was also exhibited for *C. albican* ATCC 10231 at a concentration of 0.0066*mg/ml* (6.5±0.5 mm) and 0.0333*mg/ml* (8.5±0.9mm) which is the least area of clear zone in this study for this typically fungi cell.

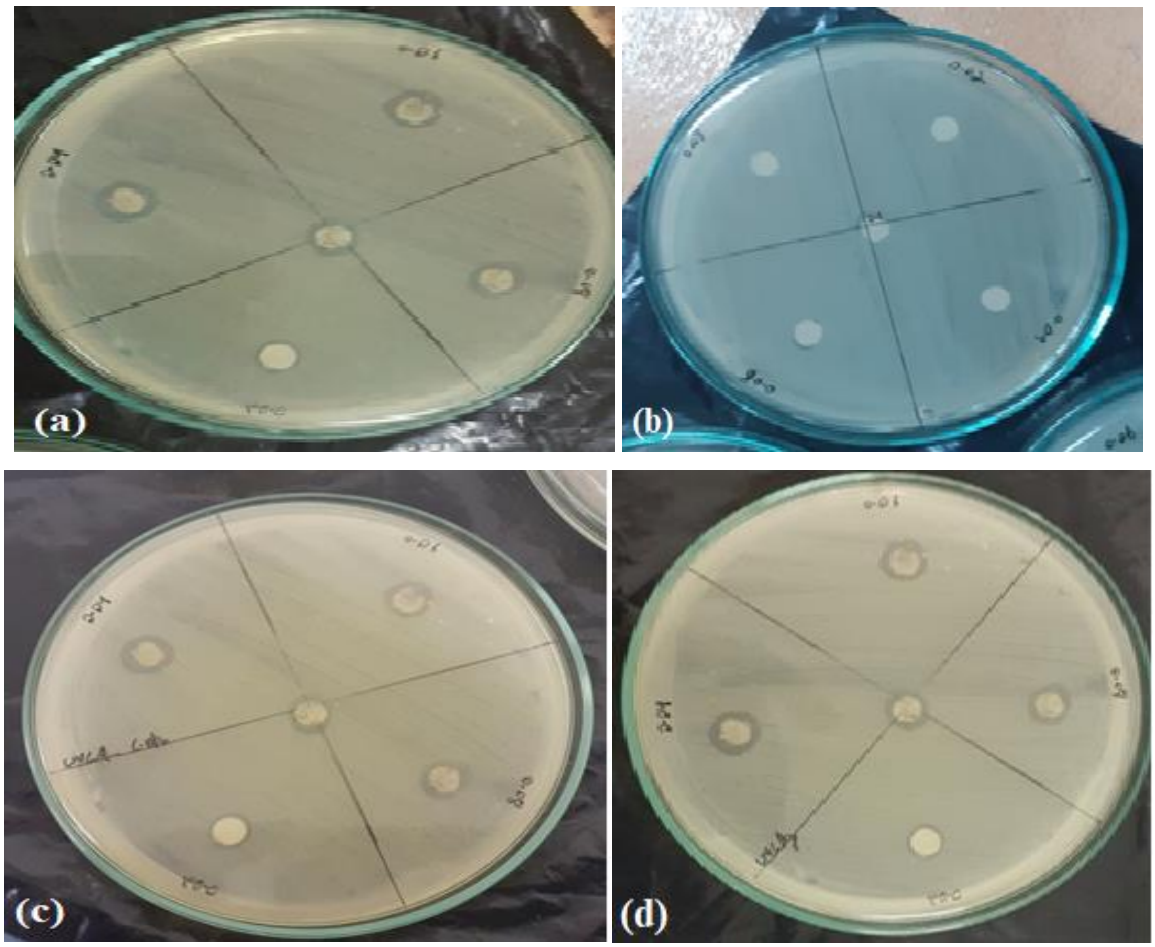

Figure 16. (a)Antimicrobial effect of ICNC (b). antimicrobial effect of MCNC. (c) Antimicrobial effect of ACNC. (d) Antimicrobial effect of AgCNC.

