CHEMICAL MODIFICATION OF THE EXTRACTED NANOCELLULOSE FROM BARK OF *EDGEWORTHIA GARDNERI* PLANT AND STUDY OF ITS ANTIMICROBIAL ACTIVITY

A DISSERTATION WORK SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE DEGREE IN CHEMISTRY

SUBMITTED BY

Name: DEVASHISH BIKRAM KARN

T.U. Examination Roll No.: 1875/2076

T.U. **Registration No.:-** 5-2-2-103-2015

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BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation entitled, **"Chemical Modification** of Extracted **Nanocellulose from the Bark of *Edgeworthia gardneri* Plant and Study** of their **Antimicrobial Activity"** by **Mr. Devashish Bikram Karn** under the supervision of Assoc. Prof. Dr. Arun Kumar Sharma, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, and under co-supervision of Asst. Prof. Hari Bhakta Oli, Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal, hereby submitted has been approved for partial fulfillment of the requirement for completion of her Master of Science (M.Sc.) Degree in Chemistry. This dissertation has not been submitted to any other university or institution previously for the award of a degree.

Tribhuvan University
Amrit Campus
Department of Chemistry

.....

Supervisor

Assoc. Prof. Dr. Arun Kumar Sharma

Department of Chemistry

Amrit Campus, TU, Kathmandu, Nepal

.....

Co-supervisor

Asst. Prof. Hari Bhakta Oli

Department of Chemistry

Amrit Campus, TU, Kathmandu, Nepal

.....

External Examiner

Asst. Prof. Dr. Bhanu Bhakta Neupane

Central Department of Chemistry

Tribhuvan University, Kathmandu, Nepal

.....

Internal Examiner

Asst. Prof. Dr. Deval Prasad Bhattarai

Department of Chemistry

Amrit Campus, TU, Kathmandu, Nepal

.....

M.Sc. Chemistry Coordinator

Assoc. Prof. Dr. Bhushan Shakya

Department of Chemistry

Amrit Campus, TU, Kathmandu, Nepal

.....

Head of the Department

Assoc. Prof. Kanchan Sharma

Department of Chemistry

Amrit Campus, TU, Kathmandu, Nepal

Date:2023/09/08

LETTER OF RECOMMENDATION

This is to recommend that the dissertation work entitled **"Chemical Modification of Extracted Nanocellulose from the Bark of *Edgeworthia gardneri* Plant and Study of their Antimicrobial Activity"** has been carried out by **Mr. Devashish Bikram Karn** as partial fulfillment for the requirements of Master of Science Degree in Chemistry. This is his original work and has been carried out under our guidance and supervision. To the best of our knowledge, this research has not been submitted for any other degree at this institute.

Supervisor

Assoc. Prof. Dr. Arun Kumar Sharma

Department of Chemistry

Amrit Campus, T.U.,

Kathmandu, Nepal

Co-Supervisor

Asst. Prof. Hari Bhakta Oli

Department of Chemistry

Amrit Campus, T.U.,

Kathmandu, Nepal

Date: 2023/08/10

DECLARATION

I, Devashish Bikram Karn, hereby declare that the work entitled **"Chemical modification of extracted nanocellulose from the bark of *Edgeworthia gardneri* plant and study of its Antimicrobial activity"** submitted to the Institute of Science and Technology Tribhuvan University as partial fulfillment for the requirements of Master of Science Degree in Chemistry has been done by myself and has not been submitted earlier in part or full in this or any other form to any other university/institute, here or elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

.....

Devashish Bikram Karn

TU Registration No.:- 5-2-2-103-2015

Examination Roll No.: CHE1875/076

SEPTEMBER, 2023

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Devashish Bikram Karn

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शोधसार

नानोसेलुलोजको वृहत्तर उपयोगिताको कारणले जिवाशेषबाट यसको निकासि गर्नेकार्य बढिरहेको छ। यस अनुसन्धानमा अर्गेलीको बोक्राबाट ३४.३ % नानोसेलुलोजको सफलतापूर्वक निकासी गरिएको छ। उक्त सेलुलोजका मणिभहरुमा चाँदीका नानोकणहरु सफलता पूर्वक टाँसिएकोकुरा एक्स रे विवर्तन तथा प्रकाशिय वर्णपट अध्ययन विधिबाट गरिएको छ। उक्त मणिभहरुको विषाणु प्रतिरोधि क्षमता परिक्षण गर्दा केहि विषाणुहरु विरुद्ध उत्कृष्ट प्रभाव देखाएका छन।

Keywords: *Edgeworthia gardneri*, alkali treatment, cellulose, acid hydrolysis, cellulose nanocrystal, AgNPs, Antimicrobial test

ABSTRACT

Interest in the extraction of nanocellulose from biomass for various applications is expanding recently. The blending of such nanocellulose with different metal nanoparticles will enhance the property of the material and help to explore the materials for new areas of application. In this research, the extraction of nanocellulose from *Edgeworthia gardneri* bark was carried out. Nanocellulose was extracted through a series of steps of alkali treatment, bleaching,and acid hydrolysis. The quantity of nanocellulose extracted was 34.30%. The extracted nanocellulose was blended with silver nanoparticles and was characterized using X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. Furthermore, Antimicrobial test of these prepared material showed an excellent effect against *Escherichia coli, Bacillus subtilis,* and *Candida albicans,* respectively.

Keywords: *Edgeworthia gardneri*, alkali treatment, cellulose, acid hydrolysis, cellulose nanocrystal, AgNPs, Antimicrobial test

LIST OF ACRONYMS AND ABBREVIATIONS

AgNPs	**:** Silver Nanoparticles
BNC	**:** Bacterial Nanocellulose
C-AgNPs	**:** Chemically Synthesized Silver Nanoparticles
G-AgNPs	**:** Green Synthesized Silver Nanoparticles
CI	**:** Crystallinity Index
CNC	**:** Cellulose Nanocrystals
CNFs	**:** Cellulose Nanofibrils
C-Ag@CNC-1	**:** Incorporation of Chemically Synthesised Silver Nanopaticles into Nanocellulose
G-Ag@CNC-1	**:** Incorporation of Green Synthesised Silver Nanoparticles into Nanocellulose
C-Ag@CNC-2	**:** Insitu Generation of Chemically Synthesised Silver Nanoparticles into Nanocellulose
FTIR	**:** Fourier Transform Infrared
NPs	**:** Nanoparticles
TEMPO	**:** 2, 2, 6, 6-tetramethylpiperidine-1-oxyl
XRD	**:** X-ray diffraction

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CHAPTER 1

INTRODUCTION

1.1. Background of study

Cellulose is a naturally occurring polymer that acts as a fundamental structural unit of all plants and can be obtained from a variety of bio-resources (Klemm et al., 2005). It is composed of the linear homopolysaccharide of β-1,4- linked anhydro-D-glucose units with the repeating unit of cellobiose (Moon et al., 2011). Cellulose chains form microfibrils and act as a stabilizer for both inter and intra-molecular hydrogen bonding (Alemdar & Sain, 2008). There are three main approaches for the extraction of cellulose and they are mechanical, chemical, and bacterial techniques.

Mechanical ways involve grinding (Nair et al., 2014a), crushing (Mocchiutti et al., 2016), steam explosion (Zeng et al., 2014), and high-pressure homogenization (Tian et al., 2016). Chemical methods comprise alkali treatment (Zeng et al., 2014), and degumming (Li et al., 2015). Because of its availability, biodegradability, renewability, low cost, reasonable strength, and lightweight (Chowdhury et al., 2013; Ilyas et al., 2018), cellulose has been used in a number of products such as food, pharmaceuticals, textiles, paints, etc. (Nasir et al., 2015).

During acid hydrolysis, cellulose gets reduced to nanoscale and is typically referred to as nanocellulose (Eyley & Thielemans, 2014). Nanocellulose has unique properties such as high surface area, anisotropic mechanical properties, and surface richness in hydroxyl groups, biocompatible and biodegradable, and also offers low cost (Jiang & Hsieh, 2015; Zeng et al., 2014). Therefore, interest is increasing on a global scale to modify nanocellulose for a variety of applications.

1.2. Nanocellulose

A natural fiber with one dimension in the nanometer range is called nanocellulose. They can be distinguished into three basic forms: bacterial nanocellulose, nanofibrillated cellulose, and nanocrystalline cellulose. Although all varieties share a similar chemical composition, differences in sources and extraction techniques have led to differences in morphology, particle size, crystallinity, and other features (Khalil

et al., 2012).

Bacterial nanocellulose (BNC) is a type of nanocellulose that is formed by the accumulation of low molecular weight sugars by bacteria, primarily *Gluconacetobacter xylinus*, over a few days to two weeks (Jozala et al., 2016). The bacterial nanocellulose is always present in its pure state, devoid of lignocellulosic biomass such as lignin, hemicellulose, pectin, and so on (Fu et al., 2013). It takes the shape of twisting ribbons with 20 to 100 nm typical diameters, micrometer lengths, and significant surface per unit area (Abitbol et al., 2016).

Nanofibrillated cellulose is often referred to as micro fibrillated cellulose, cellulose nanofiber, cellulose nanofibril, or nanofibrillar cellulose. Mechanical techniques can be used to separate the long, flexible, and entangled nanocellulose from the cellulose fibrils. Its lengthy fibril forms range in size from 500 to 2000 nm in length and 1-100 nm in diameter (Nechyporchuk et al., 2016). Additionally, it has a chemical composition of 100% cellulose with both crystalline and amorphous parts (Lu et al., 2008). Nanofibrillated cellulose can be recovered from cellulose chains by cleaving fibrils along their longitudinal axes in response to mechanical stress as in Figure 1 (Moon et al., 2011). When compared to nanocrystalline cellulose, nano-fibrillated cellulose has a longer length, a higher aspect ratio (length to diameter), a larger surface area, and a higher concentration of hydroxyl groups, making it easier to modify the surface (Lavoine et al., 2012).

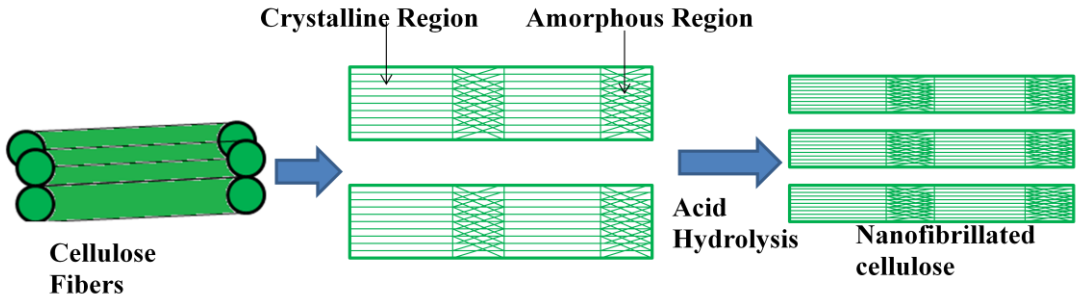

Figure 1.1:- Schematic isolation of nanofibrillated cellulose via mechanical treatment from cellulose chains.

