Preparation and characterisation of polycaprolactone/nanocellulose extracted from *Eucomis autumnalis* for bone tissue engineering

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BLOEMFONTEIN

September 2023

DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO INDEPENDENT WORK

I, Dolly Selikane, identity number _____, and student number, _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Master of Health Science in Environment, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself of any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification

30 September 2023

SIGNATURE OF STUDENT **DATE**

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Abstract

This study involved the preparation and characterization of polycaprolactone (PCL)/*Eucomis autumnalis* (EA) nanocellulose through melt blending. The cellulose was extracted from the plant material through a delignification process followed by isolation and treatment. The prepared nanocellulose was blended with PCL at a weight ratio of 97/3, and the resulting composite was characterized for its thermal and mechanical properties. The Fourier-transform infrared (FTIR) spectroscopy results affirmed the successful composite fabrication, highlighting the absence of chemical reactions during melt-compounding. Scanning electron microscopy (SEM) unveiled distinct morphologies, with PCL forming a continuous phase and EA cellulose exhibiting a fibrous network. Despite successful embedding of EA cellulose fibers in the composite, fractured surfaces indicated poor interfacial interaction, potentially leading to fiber pull out. Thermogravimetric analysis (TGA) revealed enhanced thermal stability in the composites, while differential scanning calorimetry (DSC) indicated minimal impact on PCL melting behavior. X-ray diffraction analysis (XRD) further demonstrated enhanced crystallinity in the composites, highlighting increased order in PCL crystals. Mechanical testing revealed a modest increase in stiffness attributed to the rigid cellulose fibers. However, a decrease in yield strength, tensile strength of -31,62%, and elongation at break suggested reduced ductility and inferior mechanical properties, consistent with poor interfacial adhesion observed in SEM. However, the Young's modulus was improved by 2.63% (from 380 to 390 MPa). Overall, this study contributes valuable insights into the structural, thermal, and mechanical characteristics of PCL/EA cellulose composites, offering a foundation for potential applications in biomedical and packaging materials. The findings of this study suggest that the nanocellulose from EA plant has the potential to improve the mechanical properties of PCL. The outcomes of this research hold immense potential for advancing the field of bone tissue engineering, offering a novel biomaterial with enhanced mechanical strength, and eco-friendly characteristics.

Table of Contents

List of symbols and abbreviations

AFM	Atomic Force Microscopy
CMC	Carboxymethyl cellulose
CNC	Cellulose nanocrystals
CNFs	Cellulose nanofibrils
CNWs	Cellulose nanowhiskers
CO	Cotton
DTG	Derivative thermogravimetric
DSC	Differential Scanning Calorimetry
DMSO	Dimethylsulfoxide
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier-Transform Infrared spectroscopy
HJS	*Humulus Japonicus*
HCE	Hydrolyzed-cellulose
MFC	Microfibrillated cellulose
PBS	Poly(butylene succinate)
PHA	Poly(hydroxyalkanoates)
PHB	Poly(hydroxybutyrate)
PHBV	Poly(hydroxybutyrate-co-hydroxyvalerate)
PPC	Poly(propylene carbonate)
PVA	Poly(vinyl alcohol)
PCL	Poly(ε-caprolactone)
PGA	Polyglycolic acid
PLA	Polylactide acid
PVA	Polyvinyl alcohol
NR	Rubber
SCB	Sugarcane bagasse
SH-CNCs	Sulfuric acid-hydrolyzed CNCs
SO-CNCs	Surface-oxidized cellulose nanocrystals
TGA	Thermo-Gravimetric Analyzer

List of Figures

List of Tables

CHAPTER 1: Introduction

Part of chapters 1 and 2 has been published as a conference proceeding:

Dolly Grace-Ann Selikane[1], Thandi Patricia Gumede[1], Katekani Shingange[2] and Thembinkosi Donald Malevu[3]. A Brief Overview on the Extraction of Cellulose from Medicinal Plants for Advanced Applications. Materials Science Forum, ISSN: 1662-9752, Vol. 1059, pp 81-85. DOI:10.4028/p-9hut2u.*