According to Kanagalingam *et al*. (2015), the sequential and precise method in which iodine attacks and denatures the essential cellular mechanism is yet to be clarified but the broad understanding is iodine is involved in inhibition of the vital bacterial and fungal cellular mechanism and structure. The oxidation of nucleotides fatty acids and amino acids on cell membranes of bacteria, the denaturing and deactivation of cytosolic enzymes which are required for the respiration chain (Kanagalingam *et al*., 2015).

In this study, it was found that MCNC has little effect as antibacterial and antifungal with p value 0.0048 < 0.05 (Table 4). MCNC showed none antibacterial and antifungal effect, despite the fact that there is an increasing concentration from 0.0066*mg/ml* to 0.013*mg/ml*. Table 4 indicated that bactericidal effect was detected for *E. coli* ATCC 25922*(*7.7mm±0.3) and *S. aureus* ATCC 25923 (7.3mm±0.3) and with a maximum zone of inhibition at concentration of 0.0333*mg/ml*.The anti-fungal activities were also performed. In this stuy, it was observed that the zone of inhibition due to MCNC against *C. albican* ATCC 10231 higher when compared to *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 with a maximum zone of inhibition (8.7±0.6mm) and with minimum zone of inhibition (7±0.5mm) at a concentration of 0.0333 and 0.02*mg/ml* respectively using Methyl Cellulose Nano Crystals (MCNC). In this experiment, MCNC has shown the least effective against these microbial cells. This might be due to the presence of methyl group within cellulose Nano crystal. In agreement with this study, Morton, (1950) reported that methyl is the least effective alkyl that shown antimicrobial activities against some pathogenic microbial cells. The same author further stated that CNC was not able to shown inhibition effect on microbial cells.

In the current study, the antibiotic effect of ACNC was determined using the inhibition zone method against *E. coli* ATCC 25922, *S. aureus* ATCC 25923 *and C. albican* as supported by p value 0.0042 < 0.05. As shown in (Table 4), ACNC has found to be more effective on *S. aureus* ATCC 25923 *and E. coli* ATCC 25922 than *C. albican*. The minimum zone of inhibitions were detected at a concentration of 0.0066 *mg/ml* for *S. aureus* ATCC 25923 *(*7±0.4mm) and *E. coli* ATCC 25922 (7.5±0.4 mm), respectively. However, there is no zone of inhibition against *C. albican* ATCC 10231 *at* a concentration of 0.02 *mg/ml.* The maximum zone of inhibition against *S. aureus* ATCC 25923 (10±0.8 mm), *E. coli* ATCC 25922 (9.7±0.9mm) and *C. albican* (9±0.6mm) and were observed at a concentration of 0.0333 *mg/ml.* using ACNC that show bactericidal and fungicidal activities.

The antibacterial property of acetic acid has been used as antibacterial for thousands of years. However, the mode of action has been proved only for planktonic bacterial cells (Fraise *et al*., 2013). The protonated form of acetic acid can diffuse through the cell wall of the bacteria and resulting in acidifying the cytoplasm. This used to halting microbial cellular function. According to Marquis *et al*. (2003), highly lipophilic acid and base have the ability to permeate through the cell membrane in both the dissociated and undissociated state. This result in aproton shuffle that travel back and forth, unbalancing the chemical gradient of protons of the membrane potential and total proton-motive force. Acetic acid able to cause pore in microbial cells and results in a misbalance the membrane potential which increments the permeation of proton in the cell membrane. This able to increase the H^+ accumulation causing the acidification of cytoplasm since cytoplasm is not rapid enough to alkalinize the intracellular Ph.