Nanocrystalline cellulose, also referred to as cellulose nanocrystals, or cellulose nanowhiskers, is obtained through acid hydrolysis and has a high degree of strength (Khalil et al., 2012)**.** It is shaped like a short rod or a whisker and measures 2–20 nm

in diameter by 100–500 nm in length. Additionally, it is entirely composed of cellulose, mostly in the crystalline portions. Through acid hydrolysis, amorphous domains are split apart, and local crystalline connections between nanofibrils are broken, to produce nanocrystalline particles as shown in Figure 1.2 (Moon et al., 2011).

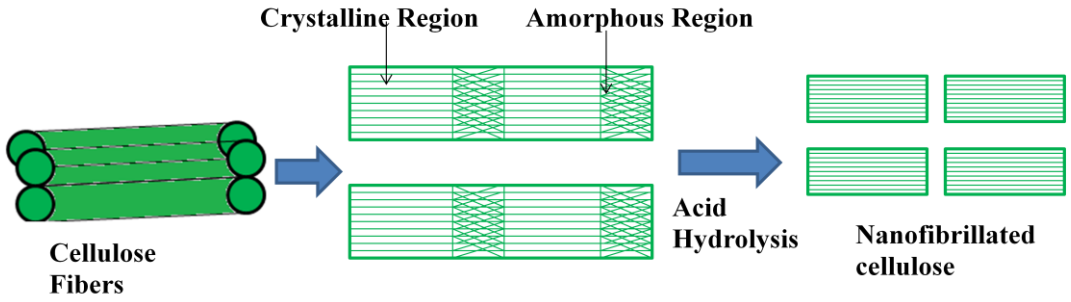

Figure 1.2:- Schematic isolation of Nanocrystalline cellulose via acid hydrolysis from cellulose chains.

1.3. Extraction techniques of nanocellulose

In a broad sense methods of extraction of nanocellulose can be divided into two parts and they are Mechanical and Chemical methods.

1.3.1. Mechanical methods

Mechanical methods are commonly used for the extraction of nanocellulose due to their simplicity and scalability. The major mechanical methods for extracting the nanocellulose are given below:-

1.3.1.1. High-pressure homogenization

High-pressure homogenization is a relatively fast and efficient used technique for the extraction of cellulose nanofibrils (CNFs). In this technique pulp, slurry, or suspension is allowed to flow through extremely small openings due to which shearing action and molecular collisions between the fibers take place, resulting in nanoscale particles of cellulose fibers. High pressure (50-2000 MPa) and velocity is used while the suspension moves through the gaps. The velocity and pressure depend upon the confinement of the gaps (Khalil et al., 2014).

1.3.1.2. Microfluidization

The theory behind micro fluidization is the creation of high-shear forces by turbulent flow in a microfluidizer or other similar devices. Cellulose suspensions are pumped through a narrow channel or chamber, where intense shearing occurs. The shearing forces disintegrate cellulose fibers into nanofibrils. The process is repeated to create finer nanocellulose structures (Kargarzadeh et al., 2017).

1.3.1.3. Ultrasonication

Ultrasonication is a relatively simple and low-cost method for nanocellulose extraction. This method involves the use of high-frequency sound waves to disrupt cellulose fibers and produce nanocellulose. Cellulose suspensions are subjected to ultrasonic waves, which create cavitation bubbles. These bubbles burst, creating strong shear forces that shred the fibers into smaller pieces (Tang et al., 2013).

1.3.1.4. Ball mining

Ball mining is a mechanical grinding process employed to extract nanocellulose. Shear forces are generated among the balls as well as between the balls and the jar's surface as a result of the centrifugal force from the revolving jar. These forces break down the fibers into smaller sizes, resulting in nanofibrils (Baheti et al., 2012).

1.3.2. Chemical methods

Chemical techniques are frequently employed for the extraction of nanocellulose from cellulose fibers. These procedures use chemicals to dissolve or change the structure of the cellulose to extract nanocellulose. The major chemical methods for extracting the nanocellulose are given below:-

1.3.2.1. Acid hydrolysis

Acid hydrolysis is a common technique for extracting nanocellulose. Strong mineral acids such as sulfuric acid or hydrochloric acid are treated with cellulose fibers. The acid breaks down the amorphous regions of the cellulose, leading to the formation of nanocellulose crystallites. The resulting suspension is then neutralized and extensively washed to remove residual acid and other impurities. The properties of obtained

nanocellulose depend upon acid concentration, reaction time, and temperature (Lavoine et al., 2012).

1.3.2.2. TEMPO mediated oxidation

TEMPO-mediated oxidation is a technique that selectively modifies the surface of cellulose fibers. It uses TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl), a stable radical, and a co-oxidant, often sodium hypochlorite (NaClO) to oxidize primary hydroxyl groups of cellulose into carboxyl groups. The oxidation process adds negative charges to the surface of the cellulose, which causes repulsion between the fibrils or crystals and makes it easier for them to break apart into nanocellulose. The oxidized cellulose is typically broken down into nanoscale by mechanical disintegration or sonication (Saito et al., 2009).

1.3.2.3. Enzymatic hydrolysis

Enzymatic hydrolysis is an environmentally friendly method that involves the use of specific enzymes, such as Cellulases (Beltramino et al., 2015), Endoglucanases (Zhao et al., 2017), Pectinases (Hideno et al., 2014) and ligninases (Shak et al., 2018) to degrade cellulose into nanocellulosic components. Cellulases can attack and dissolve the 1,4-glycosidic bonds in cellulose. The enzymatic hydrolysis process is relatively milder compared to acid hydrolysis and offers better control over the final product. It preserves the crystalline structure of cellulose, resulting in nanocellulose with higher crystallinity (Khalil et al., 2014).

1.4. Application of nanocellulose

Nanocellulose has drawn a lot of attention in recent years due to its distinct features and has applications in various fields such as nanocomposites (Abitbol et al., 2016), barrier properties and packing (Nair et al., 2014b), biomedical applications (Grishkewich et al., 2017), electronic applications (Hoeng et al., 2016), energy conservation and production (Zhu et al., 2016) and many more.

1.4.1. Nanocomposite

Nanocellulose fibers have high mechanical strength (Dufresne, 2012), and high thermal properties with lightweight and transparency (Abitbol et al., 2016) which

helps them to act as a promising candidate for Nanocomposite materials. The composites are created by mixing nanocellulose with polymers, plastics, and other matrix materials which results in enhancing performance and reducing the weight of the composite. When soya bean nanocellulose was combined with synthetic polymers, it demonstrated a significantly higher tensile strength and stiffness compared to pure base polymers (B. Wang & Sain, 2007). When Poly lactic acid and nanocellulose were mixed, a novel composite material with improved thermal and crystallinity properties was formed (Robles et al., 2015).

1.4.2. Barrier properties and packing

Nanocellulose is highly used in food packaging due to its low permeability and ability to construct dense percolating networks (Nair et al., 2014b). Nanocellulose incorporation with films, coatings, and additives improves the barrier characteristics of packaging as well as reduces the permeability of gas (H. Wang et al., 2013) and water (Minelli et al., 2010). Additionally, nanocellulose can replace non-biodegradable plastic coatings from the packaging by forming biodegradable coatings with or without additives (Honorato et al., 2015).

1.4.3. Biomedical applications

Modified surfaces of nanocellulose are utilized as antibacterial agents, biosensors, and scaffolds for biocatalyst and tissue engineering (Grishkewich et al., 2017). Nanocellulose is utilized as a drug delivery medium due to its small size, biocompatibility, hydrophilicity, etc. (George & Sabapathi, 2015). Control of dosing is also achieved when nanocellulose was blinded with tetracycline and doxorubicin, as it has a large surface area as well as has the potential to gain a negative charge during hydrolysis (Pachuau, 2017). Aero-gels created using nanocellulose have built up bioavailability and better drug-loading capacity (García-González et al., 2011). Nanocellulose is also used in tissue culture to support cell growth (Hua et al., 2014).

1.4.4. Electronic applications

Paper made from nanocellulose is translucent, optically clear, and bendable and can be used for flexible circuits, flexible displays, and electrical devices (Okahisa et al., 2009). Carbon nanofibers act as a precursor for making anode of sodium-ion batteries

(Luo et al., 2013). When carbon nanofibers are coated with indium tin oxide, an organic limiting diode is created (Legnani et al., 2008). When tin oxide was coated on the surface of nanocellulose, flexible organic field effect transistors were formed (Valentini et al., 2014). Similarly, zinc oxide's thermal characteristics were enhanced when it was coated over TEMPO-oxidized nanocellulose (Jin et al., 2017).

1.4.5. Energy conservation and production

Nanocellulose has gained interest as an efficient material for super capacitor electrodes due to its specific characteristics such as high permanent electric dipole moment, lightweight, good mechanical properties, good optical transparency, minimal porosity, thermal expansion coefficient, and air permeability (Muhd Julkapli & Bagheri, 2017). When nanocellulose was incorporated with polypyrrole, excellent conductance and high capacitance with a coating of graphene oxide sheets were observed (L. Ma et al., 2016). Flexible batteries with better energy densities were made possible by coating carbon nanofiber paper with lithium cobalt oxide (Zhou et al., 2017). Nanocellulose-based materials are used to make polymer electrolyte fuel cells (Vilela et al., 2019).

1.5. Antimicrobial Activity

One of the biggest threats to global health is antibiotic resistance, which is accelerating faster than the rate at which new, potent medications are being developed. Metal-based antimicrobial compounds, such Ag-NPs, with easily adjustable physicochemical properties, can be made to combat these antibiotic-resistant microbes. Ag NPs have a broad antibacterial effect due to their capacity to harm bacteria's internal and extracellular components (Z. Wang et al., 2020). A study suggests that the mechanism underlying AgNPs' antibacterial activity may include the production of free radicals. When these nanoparticles engage with bacteria, they release silver ions inside the cell, which causes the contents of the cell to seep out and ultimately causes protein denaturation. AgNPs can therefore be thought of as a superior alternative for treating infectious diseases brought on by microorganisms (Parthiban et al., 2019). Impregnation of such nanoparticles will enhance the antimicrobial properties of cellulose nanocrystals.

1.6. ***Edgewrothia gardneri*** **Plant**

In Nepal, the Thymelaraceae family plant *Edgeworthia gardneri* is locally referred to as Argeli. These plants flourish gregariously on the southern slopes of Nepal's Himalayan forests between 1,600 and 4,000 meters above sea level (Biggs & Messerschmidt, 2005). It is being used as raw material for the production of handmade paper in Nepal. It is assumed that its bark contains a large amount of cellulosic fiber.