1.1 Overview

For over a thousand years, cellulose has been known as a polysaccharide that is readily available in nature. It is branded as a main constituent of the cell wall. Since the cell wall is produced by all plants, it is probably the amplest organic compound on Earth. The extraction of cellulose from medicinal plants is becoming a topic of interest. This is because compounds extracted from medicinal plants including cellulose are used as additives in pharmaceutical, neutraceutical, toxicology, and other chemical industries, for treating syphilis, kidney disorders, wound healing, ulcers, skin rash, gonorrhoea, and piles [1,2,3,4]. Also, cellulose has been identified as useful for reinforcement and load-bearing purposes in composite materials due to its intrinsic stiffness and a high degree of crystallinity. However, the process of extracting cellulose, as well as the extent of the purity needed strongly depend on the application of the used polymers. This chapter focuses on the extraction of cellulose from medicinal plants as potential materials in the fabrication of polycaprolactone.

1.2 Background

Medicinal plants are plants that have extractable substances in their leaves, stems, roots, flowers, and fruits and are utilized in herbalism. These extracts are employed in a variety of industries, including pharmaceutical, nutraceutical, pesticide, and other chemical industries, among others, in their natural form or as additives. Medicinal plants are traditionally used for treating toothaches, syphilis, kidney disorders, wound

healing, ulcers, skin rash, gonorrhoea, piles, and others [1,2,3,4]. Furthermore, the usage of medicinal plants in industrialized cultures has shown trends in the production of various medicines, chemotherapeutics, as well as biotechnological uses, such as biosensors and drug delivery systems [5,6,7].

All plants contain three major components: cellulose, lignin, and hemicellulose [8]. Cellulose is the most abundant naturally occurring organic compound and a key component in the cell wall of trees and plants [9]. It is a complex carbohydrate, or polysaccharide, consisting of about 3 000 or more glucose units. Cellulose is an organic compound consisting of a linear chain of numerous glucose units and its chemical formula is represented as $(C_6H_{10}O_5)_n$. Furthermore, cellulose plays a major economic role, as it can be processed to produce paper and fibres [10,11]. The following are the three fundamental requirements of biomaterials: (i) biocompatibility, (ii) bioactivity, and z(iii) biomechanics [12,13,11]. Owing to its tunable chemical, physical, and mechanical properties, cellulose has been considered for bio-material manufacturing. Moreover, cellulose is readily available and has unlimited sources, resulting in low/reduced costs for tissue engineering. Since cellulose is insoluble in water, it is effortlessly separated from the other constituents of the plant [11,14]. Cellulose can be easily produced; a classic production process of cellulose includes acid hydrolysis, washing, centrifugation, dialysis, and sonication to form a suspension, followed by drying. However, isolation methods are subjective to the tissue of the plant that is utilized as the source of cellulose [15]. Cellulose has been extensively used in fabricating biopolymers and composites.

Biopolymers are polymers that are produced by living organisms. They include many types of plants, and various types of trees, and bacteria. Natural processes, microorganisms and enzymes can break biopolymers down, thus allowing their re-absorption in the environment [16,17,18]. Biopolymers have tremendous potential in green electronics, dye and heavy metal removal, oil or water separation, drug delivery

systems, tissue engineering scaffolds, biological devices, optics, and biosensing [17,18,12]. Biopolymers include poly(ε-caprolactone) (PCL), poly(butylene succinate) (PBS), polylactide (PLA), poly (glycolic acid) (PGA), polyvinyl alcohol (PVA), poly(hydroxyalkanoates) (PHA), poly(hydroxybutyrate) (PHB), and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV). Amongst the most used biopolymers, PCL has received considerable attention due to its elasticity, biocompatibility, and good ductility caused by its low glass transition temperature of -60^{o}C. It is also affordable and easy to be melt-processed by extrusion, melt-spinning, film blowing, and injection moulding [13, 19,20,21,22]. It is for these reasons that PCL is used for easy production of scaffolds for tissue engineering, bone and cartilage repair, surgical sutures, and drug-delivery systems [23].