In the current study, the antibiotic effect of AgCNC was detected against *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *C. albican*, as supported by p value $0.002 < 0.05$. In this study, it was confirmed that a maximum zone of inhibition (13.5±0.8 mm) was observed at 0.0333 *mg/ml* concentration of AgCNC against gram negative pathogenic *E. coli* ATCC 25922 as seen in (Table 4). In this study, minimum (8.2±0.6 mm) and maximum (12±0.8mm) zone of inhibition was also detected for *S. aureus* a concentration of 0.0066 and 0.0333*mg/ml* against AgCNC, respectively. In this study, it was realized that *C. albican* ATCC 10231 is the least affected with minimum zone of inhibition (7.5±0.25mm) at a concentration of 0.0066 *mg/ml*. However, the maximum zone of inhibition (11.5±1.1mm) at a concentration of 0.0333*mg/ml*. No zone of inhibition was observed for CNC which indicating that the antimicrobial activity of AgCNC is attributed to the Ag nanoparticle on the CNC rather than the CNC itself. It was found that AgCNC is by far the most effective for antibacterial and antifungal even with a low concentration. It was showing better inhibition zone when compared to other derivatives. In agreement with the current study, Xu *et al*. (2016) it was found that AgCNC was effective against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 with maximum zone of inhibition having 0.8cm and 0.7cm clearing area, respectively. Other study performed by Sondi and Salpoek (2004) indicating that the antimicrobial activity of Ag nanoparticles inhibiting about 70% bacterial growth at a concentration of 10 ng cm^{-3} and at concentration of 50-60 ng cm^{-3} . This resulted in complete inhibition of bacterial growth. It was confirmed that variance in terms of effectiveness of Ag nanoparticles against microbial cells are documented due to

method of investigation, method of analysis, and type of Ag nanoparticles (Lee *et al*., 2008), which could be a possible suggestion for our current variation in terms of zone of inhibition. According Hong *et al*. (2016) statement, the size and shape of Ag nanoparticle are determinant factor for variation of zone of inhibition and hence variation in antimicrobial activities.

Although the exact mechanism of silver nanoparticles action against microbial cells are not yet known, various antibacterial activities have been proposed due to silver metals and other heavy. For instance, the increasing permeability of the cytoplasm membrane and cell membrane damages toward the influx of silver ions can be resulted in deleterious effects to microbial cells. This can be due to ribosomes denaturation that finally halt the synthesis of protein (Durán *et al*., 2016). The other hypothesis is an accumulation of silver ions on a cell wall that able to kill the cell by denaturing the cell membrane and results in cell lysis (Zhen *et al*., 2019).

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusions

Cellulose Nano crystals (CNC) were performed in this study. These particles were also characterized. Generally, the following points are concluded from this study:.

- Four concentrations of H_2SO_4 were used for the preparation of CNC, based on initial observation on color change and coagulation of the solutions containing HWTP and H_2SO_4, 45% concentration of H_2SO_4 was found to be suitable for hydrolysis of WTP for the production of CNC.
- The result of the initial observation was supported by the XRD result, obtaining a CNC with a Crystallinity size of 15.39nm and Crystallinity index of 81.8%. As shown in the FTIR results all derivatives were successful.
- The antimicrobial test of the derivatives AgCNC has shown the most promising result, but environmental contamination of silver metal has one of the most deleterious effects when consumed, despite its antimicrobial property the use of AgCNC is has a deleterious effect.
- MCNC has shown the least antimicrobial property which coincides with the property of methyl which is the list of effective alcohol indicating the antimicrobial property of CNC can only improve in corresponding to the compound to be added on the CNC.
- As shown in the study CNC was used as a negative control, several studies have shown CNC alone doesn't exhibit antimicrobial properties. ICNC and ACNC have shown promising results, with ICNC having better results when compared with ACNC.

6.2. Recommendations

Based on the study, the following recommendations were made:

- Finding a procedure that is capable of bleaching and hydrolyzing different types of waste paper (cardboard, newspaper, office paper, etc.) with different contamination content (oil, ink, organic matter, etc.).
- Finding an environmentally friendly method to bleach and hydrolysis the paper, which is capable of producing CNC with high Crystallinity and smaller size.
- Finding a way to make polymer without losing the modifying group to solvent.

7. REFERENCES

Abdel-Halim, E. S. (2012). An effective redox system for bleaching cotton cellulose. Carbohydrate polymers, 90(1), 316-321.

Abera, B., Kibret, M., & Mulu, W. (2014). Knowledge and beliefs on antimicrobial resistance among physicians and nurses in hospitals in Amhara Region, Ethiopia. BMC Pharmacology and toxicology, 15(1), 1-7.

Abitbol, T., Kam, D., Levi-Kalisman, Y., Gray, D. G., & Shoseyov, O. (2018). Surface charge influence on the phase separation and viscosity of cellulose Nano Crystals. Langmuir, 34(13), 3925-3933.

Agarwal, U. P., Ralph, S. A., Baez, C., Reiner, R. S., & Verrill, S. P. (2017). Effect of sample moisture content on XRD-estimated cellulose crystallinity index and crystallite size. Cellulose. 24 (5): 1971-1984., 24(5), 1971-1984.