The Argeli plant is classified scientifically as:

Systemic Classification

Kingdom: Plantae

Phylum: Tracheophyta

Family: Thymelaeaceae

Class: Magnoliopsida

Order: Malvales

Genus: *Edgeworthia*

Species: *gardneri*

Figure 1.3:- *Edgeworthia gardneri* (Argeli)

1.7. Objectives

1.7.1. General objective

The main objective of this research is extraction and characterization of cellulose nanocrystals from the bark of *Edgeworthia gardneri* (Argeli plant) followed by incorporation of silver nanoparticles and studies its antimicrobial effect.

1.7.2. Specific objectives

The specific objectives of the study can be outlined as follow:-

- Extraction of nanocellulose from the bark of *Edgeworthia gardneri* (Argeli plant).
- Synthesis of silver nanoparticles by chemical and green methods.
- Incorporation of as-synthesized AgNPs in nanocellulose.
- Characterization of the nanocellulose, modified nanocellulose, and silver nanoparticles by using FTIR and XRD.
- Study of antimicrobial activity of AgNPs incorporated cellulose nanocrystals.

CHAPTER 2

LITERATURE REVIEW

Various studies have been carried out to extract and modify nanocellulose from plants. Some of the recent works on this topic have been reviewed below:

Mehanny et al. (2021) used Spanish poplar wastes as the starting point for the successful extraction of crystalline nanocellulose. Spanish poplar was treated with a 10% sodium hydroxide (wt/wt) solution, sodium chlorite, and acid hydrolysis. According to the findings, nanocellulose has dimensions of 219 nm in length and 69 nm in breadth. Additionally, the crystallinity index (CI) was dramatically increased by 7-8% by acidic hydrolysis.

Using the most recent technology, Khan et al., (2020) recovered nanocellulose from dunchi fiber. Nanocellulose possessed a high degree of crystallinity up to 66.7% and an average length and width of 202.87 nm and 20.67 nm, respectively.

Júnior et al. (2019) used soft chemical and steam explosion treatments to extract nanocellulose from yerba mate sticks. The characterization of nanocellulose was carried out using several methods. Nanocellulose exhibited dimensions between 11 and 15 nm, aspect ratios between 12 and 24, and a 35% rise in the index of crystallinity.

Nanocellulose was effectively produced from apple steam by Phanthong et al. (2015) The apple stem underwent standard cellulose extraction as a pretreatment, and then cellulose was mildly acid hydrolyzed to produce nanocellulose. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TG) were used for characterization. The outcomes proved that nanocelluloses with dimensions ranging from 10 to 20 nm were produced. The nanocellulose had a whisker-like form and had a higher degree of crystallinity.

From Vietnamese agricultural wastes (Nypa Fruticans trunk, coconut husk fiber, and rice husk), Nhan An et al. (2020) extracted cellulose nanocrystals. Acid hydrolysis, hydrogen peroxide/sodium hydroxide treatment, and formic/peroxyformic acid pre-

treatment were all used. Following each stage of treatment, the structure and thermal characteristics of the produced materials were identified by XRD, TGA, TEM, and FT-IR analyses. Nanocellulose had a high aspect ratio and crystallinity of up to 80%. Its dimensions were 200-500 nm in length and 10-15 nm in diameter.

By using microwave treatment, bleaching, acid-hydrolysis, and ultrasonic treatment, Song et al. (2018) were able to isolate nanocellulose from kenaf bast. Nanocellulose's characterization revealed a length distribution of about 300 to 600 nm and a diameter spread of 10 to 60 nm, with a crystallinity index of 79.3%.

Ball-milling-assisted acid hydrolysis technique was used by Plermjai et al. (2018) to produce nanocellulose from sugarcane bagasse. The elimination of hemicellulose and lignin from the sugarcane bagasse was verified by the FTIR spectrum analysis. According to XRD data nanocellulose had a higher crystallinity than untreated cellulose.

Raw oil palm frond leaves were effectively used to make nanocellulose by Hussin et al. (2020). To create nanocellulose, leaves were chemically treated with bleach, alkali, and acid. According to X-ray diffraction, transmission, and field emission scanning electron micrographs, isolated Nanocellulose crystallized into needle-like structures with a crystallinity index of more than 45.5% and sizes between 10 and 30 nm.

The husks of *Xanthoceras sorbifolia* were subjected to several chemical processes to extract the nanocellulose Ma et al. (2015). The nanocellulose from the husks of *Xanthoceras sorbifolia* had a rod-like shape and a crystallinity index of 71.5%. Its diameter was about 38 nm. According to FTIR data, cellulose fibers from the husks of *Xanthoceras sorbifolia* possessed high cellulose purity.

Through the use of benzyl alcohol extraction, sodium chlorite bleaching, potassium hydroxide alkaline treatment, and ultrasonic crushing, Zhang et al. (2023) successfully extracted nanocellulose from the banana pseudo-stem. By adding konjac glucomannan (KGM) to 0.8-weight percent nanocellulose dispersion and freeze-drying it, composite aerogel was created. Different ratios of nanocellulose and KGM were used to control how effectively aerogels performed. The results demonstrated that aerogel with an 8/2 mass ratio of nanocellulose to KGM had commendable inhibitory effects on *E. coli* and *S. aureus*. The results also demonstrated that aerogel

with an 8/2 mass ratio of nanocellulose to KGM had good hemolysis and no cytotoxicity, with a hemolysis rate of 3.38% and a cell survival rate of over 90%.

Nanocellulose was produced from banana pseudostems by Shrestha et al. (2021) using the acid hydrolysis technique. Scanning electron microscopy, Fourier-transformed infrared spectroscopy, and X-ray diffraction were used to analyze the production of nanocellulose. Nanocellulose had an average size of 18.79 nm in diameter, 202.12 nm in length, and a high crystallinity of 81.67%. Tetracycline was used to create chitosan-nano cellulose-based antimicrobial nanocomposite films, which demonstrated a well-defined zone of inhibition against *Staphylococcus aureus* and *Escherichia coli*.

Using an ultrasonic and sulfuric acid hydrolysis combination technique, nanocellulose was isolated from sugarcane bagasse. Investigations were made into the mechanical properties of adding nanocellulose to chitosan. The morphology of chitosan, chitosan-nanocellulose biocomposites, and nanocellulose was characterized using scanning electron microscopy (SEM). The formation of biocomposites was successfully demonstrated by X-Ray Diffraction and Fourier-transformed infrared spectroscopy data. Particles of nanocellulose had an average size of 132.67 nm. Compared to other biocomposite ratios, nanocellulose-chitosan biocomposites with a ratio of 10:2 exhibit the strongest antibacterial activity against *Escherichia coli* (Caschera et al., 2020)

In Nepal, the bark of the Argeli plant is heavily used for manufacturing Nepali Kagaj (Paper) but the estimation of cellulose is not reported yet. Manufacturing of Nepali paper with traditional method can be found however study for quality enhancement has not been observed. No study has been reported yet about the paper with Antimicrobial effect.

CHAPTER 3

MATERIALS AND METHOD

3.1. Chemicals

All the required chemicals were used in the laboratory under the Department of Chemistry, Amrit Campus, Tribhuvan University. Chemicals like sodium hydroxide pellets (Emplura, 97%), glacial acetic acid (Qualigens,1.049g/mL, 99.5%), nitric acid (Qualigens, 1.42 g/mL,69-71%), sulfuric acid (Fisher Scientific,1.8gmL, 97%), ethanol (changshu hongsheng fine chemicals, 99.9 %), benzene (Emplura, 0.88g/mL, 99%), Trisodium citrate (British Drug Houses, 99-100%), and Silver Nitrate (Qualigens, 99.8%) were used in as received condition without further purification.

3.2. Instruments

Mechanical grinder (Herbal Medicine Disintegrator, FW117), digital weighing balance (Phoiex, PH2202), magnetic stirrer, centrifuge (CENTURION Centrifuge model 1020 series), UV-visible spectrophotometer (labtronics LT-2802), FTIR spectrometer (PerkinElmer Spectrum IR Version 10.6.2), sonicator bath (MRC DC-150H), X-ray diffraction (Rigaku SmartLab, 9 kW rotating anode) were used during this work.

3.3. Collection of plant materials

The bark of *Edgeworthia gardneri* was collected in Phaktep, Panchthar district (Latitude: 27.1096° N, Longitude: 87.8157° E), Nepal. Sample collection site is shown in Figure 3.1.

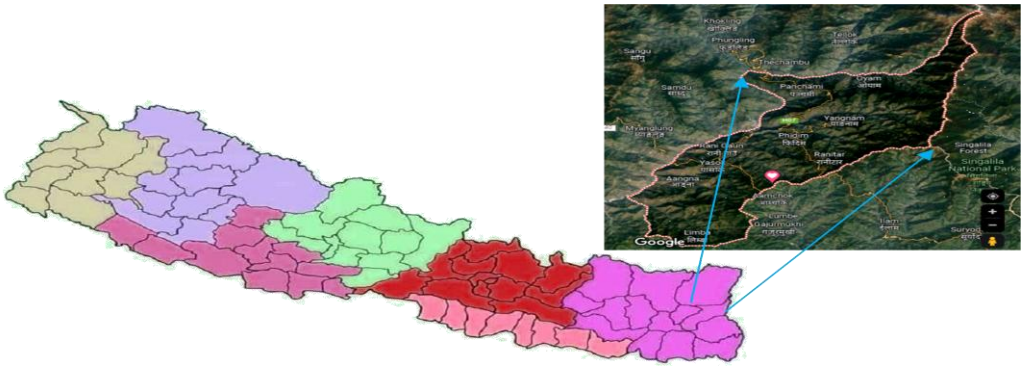

Figure 3.1:- Google map of the sample collection area.

3.4. Processing of plant materials

The collected bark were cut into small pieces, washed with distilled water, and dried in the shade. The dried sample was ground and converted into Fibre by using an herbal grinder machine. Then the fiber was placed in a dry place in a plastic container.

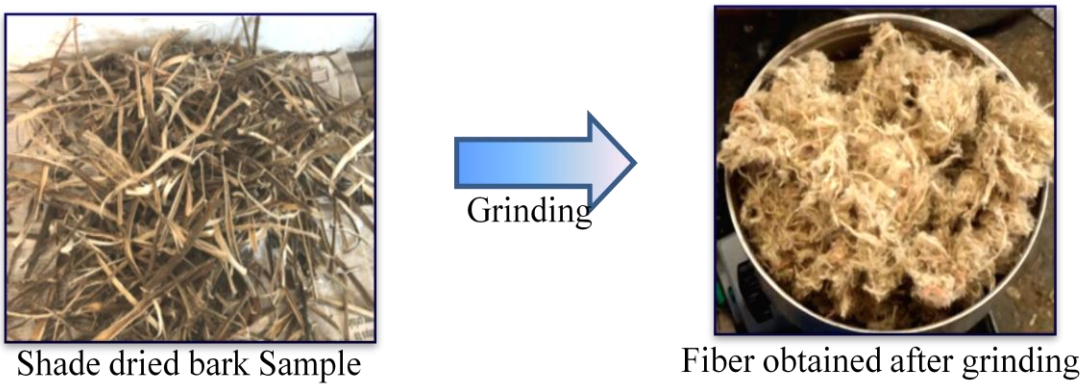

Figure 3.2:- Schematic conversion of bark into fiber via grinding machine.