Despite the advantages of PCL, it has relatively low mechanical strength, which limits some of its practical applications [17]. The mechanical properties and thermal stability of PCL can be improved by blending it with natural biomaterials such as cellulose [17,24]. Various researchers have therefore studied the potential reinforcement properties of cellulose extracted from various medicinal and/or non-medicinal plants [25,26,27].

For PCL/cellulose extracted from non-medicinal plants such as, the ramie plant and the pineapple crown, the cellulose nanocrystals (CNC) were obtained through acid hydrolysis [25,26]. The electrospun CNC fibers obtained from the ramie plant showed minimal changes in the thermal characteristics of PCL/CNC composites. However, the mechanical properties of the PCL were greatly improved with the addition of CNC [25]. In addition, during the electrospinning process the individual nanofibers became tough, which caused the CNC to have an undesirable outcome on the morphology [25]. In the case of the CNC extracted from the Pineapple crown, the high moisture content and absorption of CNC allowed the use of CNC as reinforcements in polymer nanocomposites [26].

In various studies in which PCL/cellulose composites were studied, the cellulose was extracted from medicinal plants; the cellulose extracts were obtained from the *Humulus Japonicus (HJS)* plant and from onion skin [2,28]. The CNC obtained from the *HJS* plant were blended with PCL using the solution mixing method to fabricate CNC/PCL composites for 3D printing [27]. The PCL/CNC composites demonstrated enhanced biological, and mechanical properties, as well as good biocompatibility as compared to the pure PCL [27]. The CNC extracts from the onion

skin showed high hydrophilicity which allowed them to be potential fillers in bionanocomposite films [28].

Although tremendous research has been done in developing cellulose based materials from non-medicinal plants; to date, limited information is available on the extraction of cellulose from medicinal plants as a potential material in the fabrication of biopolymers such as polycaprolactone for advanced applications in polymer science, medicine, and engineering fields. In this study, the aim is to investigate the preparation process of the PCL/nanocellulose composite, optimize its composition, and comprehensively characterize its physical properties. The study will focus on improving the properties of PCL by melt blending it with nanocellulose obtained from EA for advanced applications in bone tissue engineering. The preparation and characterization of a composite material based on PCL/nanocellulose extracted from EA hold significant promise for bone tissue engineering applications. Moreover, the utilization of nanocellulose from EA provides a sustainable and environmentally friendly approach to the development of biomaterials.

1.3 Problem statement

The use of polymers has increased to a great end worldwide. Polymers can be used for packaging, in agriculture and for medicinal purposes. Consequently, plastics not properly disposed of pollute the environment. Often the natural environment has to work as a waste reservoir, either by having the waste absorbed or recycled into valuable or harmless substances. The inability of waste to be absorbed by the environment often results in environmental pollution, which ultimately poses a threat to lifeforms. Biodegradable polymers, however, are subject to biological degradation [29]. Polycaprolactone (PCL) generally degrades slowly [13]. though its properties can be enhanced by adding natural biomaterials to it [30]. To avoid having material which can take time to degrade in the environment and potentially have it polluted, measures are taken to have the material recycled and used for other purposes. Therefore, PCL with be blended with cellulose in order to investigate their properties and ultimately be used for bone tissue engineering.

1.4 Research aims

This study seeks to improve the properties of PCL by melt blending it with nanocellulose extracted from EA for advanced applications in bone tissue engineering.

1.5 Research objectives

- ✓ To collect *E. autumnalis* plant then wash, dry, and grind its leaves into fine powder for chemical analysis;
- ✓ To extract nanocellulose from the plant leaves using the alkali and bleaching treatment;
- ✓ To prepare nanocomposites by melt-mixing the extracted nanocellulose with PCL; and
- ✓ To characterise the PCL/nanocellulose composites using different analytical techniques and study the morphology, structural, thermal, thermo-mechanical and mechanical properties of the nanocomposites; then correlating the results with the dispersion, structure, and morphology of the cellulose on the PCL matrix.