An, X., Wen, Y., Cheng, D., Zhu, X., & Ni, Y. (2016). Preparation of cellulose Nano Crystals through a sequential process of cellulase pretreatment and acid hydrolysis. Cellulose. 23(4), 2409-2420.

Arcos-Hernandez, M.V. Gurieff, N. Pratt, S. Magnusson, P. Werker, A. Vargas, A. & Lant P. (2010), Rapid quantification of intracellular PHA using infrared spectroscopy: an application in mixed cultures, J. Biotechnol. 150, 372–379, https://doi.org/10.1016/j.jbiotec.2010.09.939.

Besemer, A.C., & VanBekkum, H. (1994). Dicarboxy-Starch by Sodium Hypochlorite/Bromide Oxidation and Its Calcium Binding Properties. Starch-Stärke, 46(3), 95-101.

Bloemhof-Ruwaard, J. M., Van Wassenhove, L. N., Gabel, H. L., & Weaver, P. M. (1996). An environmental life cycle optimization model for the European pulp and paper industry. Omega, 24(6), 615-629.

Boateng, J., & Catanzano, O. (2015). Advanced therapeutic dressings for effective wound healing—a review. Journal of pharmaceutical sciences, 104(11), 3653-3680.

Börjesson, M., & Westman, G. (2015). Crystalline nanocellulose—preparation, modification, and properties. Cellulose-fundamental aspects and current trends, 159-191.

Boufi, S., & Belgacem, M. N. (2006). Modified cellulose fibres for adsorption of dissolved organic solutes. Cellulose, 13(1), 81-94.

Chandra, J., George, N. & NarayACNCkutty, S. K. (2016). Isolation and characterization of cellulose nanofibril from arecanut husk fibre. Carbohydrate polymers 142, 158–166.

Coseri S, Spatareanu A, Sacarescu L, Rimbu C, Suteu D, Spirkd S, Harabagiu V (2015) Green synthesis of the silver nanoparticles mediated by pullulan and 6-carboxypullulan. Carbohydr Polym 116, 9–17.

Czernik, S., & Bridgwater, A. V. (2004). Overview of applications of biomass fast pyrolysis oil. Energy & fuels, 18(2), 590-598.

Deepa, B., Abraham, E., Cherian, B. M., Bismarck, A., Blaker, J. J., Pothan, L. A., ...& Kottaisamy, M. (2011). Structure, morphology and thermal characteristics of bACNCa nano fibers obtained by steam explosion. Bioresource Technology. 102(2): 1988-1997.

Dong, S., Bortner, M. J., & Roman, M. (2016). Analysis of the sulfuric acid hydrolysis of wood pulp for cellulose nanocrystal production: a central composite design study. Industrial Crops and Products, 93, 76-87.

Durán, N., Nakazato, G., & Seabra, A. B. (2016). Antimicrobial activity of biogenic silver nanoparticles, and silver chloride nanoparticles: an overview and comments. Applied microbiology and biotechnology, 100(15), 6555-6570.

Ebise, G. B., & Hundessa, D. D. (2021). Extraction and Characterization of Nanocellulose from Eragrostis Teff Straw.1-9.

Eichhorn, S. J., Dufresne, A., Aranguren, M., Marcovich, N. E., Capadona, J. R., Rowan, S. J., & Gindl, W. (2010). Current international research into cellulose nanofibers and nanocomposites. Journal of materials science. 45(1), 1-33.

EPA (Ethiopian Environmental Protection Agency) (2004) State of Environment Report for Ethiopia. Addis Ababa. 1-143.

FAO,2018 Global Forest products (including paper) facts and figures FAO 2018. http://www.fao.org/3/CA2839EN/ca2839en.pdf (accessed December 2019).

Festucci-Buselli, R. A., Otoni, W. C., & Joshi, C. P. (2007). Structure, organization, and functions of cellulose synthase complexes in higher plants. Brazilian Journal of Plant Physiology, 19, 1-13.

Foster, E. J., Moon, R. J., Agarwal, U. P., Bortner, M. J., Bras, J., Camarero-Espinosa, S., ... & Youngblood, J. (2018). Current characterization methods for cellulose nanomaterials. Chemical Society Reviews, 47(8), 2609-2679.