3.5. Preparation of cellulose nanocrystals

3.5.1. Preparation of Sodium Hydroxide (NaOH) Solution

Sodium hydroxide solutions of 2 wt% and 7.5 wt% concentration were used in the experiment. 2 wt% NaOH solution was prepared by dissolving 2 g of the compound in 100 mL of distilled water in a volumetric flask. Similarly, 7.5 g NaOH pellets were used for the preparation of 7.5% wt NaOH in 100 mL of distilled water.

3.5.2. Preparation of Solution 32 wt% sulphuric acid Solution

32.65 mL of sulphuric acid was dissolved in 100 mL of distilled water in a volumetric

flask for the preparation of 32 wt% sulphuric acid.

3.5.3. Isolation of cellulose nanocrystals

The following processes were taken to extract cellulose nanocrystals: extractives, pre-alkalization, alkalization, acetylation, and acid hydrolysis (Mondragon et al., 2014). Fibers (5 g in each of two beakers) were treated with a benzene/ethanol (2:1) solvent mixture for 24 hours at room temperature to remove extractives (waxes and oils). After filtration, the residue was treated with 2 wt% NaOH solution in a round bottom flask for 12 hours at 41 °C. Alkali treatment was done by refluxing the fibers with 7.5 wt% NaOH in a round bottom flask for 90 min to remove hemicelluloses and lignin. After this step, the fibers obtained from the alkali treatment were washed with distilled water to neutralize them. The obtained fibers were again refluxed in a round bottom flask with acetic and nitric acid (6:1 v/v) placing 25 mL in each sample for 30 minutes, here after distilled water was used to wash these fibers until pH 6. Acetylated nanofibres were treated with 32 % sulphuric acid at 45 °C for 35 minutes under continuous stirring. Then, the reaction was stopped by adding distilled water. The surplus of sulfuric acid was separated by centrifugation process at the speed of 3000 RPM for 45 minutes followed by washing until pH becomes neutral. After completion of this step, the turbid was dried in a hot air oven at 40-50 °C for complete removal of moisture and was stored in a vial.

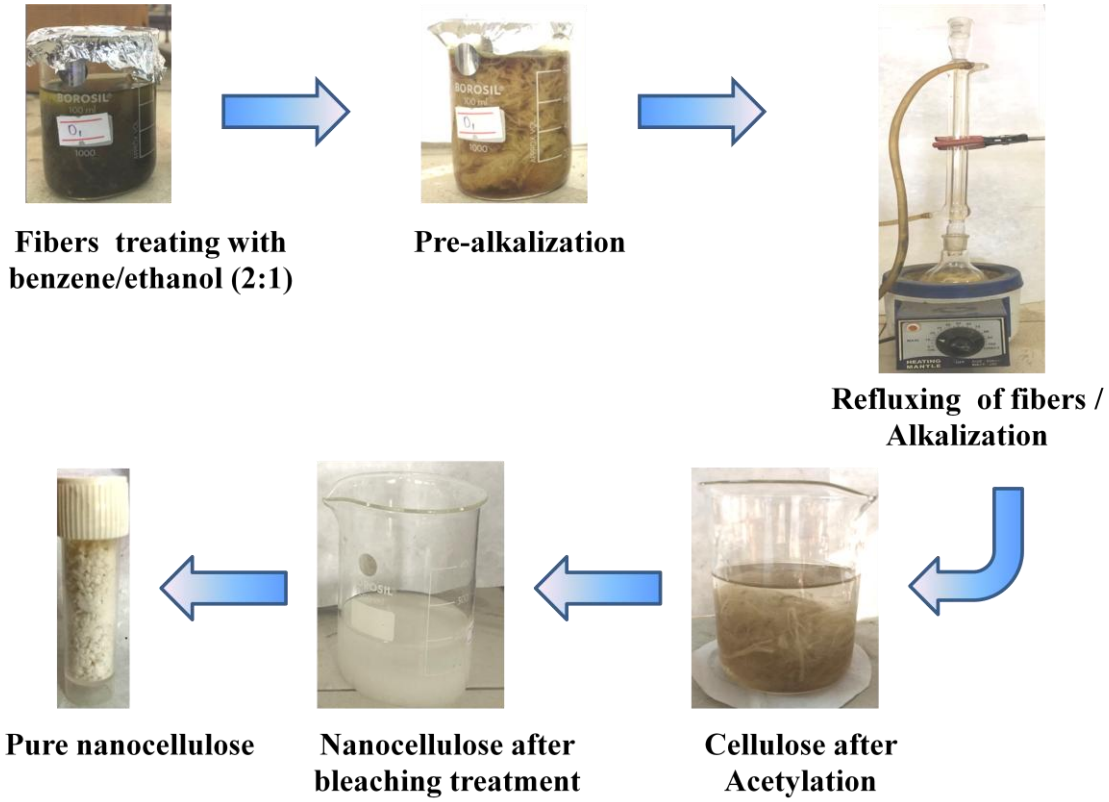

Figure 3.3:- Extraction of nanocellulose (process detail).

3.6. Synthesis of Silver Nanoparticles

3.6.1. Preparation of Silver Nitrate Solution

A 0.1 M $AgNO_3$ solution was produced by dissolving 1.69 g of the compound in 100 mL of distilled water. The solution was shielded from sunlight by wrapping the volumetric flasks in carbon paper and storing them in a refrigerator.

3.6.2. Preparation of 0.1 M Trisodium Citrate Solution

2.58 g of the trisodium citrate was dissolved in 100 ml of distilled water in a volumetric flask to produce 0.1 M of trisodium citrate.

3.6.3. Preparation of Extract

About 3 g of finely powdered bark of *Edgeworthia gardneri* was boiled in 50 mL distilled water at 60 °C for 10 minutes in a beaker. After cooling, it was filtered through Whatman No. 1 filter paper. The light-brown filtrate was kept in the refrigerator at 4 to 6 °C for later use.

3.6.4. Green Synthesis of Silver Nanoparticles

First, 10 mL of extract was taken in a 250 mL beaker and 10 mL of 0.1 M $AgNO_3$ was added from the burette by continually stirring in a magnetic stirrer at room temperature. The reddish-brown suspension was gradually produced. The alteration in color demonstrated the emergence of silver nanoparticles. The acquired suspension was centrifuged at 800 rpm for 10 minutes, the supernatant liquid was decanted off, and the residue was washed with ethanol followed by centrifugation and decantation. This process was repeated three times to remove any impurities. After drying the precipitation, Green synthesized silver nanoparticles (G-AgNPs) were collected and placed in a vial.

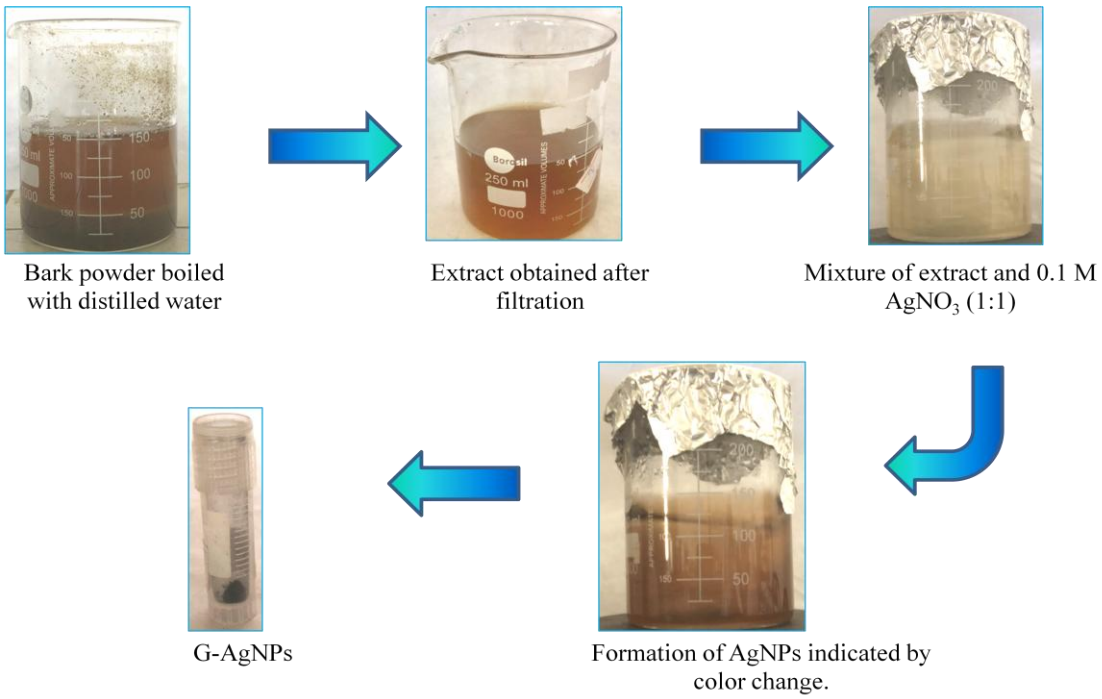

Figure 3.4:-Synthesis of G-AgNPs by a green method.

3.6.5. Chemical Synthesis of Silver Nanoparticles

10 mL of 0.1 M $AgNO_3$ was dropped into 10 mL of 0.1 M trisodium citrate solution in a beaker while being magnetically stirred. The resulting mixture was left in the sun for 4 hours for the development of nanoparticles. The obtained nanoparticles were washed with water and ethanol to remove impurities using a centrifuge. Thus obtained

chemically synthesized silver nanoparticles (C-AgNPs) were allowed to dry at room temperature and were stored in a vial.

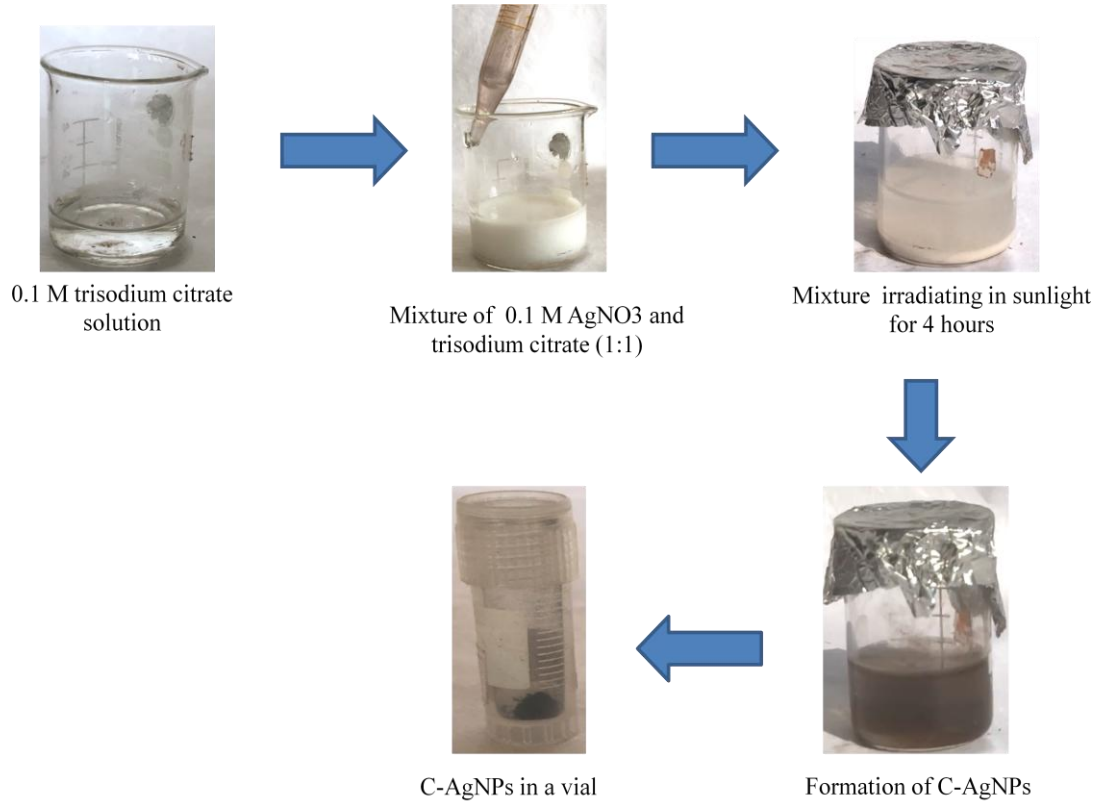

Figure 3.5:- Synthesis of C-AgNPs by chemical method.

3.7. Incorporation of Silver Nanoparticles into Nanocellulose

3.7.1. Ex-situ incorporation of Silver Nanoparticles into Nanocellulose

First, cellulose nanocrystals and G-AgNPs were dispersed by sonicating 0.5 g of nanocellulose in 20 mL of distilled water in a beaker and 0.2 g G-AgNPs in 50 mL of distilled water for 30 minutes separately. The two solutions were then combined and swirled magnetically for 45 minutes. After that, the material was left for 24 hours, which caused the development of dark brown nanoparticle incorporated nanocellulose. It was dried and collected in a vial and labeled as G-Ag@CNC-1. A similar process was carried out to incorporate the chemically synthesized silver nanoparticles into cellulose nanocrystals. The obtained nanoparticle incorporated nanocellulose was labeled as C-Ag@CNC-1.

3.7.2. Insitu generation Silver nanoparticles into Nanocellulose

First, 0.5 g of nanocellulose was sonicated for 30 minutes in 20 mL of distilled water to disperse the cellulose nanocrystals. Then, 10 mL of 0.1 M $AgNO_3$ was added to it, and the combined mixture was continuously stirred at room temperature for an hour. A burette was then used to add 10 mL of 0.1 M trisodium citrate while continuously stirring. Silver nanoparticles incorporated cellulose nanocrystals were formed after the solution was exposed to sunlight for four hours. It was dried at 40-50 °C in an oven until the removal of moisture and was collected in a vial. The obtained nanoparticle cellulose nanocrystal was labeled as C-Ag@CNC-2.

3.8. Antimicrobial test

3.8.1. Media preparation

3.8.1.1. Preparation of Muller Hinton broth

21 g of Muller Hinton broth was dissolved in 1 L of distilled water in a conical flask and was sterilized by autoclaving at 121 °C for 15 minutes.

3.8.1.2. Preparation of nutrient broth

13 g of nutrient broth was thoroughly dissolved in 1 L of distilled water after being mildly boiled. It was then autoclaved for 15 minutes at 121 °C to sterilize it.

3.8.2. Microbial culture preparation

With the aid of a sterilized inoculating loop, a tiny quantity of the Gram-negative bacteria *Escherichia coli* ATCC 8739, the Gram-positive bacteria *Bacillus subtilis* ATCC 6051, and the fungus *Candida albicans* ATCC 2091 was removed from a pure respective plate. These microbes were then dissolved in a tube containing 10 mL of nutritional broth. The tube was then incubated for 24 hours at 36 degrees in an incubator.

3.8.3. Inoculation of the culture plate

Three Petri disc was covered with a thin coating of hot Muller Hinton broth solution, which was then allowed to cool inside a biosafety cabinet. With the aid of a marker

and scale, various portions were then created and labeled. Then, using a cotton swab stick that had been sanitized, the cultured bacteria as well as fungus were distributed inside each Petri disc. The injected plate was covered after inoculation.

3.8.4. Sample Preparation, placement, and Incubation

50 µg of the reference antibiotic *kanamycin* and 10 µg of the samples were added to specific areas of the cultivated plate. After that, these plates were incubated for 24 hours at 37 °C inside the incubator. The zone of inhibition was measured and compared after 24 hours of incubation. The test samples used for the antimicrobial were G-AgNPs, C-AgNPs, G-Ag@CNC-1, C-Ag@CNC-1, and C-Ag@CNC-2, respectively.

3.9. Characterization techniques

Characterization of nanocellulose chemically modified nanocellulose and synthesized silver nanoparticles were done by various techniques.

3.9.1. Visual Observation (Color Change Test)

The initial visual verification of the chemically and environmentally friendly synthesized silver nanoparticles was done. The alteration in color indicated the existence of nanoparticles. When exposed to the plant extract (green method) and sunlight (chemical method), silver ions were converted into silver particles, which is followed by a color shift.

3.9.2. UV-Vis Spectroscopy

The generation of Ag NPs was confirmed using UV-vis Spectroscopy analysis to identify the absorption band. Using, UV-Visible spectroscopy, labtronics LT-2802 double beam ultraviolet-visible spectrometer reduction of pure Ag^+ ions was observed. The samples were scanned between 200 - 800 nm. The baseline correction of the spectrophotometer for the experiment was performed using ethanol as a blank solution. A quartz cuvette was used to load all of the samples for UV measurement.

3.9.3. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectrophotometer is an effective tool for identifying the type of bonding, bond

conjugate system, aromatic and aliphatic structures, and other features of compounds. FTIR spectrum shows the presence of various functional groups in the sample as well as the molecular structure and conformation of organic compounds. Using a PerkinElmer Spectrum IR Version 10.6.2 FTIR spectrometer, scanning was carried out in the wavelength range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} in Attenuated Total Reflectance (ATR) mode.

3.9.4. X-Ray Diffraction (XRD)

X-ray diffraction has mostly been used to characterize the fingerprint of crystalline materials and identify their structural characteristics. The distinctive X-ray diffraction pattern of each crystalline solid can be used as a "fingerprint" to identify it. Once the material has been identified, X-ray crystallography may be used to ascertain its structure, including the interatomic distance and angle, how closely the atoms are packed together in a crystalline state, etc. In this case, nanocellulose, nanocellulose incorporated with Ag NPs, and AgNPs powder's crystal structure was revealed by X-ray diffraction. The working conditions were typically 2θ scanning between 5 to 90^{o}.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Visual characterization

The visual characterizations for extracted nanocellulose and Silver nanoparticles synthesized by green and chemical methods were performed. A white gelatinous solution of the nanocellulose was extracted which ondrying became white powder as shown in Figure 6. The formation of nanoparticles in the green synthesis method was ascertained by the evolution of the dark brown color of the mixture (extract and silver nitrate solution) after a certain time as in Figure 7. Similarly, the dark black coloration of a mixture of trisodium citrate and silver nitrate solution after exposure to sunlight indicated the formation of nanoparticles as in Figure 8.

4.2. Quantitative analysis

The extraction of nanocellulose from the bark of the *Edgeworthia gardneri* plant was carried out taking two samples. Then the extracted nanocellulose weight was measured by using a digit weighing balance. Quantitative measurement reflected 34.3% of cellulose in the bark of *Edgeworthia gardneri* plant.

Table 1:- Quantitative analysis of extracted nanocellulose

S.N.	**Plant name**	**Fiber Weight (g)**	**Fiber Mean Weight (g)**	**Nanocellulose Weight (g)**	**Nanocellulose Mean Weight (g)**	**Percentage Of Nanocellulose (%)**
1.	*Edgeworthia gardneri*	5.005	**5.004**	1.693	**1.717**	**34.30**
2.		5.003		1.740		

4.3. UV-Visible spectroscopic analysis

The spectroscopic characterization of prepared CNC, CAgNPS, GAgNPS, CAg@CNC-1, GAg@CNC-1 and CAg@CNC-2 were performed using a labtronics, LT-2802 double beam ultraviolet-visible (UV-Vis) spectrometer at the department of chemistry, Amrit Campus, Kathmandu, Nepal. The result showed that maximum absorbance for C-AgNPs, G-AgNPs, C-Ag@CNC-1, G-Ag@CNC-1, and C-Ag@CNC-2 are 325 nm, 345 nm, 310 nm, 320 nm, and 315 nm, respectively.

Similarly, Alomar et al. also synthesized AgNPs using *pegamum harmala* leaf and observed maximum UV absorption at 350 nm indicating the formation of silver nanoparticles (Alomar et al., 2020). Shankar et al.(2003) also found maximum UV absorption of AgNPs at 370 nm suggesting the formation of AgNPs (Shankar et al., 2003).

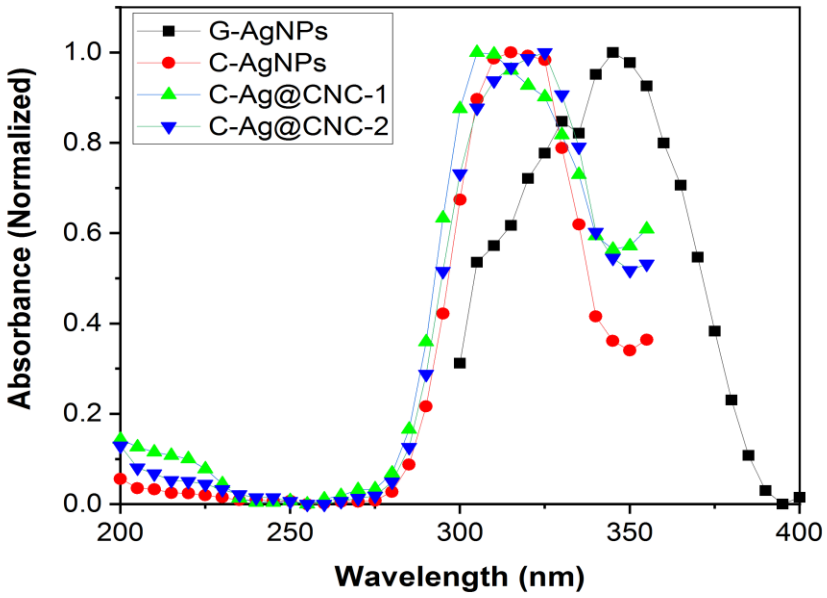

Figure 4.1:- UV–Visible absorption spectra of C-Ag@CNC-1, C-Ag@CNC-2, C-AgNPs, and G-AgNPs.