Fraise, A. P., Wilkinson, M. A. C., Bradley, C. R., Oppenheim, B., & Moiemen, N. (2013). The antibacterial activity and stability of acetic acid. Journal of Hospital Infection, 84(4), 329-331.

French, A. D. (2017). Glucose, not cellobiose, is the repeating unit of cellulose and why that is important. Cellulose, 24(11), 4605-4609.

Golmohammadi, H., Morales-Narváez, E., Naghdi, T., & Merkoçi, A. (2017). Nanocellulose in sensing and biosensing. Chemistry of Materials, 29(13), 5426-5446.

Habibi, Y., Lucia, L. A., & Rojas, O. J. (2010). Cellulose Nano Crystals: chemistry, self-assembly, and applications. Chemical reviews. 110(6), 3479-3500.

Hamer, G. (2003). Solid waste treatment and disposal: effects on public health and environmental safety. Biotechnology advances, 22(1-2), 71-79.

Hearle, J. W. S. (1958). A fringed fibril theory of structure in crystalline polymers. Journal of Polymer Science, 28(117), 432-435.

Henriksson, M., Henriksson, G., Berglund, L. A., & Lindström, T. (2007).An environmentally friendly method for enzyme-assisted preparation of microfibrillated cellulose (MFC) nanofibers. European Polymer Journal. 43(8), 3434-3441.

Hermanutz, F., Meister, F., & Uerdingen, E. (2006). Raw Materials-New developments in the manufacture of cellulose fibers with ionic liquids. Chemical Fibers International. 56(6), 342-344.

Hong, X., Wen, J., Xiong, X., & Hu, Y. (2016). Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method. Environmental science and pollution research, 23(5), 4489-4497.

Huang, C., Guo, H. J., Xiong, L., Wang, B., Shi, S. L., Chen, X. F., ...& Chen, X. D. (2016). Using wastewater after lipid fermentation as substrate for bacterial cellulose production by Gluconacetobacter xylinus. Carbohydrate polymers. 136,198-202.

Ichihara, K. I., & Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography [S]. Journal of lipid research, 51(3), 635-640.

Iwamoto, S., Nakagaito, A. N., & Yano, H. (2007).Nano-fibrillation of pulp fibers for the processing of transparent nanocomposites. Applied Physics. 89(2), 461-466.

Jonoobi, M., Oladi, R., Davoudpour, Y., Oksman, K., Dufresne, A., Hamzeh, Y., & Davoodi, R. (2015). Different preparation methods and properties of nanostructured cellulose from various natural resources and residues: a review. Cellulose. 22(2), 935-969.

Joshi, G., Naithani, S., Varshney, V. K., Bisht, S. S., & Rana, V. (2017). Potential use of waste paper for the synthesis of cyanoethyl cellulose: A cleaner production approach towards sustainable environment management. Journal of Cleaner Production, 142, 3759-3768.

Kale, R. D., Bansal, P. S., & Gorade, V. G. (2018).Extraction of microcrystalline cellulose from cotton sliver and its comparison with commercial microcrystalline cellulose.Journal of Polymers and the Environment. 26(1), 355-364.

Kanagalingam, J., Feliciano, R., Hah, J. H., Labib, H., Le, T. A., & Lin, J. C. (2015). Practical use of povidone-iodine antiseptic in the maintenance of oral health and in the prevention and treatment of common oropharyngeal infections. International journal of clinical practice, 69(11), 1247-1256.

Khalil, H. A., Bhat, A. H., & Yusra, A. I. (2012). Green composites from sustainable cellulose nanofibrils: A review. Carbohydrate polymers.87(2), 963-979.

Kiehlbauch, J. A., Hannett, G. E., Salfinger, M., Archinal, W., Monserrat, C., & Carlyn, C. (2000). Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. Journal of clinical microbiology, 38(9), 3341-3348.

Kim, T. H. (2011). Sequential hydrolysis of hemicellulose and lignin in lignocellulosic biomass by two-stage percolation process using dilute sulfuric acid and ammonium hydroxide. Korean Journal of Chemical Engineering, 28(11), 2156-2162.

Kruer, Z, N., Cantero, T. B., & Wilson, D. B. (2018). Characterization of cellulose crystallinity after enzymatic treatment using Fourier transform infrared spectroscopy (FTIR). Cellulose, 25(1), 37-48.

Kumar, V., Pathak, P., & Bhardwaj, N. K. (2020). Waste paper: An underutilized but promising source for nanocellulose mining. Waste management, 102, 281-303.

Lacerda, T. M., Zambon, M. D., & Frollini, E. (2013). Effect of acid concentration and pulp properties on hydrolysis reactions of mercerized sisal. Carbohydrate polymers, 93(1), 347-356.

Lavoine, N., Desloges, I., Dufresne, A., & Bras, J. (2012). Microfibrillated cellulose–Its barrier properties and applications in cellulosic materials: A review. Carbohydrate polymers.90(2), 735-764.

Lee, K. C., Lin, S. J., Lin, C. H., Tsai, C. S., & Lu, Y. J. (2008). Size effect of Ag nanoparticles on surface plasmon resonance. Surface and Coatings Technology, 202(22-23), 5339-5342.

Li, B., & Webster, T. J. (2018). Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. Journal of Orthopaedic Research, 36(1), 22-32.

Lin, N., Huang, J., Chang, P. R., Feng, J., & Yu, J. (2011). Surface acetylation of cellulose nanocrystal and its reinforcing function in poly (lactic acid). Carbohydrate Polymers, 83(4), 1834-1842.

Listyanda, R. F., Wildan, M. W., & Ilman, M. N. (2020). Preparation and characterization of cellulose nanocrystal extracted from ramie fibers by sulfuric acid hydrolysis. Heliyon, 6(11),1-10.

Liu, D. Y., Sui, G. X., & Bhattacharyya, D. (2014). Synthesis and characterisation of nanocellulose-based polyaniline conducting films. Composites Science and Technology. 99, 31-36.

Liu, Xuanyong, Paul K. Chu, and Chuanxian Ding (2004). "Surface modification of titanium, titanium alloys, and related materials for biomedical applications." Materials Science and Engineering: R: Reports 47, no. 3-4: 49-121.

Lokanathan, A. R., Uddin, K. M. A., Rojas, O. J., & Laine, J. (2014). Cellulose nanocrystal-mediated synthesis of silver nanoparticles: role of sulfate groups in nucleation phenomena. Biomacromolecules, 15(1), 373-379.6

Maiti, S., Jayaramudu, J., Das, K., Reddy, S. M., Sadiku, R., Ray, S. S., & Liu, D. (2013). Preparation and characterization of nano-cellulose with new shape from different precursor. Carbohydrate polymers, 98(1), 562-567.

Marquis, R. E., Clock, S. A., & Mota-Meira, M. (2003). Fluoride and organic weak acids as modulators of microbial physiology. FEMS microbiology reviews, 26(5), 493-510.

Meyer, K., & Mark, H. (1928). For the crystallizable part of cellulose. Chemische Berichte, 61, 593-614.

Mondal, S. (2017). Preparation, properties and applications of nanocellulosic materials. Carbohydrate polymers. 163, 301-316.

Morton, H. E. (1950). The relationship of concentration and germicidal efficiency of ethyl alcohol. Annals of the New York Academy of Sciences, 53(1), 191-196.

Nasatto, P. L., Pignon, F., Silveira, J. L., Duarte, M. E. R., Noseda, M. D., & Rinaudo, M. (2015). Methylcellulose, a cellulose derivative with original physical properties and extended applications. Polymers, 7(5), 777-803.

Osong, S. H., Norgren, S., & Engstrand, P. (2016). Processing of wood-based microfibrillated cellulose and nanofibrillated cellulose, and applications relating to papermaking: a review. Cellulose. 23(1), 93-123.

Pagliaro, M. (1999). New iodination of cellulose in phosphoric acid. Carbohydrate research, 315(3-4), 350-353.

Qiao, K., Tian, W., Bai, J., Wang, L., Zhao, J., Du, Z., & Gong, X. (2019). Application of magnetic adsorbents based on iron oxide nanoparticles for oil spill remediation: A review. Journal of the Taiwan Institute of Chemical Engineers, 97, 227-236.

Reddy, N., & Yang, Y. (2005). Biofibers from agricultural byproducts for industrial applications. trends in Biotechnology, 23(1), 22-27.