4.3. FTIR spectroscopy analysis

In FTIR spectra of CNC, The stretching vibrations of OH in cellulose are responsible for the peak at 3343 cm^{-1}(Ilyas et al., 2018; Saravanakumar et al., 2013). The peak at 2900 cm^{-1} corresponds to the C-H stretching vibration of alkyl groups in aliphatic bonds of cellulose (Saurabh et al., 2016). The peak at 1641 cm^{-1} corresponds to the O-H bending of water absorbed in the cellulose fiber structure (Łojewska et al., 2005). The absorption bands at 1436, 1360, 1158, 1107, and 1032 cm^{-1} are caused by stretching and bending vibrations of the CH_2, CH, OH, and CO bonds in cellulose (Ilyas et al., 2018). The band at approximately 1436 cm^{-1} indicates the amount of crystalline cellulose, whereas the band at 897 cm^{-1} indicates the amorphous region in cellulose (Hospodarova et al., 2018).

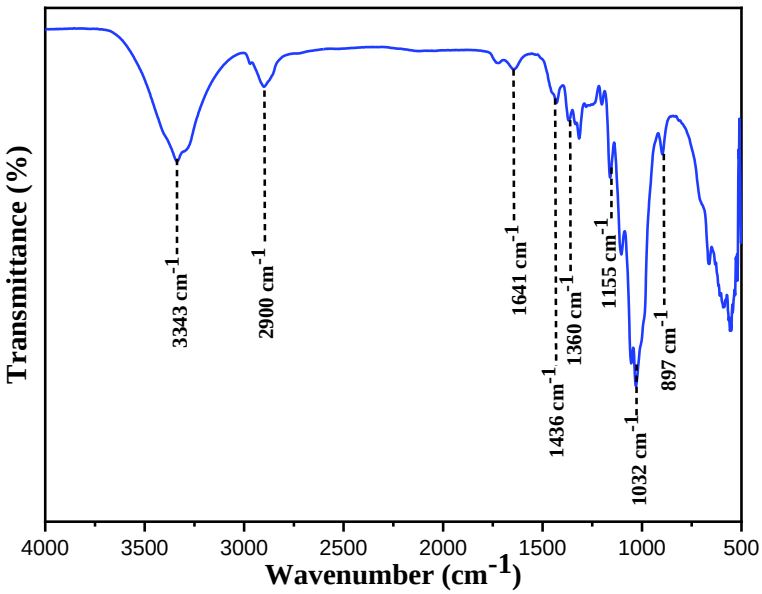

Figure 4.2:- FTIR spectra of CNC.

Similarly, FTIR spectra of G-AgNPs had a board band at 3343 cm^{-1} due to the O-H stretching vibration of the extract (Ren et al., 2019). A weak band at 2900 cm^{-1} was attributed to C-H stretching (Chahar et al., 2018) and a band at 1701 cm^{-1} represented the C=O group of carboxylic acids (Awwad & Salem, 2012). The peak at 1572 cm^{-1} to N-H deformation of the amide group (Lenormant & Blout, 1953). C-O stretching was responsible for the peak at 1284 cm^{-1} and a wide band of 1022 cm^{-1} was attributed to

aromatic ethers and polysaccharides (C-O-C stretch) phenolic groups (Szymczycha-Madeja et al., 2013).

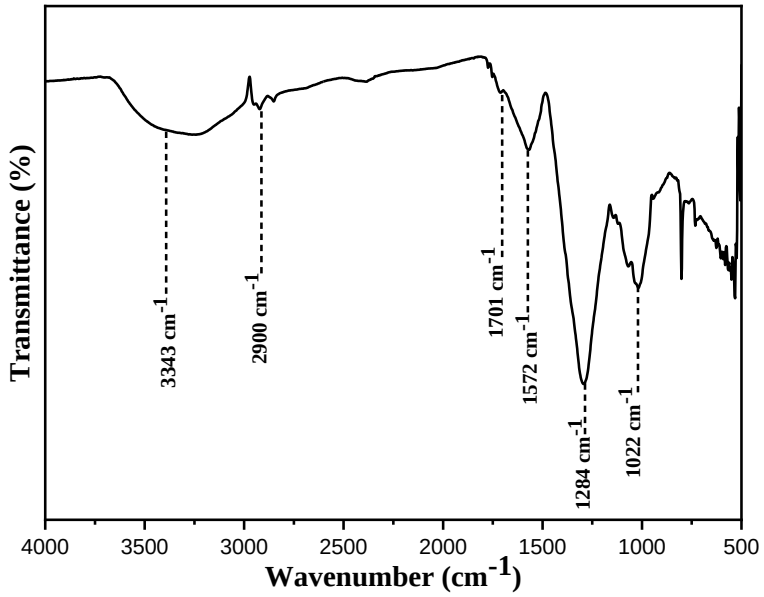

Figure 4.3:- FTIR spectra of G-AgNPs.

In the spectra of GAg@CNC-1, It was found that all the functional groups of CNC-doped silver nanoparticles are similar to CNC with a shift in the position of the respective functional group due to silver nanoparticles as shown in Figure 4.4.

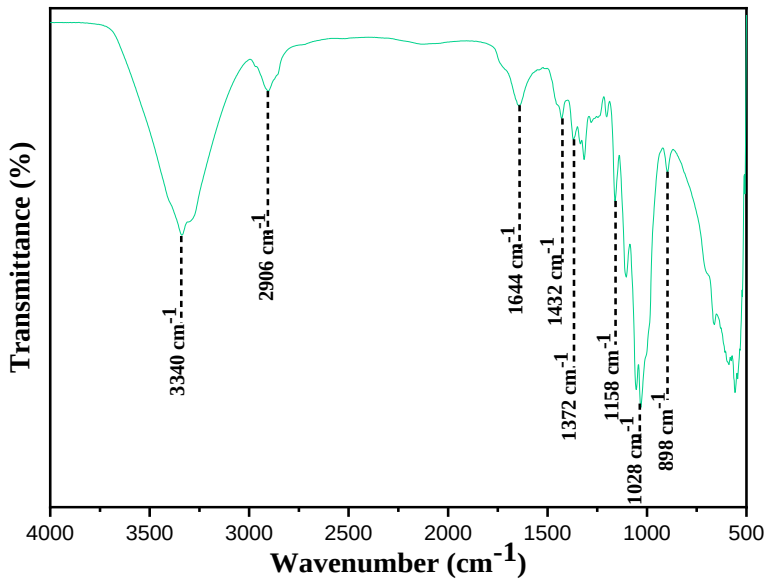

Figure 4.4:- FTIR spectra of G-Ag@CNC-1.

In the case of C-Ag@CNC-1 there were no stretching vibrations of O-H and C-H stretching vibration at 3343 cm^{-1} and 2900 cm^{-1}. This indicated that the O-H Stretching and C-H stretching of cellulose was completely blocked by silver nanoparticles. Furthermore, functional groups such as N-H deforming, CH_2, CH, OH, and CO bond in cellulose are also shifted due to silver nanoparticles incorporation in nanocellulose.

Additionally, it was discovered that there were no notable bands of vibration for any functional group while examining the spectra of C-Ag@CNC-2, showing a strong association between nanocellulose and silver nanoparticles.

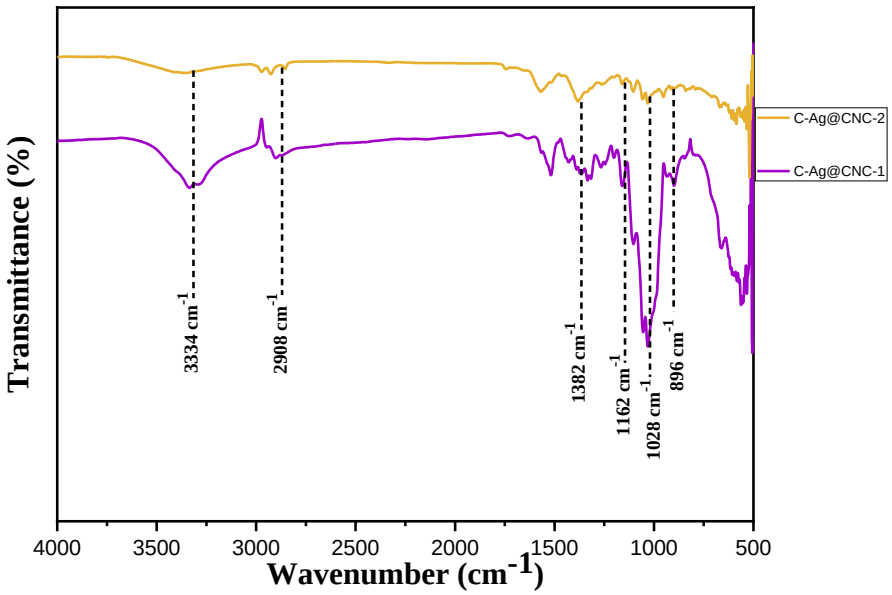

Figure 4.5:- FTIR spectra of C-Ag@CNC-1 and C-Ag@CNC-2.

In conclusion, it can be said that the in-situ synthesis of silver nanoparticles in nanocellulose demonstrated a higher contact between silver nanoparticles and nanocellulose than the ex-situ generation of silver nanoparticles in nanocellulose.