Rice, L. B. (2006). Challenges in identifying new antimicrobial agents effective for treating infections with Acinetobacter baumannii and Pseudomonas aeruginosa. Clinical infectious diseases, 43(2), 100-105.

Rodriguez, C., Alaswad, A., El-Hassan, Z., & Olabi, A. G. (2017). Mechanical pretreatment of waste paper for biogas production. Waste Management. 68, 157-164.

Segal, L., Creely, J., Martin, A. Jr, & Conrad, C. (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Textile Research Journal. 29(10), 786-794.

Sekiguchi, Y., Sawatari, C., & Kondo, T. (2003). A gelation mechanism depending on hydrogen bond formation in regioselectively substituted O-methylcelluloses. Carbohydrate Polymers, 53(2), 145-153.

Sharma, A., Thakur, M., Bhattacharya, M., Mandal, T., & Goswami, S. (2019). Commercial application of cellulose nano-composites-A review. Biotechnology Reports. 12,117-132.

Shin, Y., Bae, I. T., Arey, B. W., & Exarhos, G. J. (2008). Facile stabilization of gold-silver alloy nanoparticles on cellulose nanocrystal. The Journal of Physical Chemistry C, 112(13), 4844-4848.

Sinko, R., Qin, X., & Keten, S. (2015). Interfacial mechanics of cellulose Nano Crystals. Mrs Bulletin, 40(4), 340-348.

Sondi, I., & Salopek-Sondi, B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science, 275(1), 177-182.

Sun, B., Peng, G., Duan, L., Xu, A., & Li, X. (2015). Pretreatment by NaOH swelling and then HCl regeneration to enhance the acid hydrolysis of cellulose to glucose. Bioresource technology, 196, 454-458.

Sutton, M. A., Li, N., Joy, D. C., Reynolds, A. P., & Li, X. (2007). Scanning electron microscopy for quantitative small and large deformation measurements part I: SEM imaging at magnifications from 200 to 10,000. Experimental mechanics, 47(6), 775-787.

Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J., & Dittrich, S. (2017). Antimicrobial resistance in Africa: a systematic review. BMC infectious diseases, 17(1), 1-17.

Takagi, S. (1969). A dynamical theory of diffraction for a distorted crystal. Journal of the Physical Society of Japan, 26(5), 1239-1253.

Teshome, F. B. (2020). Municipal solid waste management in Ethiopia; the gaps and ways for improvement. Journal of Material Cycles and Waste Management, 1-14.

Thomas, B., Raj, M. C., Joy, J., Moores, A., Drisko, G. L., & Sanchez, C. (2018). Nanocellulose, a versatile green platform: from biosources to materials and their applications. Chemical reviews, 118(24), 11575-11625.

Trache, D., Tarchoun, A. F., Derradji, M., Hamidon, T. S., Masruchin, N., Brosse, N., & Hussin, M. H. (2020). Nanocellulose: from fundamentals to advanced applications. Frontiers in Chemistry.392(8), 1-33.

Xing, L., Hu, C., Zhang, W., Guan, L., & Gu, J. (2020). Transition of cellulose supramolecular structure during concentrated acid treatment and its implication for cellulose nanocrystal yield. Carbohydrate polymers, 229, 43-55.

Xu, Y., Zuo, L., Qian, X., and Wang, J. (2016). "Preparation and characterization of cellulose-silver nanocomposites by in situ reduction with alkalis as activation reagent," BioRes. 11(1), 2797-2808.

Zakani, B., & Grecov, D. (2020). Yield stress analysis of cellulose nanocrystalline gels. Cellulose, 27(16), 9337-9353.

Zannis, J., Angobaldo, J., Marks, M., DeFranzo, A., David, L., Molnar, J., & Argenta, L. (2009). Comparison of fasciotomy wound closures using traditional dressing changes and the vacuum-assisted closure device. Annals of plastic surgery, 62(4), 407-409.

Zhen, J. B., Kang, P. W., Zhao, M. H., & Yang, K. W. (2019). Silver nanoparticle conjugated star PCL-b-AMPs copolymer as nanocomposite exhibits efficient antibacterial properties. Bioconjugate chemistry, 31(1), 51-63.