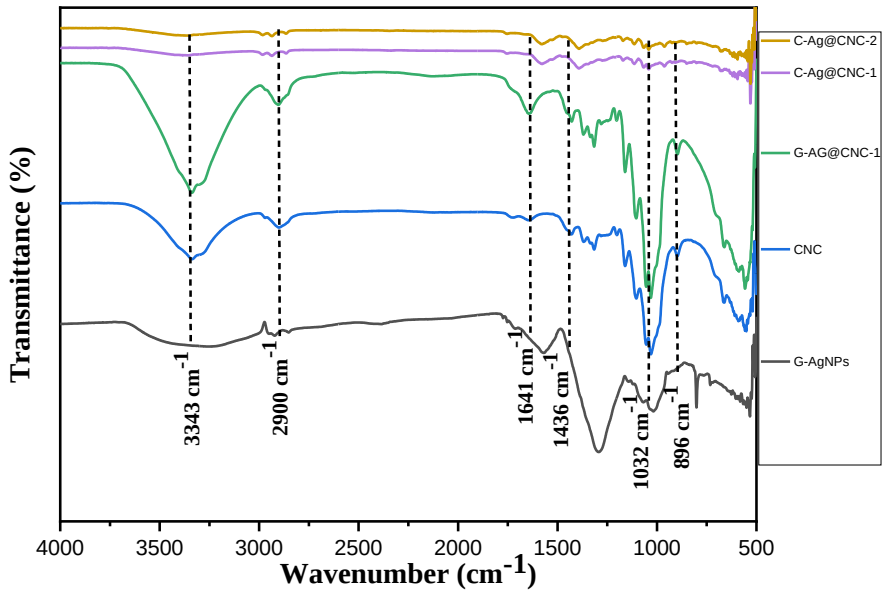

Figure 4.6:- FTIR spectra of G-AgNPs, CNC, G-Ag@CNC-1, C-Ag@CNC-1 and C-Ag@CNC-2.

4.4. X-Ray Diffraction (XRD) spectroscopic analysis

XRD patterns of C-AgNPs, G-AgNPs, CNC, G-Ag@CNC-1, C-Ag@CNC-1, and C-Ag@CNC-2 are shown in Figure 11. The XRD pattern of CNC exhibits three diffraction peaks at 2Θ values of 16.2°, 22.5°, and 34.5°, respectively. These fixed peaks correspond to the crystallographic planes of (101), (200), (040) , respectively, which are in accordance with the structural type of cellulose *Iβ* (Dai, 2012).

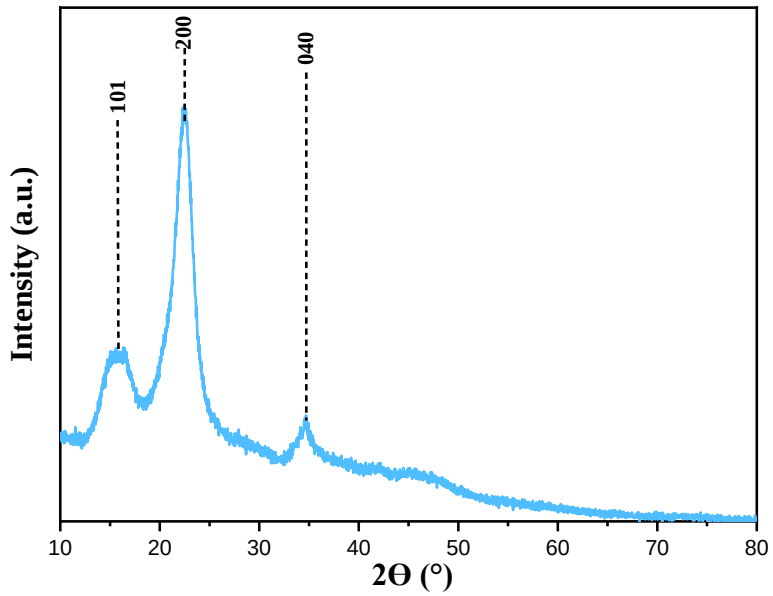

Figure 4.7:- XRD spectra of CNC.

C-AgNPs displayed four diffraction peaks at 37.8°, 44.2°, 66.5°, and 77.5°, respectively, and these peaks corresponded to the (111), (200), (220), and (311) planes of silver with cubic structure that were reported by the International Centre for Diffraction Data (JCPDS data number 04-0783 card).

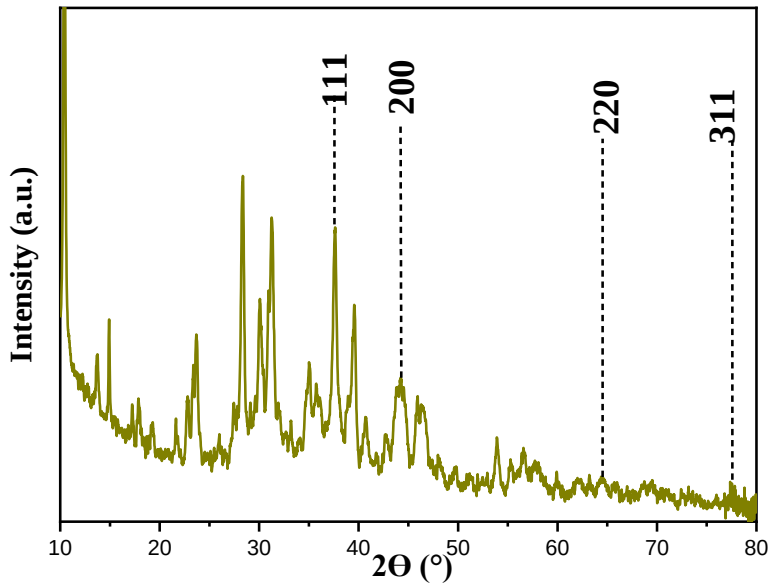

Figure 4.8:- XRD spectra of C-AgNPs.

Similarly, G-AgNPs also displayed four diffraction peaks at 38.2°, 43.7°, 64.4° and 77.4° that were associated with (111), (200), (220), and (311) crystal planes of silver with cubic structure and were represented by JCPDS card number 89-3722. The additional peaks might be due to the phytochemicals (Jeeva et al., 2014; Kumar & Yadav, 2011)

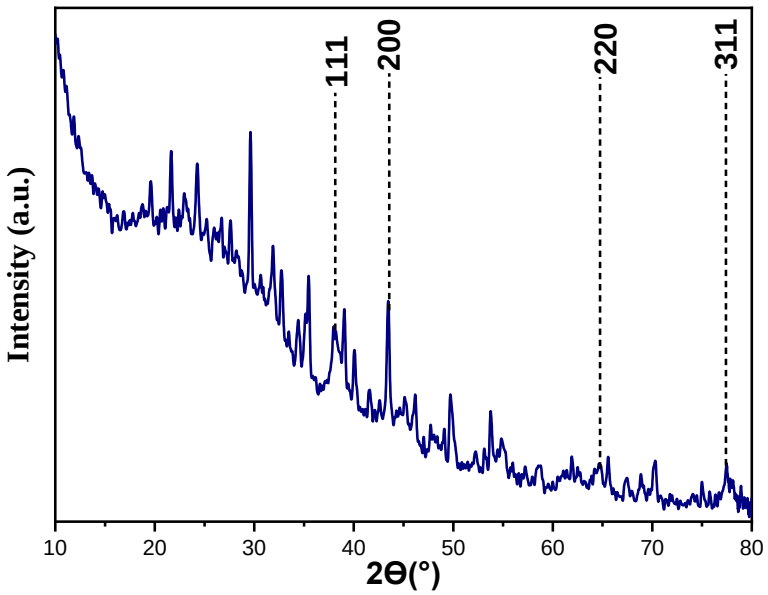

Figure 4.9:- XRD spectra of G-AgNPs.

While examining the XRD spectra of G-Ag@CNC-1, It was found that all significant peaks of nanocellulose and green synthesized silver nanoparticles were identified, confirming the formation of silver nanocellulose composite.

Furthermore, C-Ag@CNC-1 and C-Ag@CNC-2 also had all the major peaks of nanocellulose and chemically synthesized silver nanoparticles, suggesting the formation of silver nanocellulose composite.

Also, while comparing the silver nanocellulose composite G-Ag@CNC-1 and C-Ag@CNC-1 and C-Ag@CNC-2, It was found that green synthesized nanoparticles showed no effect on the significant peaks of nanocellulose whereas chemically

synthesized silver nanoparticles showed a shift on the position of nanocellulose. This might be due to the orientation of silver nanoparticles.

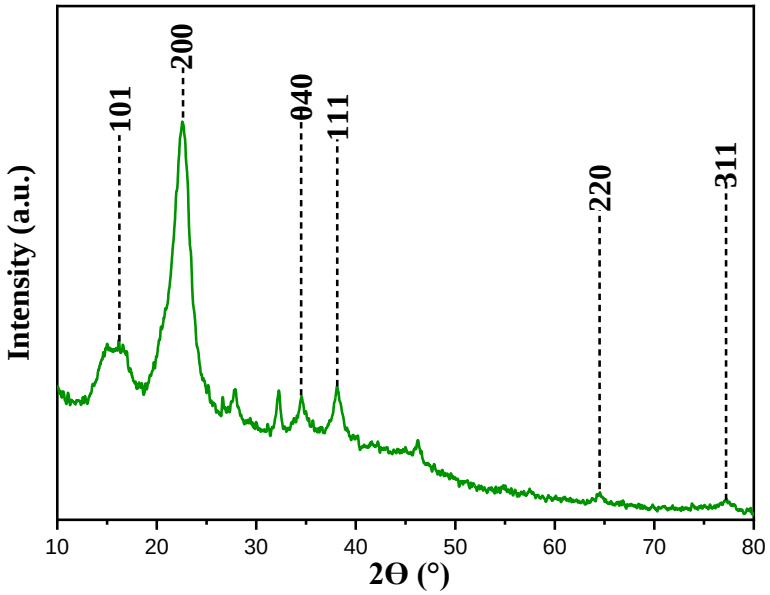

Figure 4.10:- XRD spectra of G-Ag@CNC-1.

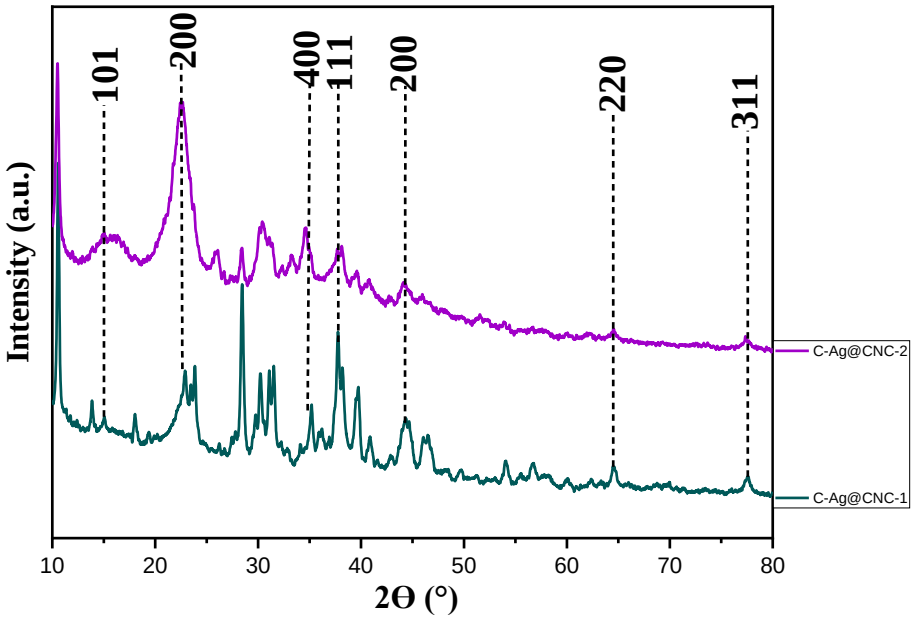

Figure 4.11:- XRD spectra of C-Ag@CNC-1 and C-Ag@CNC-2.

Similar XRD patterns were found for silver nanocellulose composite, which had cellulose peaks at 15.53°, 22.30°, and 34.71°, which corresponded to the (101), (200),

and (400) lattice planes of cellulose *Iβ*, and face-centered cubic (FCC) structure of Ag NPs crystal planes at 38.22°, 44.77°, 64.43°, and 77.44°, which was identified by the JCPDS card number 65-2871 (Toro et al., 2021). Similarly, Zhang et al also noted characteristics of XRD peaks at 15.0°, 22.6° and 33.5° corresponding to (101), (200), and (400) lattice planes of cellulose *Iβ*, and at 38.2°, 43.7°, 64.4° and 77.4 corresponding to (111), (200), (220) and (311) crystal planes of silver in nanocellulose doped silver nanoparticles (M. Zhang et al., 2023).

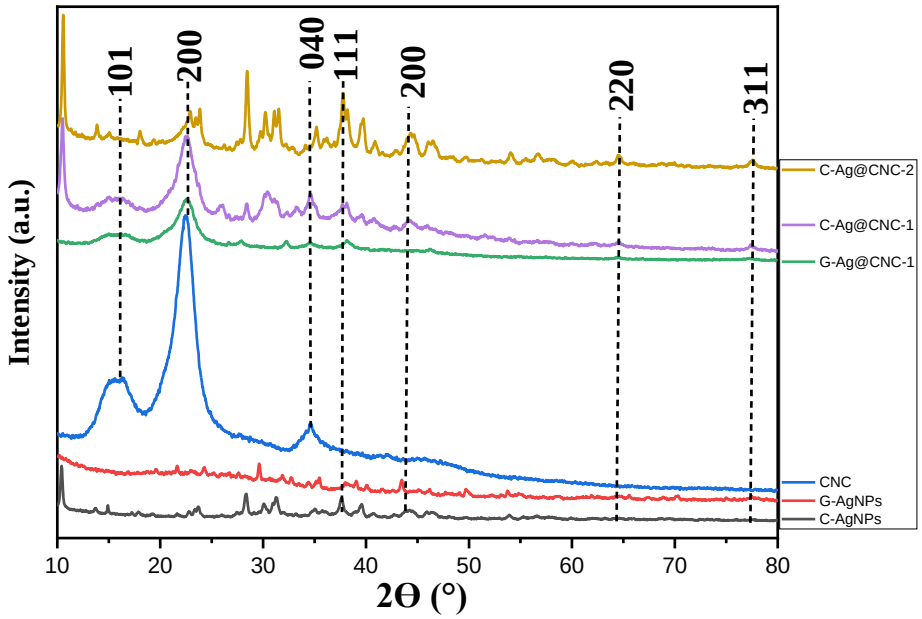

Figure 4.12:- XRD patterns of C-AgNPs, G-AgNPs, CNC, G-Ag@CNC-1, C-Ag@CNC-1 and C-Ag@CNC-2.

4.5. Antimicrobial analysis

Antimicrobial analysis for the chemically modified nanocellulose incorporated with silver nanoparticles and synthesized silver nanoparticles was done by disc diffusion method. The test was performed for three strains: - Gram-negative bacteria *Escherichia coli* (ATCC 8739), Gram-positive bacteria *Bacillus subtilis* (ATCC 6051), and fungus *Candida albicans* (ATCC 2091). The antibiotic *Kanamycin* (c+) is used as the standard reference.

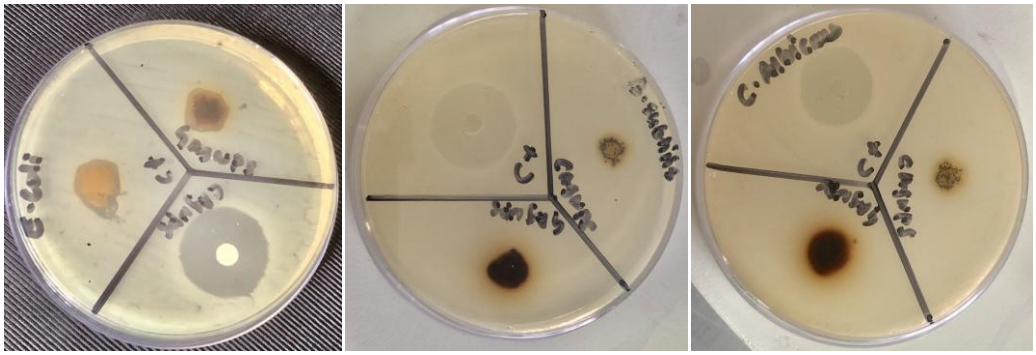

Figure 4.13:-Antimicrobial activity of G-Ag@NPs and C-AgNPs.

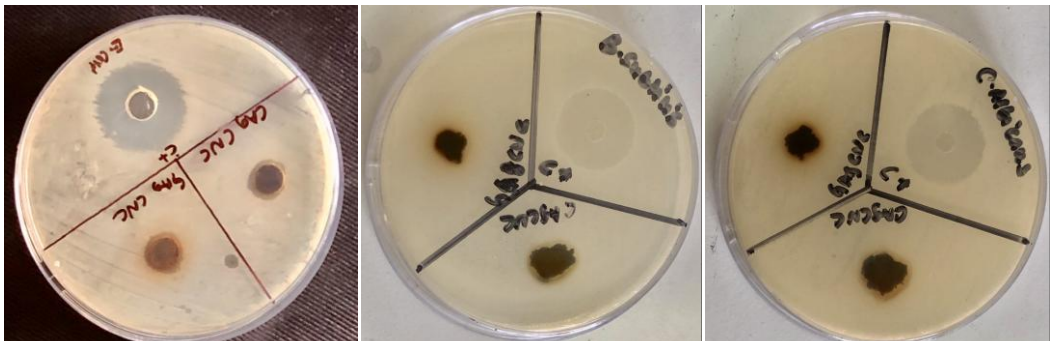

Figure 4.14:- Antimicrobial activity of C-Ag@CNC-1 and G-**A**g@CNC-1.

Figure 4.15:- Antimicrobial activity of CAg@CNC-2 (on the right side).

Table 2:- Antimicrobial analysis of samples.

Sample	**Bacterial strain**	**ATCC code**	**Zone of inhibition (ZOI)**	
			Standard (cm)	**Sample (cm)**
C-AgNPs	*Escherichia coli* (Gram-ve)	ATCC 8739	1	0.85
	Bacillus subtilis (Gram+ve)	ATCC 6051	1	0.65
	Candida albicans (human pathogenic fungi)	ATCC 2091	1	0.7
G-AgNPs	*Escherichia coli* (Gram-ve)	ATCC 8739	1	0.8
	Bacillus subtilis (Gram+ve)	ATCC 6051	1	0.5
	Candida albicans (human pathogenic fungi)	ATCC 2091	1	0.6
G-Ag@CNC-1	*Escherichia coli* (Gram-ve)	ATCC 8739	1	0.65
	Bacillus subtilis (Gram+ve)	ATCC 6051	1	0.5
	Candida albicans (human pathogenic fungi)	ATCC 2091	1	0.6
C-Ag@CNC-1	*Escherichia coli* (Gram-ve)	ATCC 8739	1	0.85
	Bacillus subtilis (Gram+ve)	ATCC 6051	1	0.65
	Candida albicans (human pathogenic fungi)	ATCC 2091	1	0.7
C-Ag@CNC-2	*Escherichia coli* (Gram-ve)	ATCC 8739	1	0.8
	Bacillus subtilis (Gram+ve)	ATCC 6051	1	0.75

	Candida albicans (human pathogenic fungi)	ATCC 2091	1	0.65

The result showed that all the samples showed significant antimicrobial activity against all the pathogens. Chemically synthesized nanoparticles and their incorporation with CNC had a greater antimicrobial effect than green synthesized nanoparticles.

Similarly, Nguyen et al. also noticed an excellent antibacterial activity of nanocellulose incorporation with silver nanoparticles against *Escherichia coli and Staphylococcus aureus* respectively (Nguyen et al., 2016). When Zhang et al. doped nanocellulose with silver nanoparticles; they observed a similar antimicrobial activity that demonstrated excellent results against *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*. Additionally, it had better antibacterial effects against *Escherichia coli* than against *Staphylococcus aureus* and *Candida albicans* (X. Zhang et al., 2019).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this study, nanocellulose was successfully extracted from the bark of the *Edgeworthia gardneri* and found to be 34.30%. Modification of thus obtained nanocellulose was done by incorporation with chemically and green synthesized silver nanoparticles. Characterization of nanocellulose and nanocellulose incorporated with silver nanoparticles was done by using UV- Visible, FTIR, and XRD spectroscopic techniques. The UV spectrum of GAg@CNC-1, CAg@CNC-1and CAg@CNC-2 showed maximum absorbance around 305-320 nm. XRD data showed that all the samples were in the nano range.

Furthermore, antimicrobial analysis of nanocellulose incorporated with silver nanoparticles was done by disc diffusion method. The results showed that all the samples showed excellent antimicrobial activity against *Escherichia coli, Bacillus subtilis,* and *Candida albicans*; measured with reference to standard *Kanamycin* antibiotics.

Thus, from this work, it can be concluded that the extraction of nanocellulose crystals can be successfully using the acid hydrolysis method and Silver nanoparticles can be successfully synthesized from green and chemical methods. Modification of CNC using silver nanoparticles can be achieved with excellent antimicrobial activity. This work provides a pathway in the field of natural polymer with numerous applications.

5.2. Limitations of Work

Some of the limitations of this work due to the inaccessibility of devices are as follows:-

- Sulfate ions can be more easily separated from nanocellulose using a high-speed centrifuge.
- Zeta potential can be used to investigate nanocellulose dispersion stability in aqueous media.
- Thermo gravimetric analysis can be used to examine the thermal stability of

nanocellulose.

- Transmission electron microscopy can be used to investigate surface characterization.

5.3. Recommendations

Some of the recommendations of this work are as follows:-

- To gain further insight, additional characterization techniques like FESEM and TEM can be used.
- Cytotoxicity of nanocellulose-doped silver nanoparticles can be studied.
- Anticancer, anti-diabetic, anti-inflammatory, and antiseptic activity can be studied.
- Electrical and optical properties can be studied for biosensor invention.
- Various parameters such as temperature, concentration, pH, etc. can be studied for nanocellulose.

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