1.6 Dissertation structure

Chapter 1: Introduction
Chapter 2: Literature Review
Chapter 3: Methodology
Chapter 4: Results and Discussion
Chapter 5: Conclusions

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CHAPTER 2: Literature Review

2.1 Overview

Medicinal plants are currently gaining a lot of attention because of the unique and interesting chemical compounds in their different parts that are responsible for their various traits [1]. These chemical compounds provide extensive leads for the development of green products across sectors. Regular screening of plant species with the objective to discover new bioactive compounds is a routine activity for many laboratories. Thus, proper identification and understanding of medicinal plants and their morphogenetic features is essential for proper analysis and application. In this chapter various medicinal plants' characteristics are discussed, including the methods of cellulose extraction, percentage yield and crystallinity. The incorporation of cellulose to biopolymers, especially PCL, is also discussed.

2.2 Cellulose extracted from medicinal plants

Generally, a number of studies extracted cellulose fibres from medicinal plants using alkali and bleaching treatments as well as acid hydrolysis [2- 14]. The cellulose obtained was characterized using morphological investigations (optical, scanning electron and atomic force microscopy), as well as physico-chemical techniques (infrared spectroscopy, X-ray diffraction and thermogravimetric analysis). The results demonstrated the successful extraction of cellulose from medicinal plants with a percentage yield ranging between 40 and 71% [2-14]. The percentage yield depends on the raw material and the process of extraction [15].

From Table 2.1 it can be seen that the cellulose obtained using the alkali and bleaching treatments revealed the highest cellulose yield and percentage crystallinity [2,3,9-13]. The high cellulose content suggests the possibility to use the cellulose obtained from medicinal plants in the production of biodegradable nanocomposites with enhanced properties [14]. Moreover, the medicinal plants from which cellulose is derived possess biological properties, which might be beneficial for biomedical applications such as tissue engineering, biosensing, scaffolds, and drug delivery applications etc. [4-8,16-37].

In a number of studies in which cellulose has been incorporated with various biopolymers, the composites have been shown to be potential materials for packaging, bioplastics and medical applications [38-41], while the cellulose extracted from non-medicinal plants, used to reinforce PCL, has shown improved mechanical and thermal properties of the composites, also proving the potential of these composites to be used in food packaging, bone scaffolds and tissue engineering applications [42-44].

The crystallinity percentage of the cellulose extracted from certain medicinal plants, such as, *Citrullus colocynthis*, *Imperata brasiliensis*, *Agave sisalana* (sisal fibers), *Luffa cylindrical* ranges between 51 and 64% [45-56,4].

Even though the use of EA derived compounds has been limited to pharmaceutical products [57,21-26], there is a need to explore their full potential such as extracting cellulose to develop new green composites and broaden its practical applications.

2.3 Biopolymer/cellulose extracted from medicinal and non-medicinal plants

Fortunati *et al.* [14] investigated nanocellulose obtained from *Phormium tenax* and incorporated it into a polylactic acid (PLA) matrix using the melt blending method to fabricate green nanocomposites. The thermal, morphological, and mechanical properties of the nanocomposites were analysed using the Differential Scanning Calorimetry (DSC), Thermo-Gravimetric Analyzer (TGA), Fourier-Transform Infrared (FTIR) spectroscopy, Field Emission Scanning Electron Microscope (FESEM), and tensile test. The thermal properties of PLA, such as crystallinity, were improved by adding cellulose. The reason for the improved crystallinity of PLA is that CNC act as nucleating agents for PLA. As a result, the addition of CNC can be attributed to the anchoring effect of the cellulosic filler. The extracted cellulose has been shown to be

used as reinforcement for composites, as well as being suitable as active food packaging materials [14,27].

In a study by Benini *et al.* [30] cellulose nanofibrils (CNFs) obtained from *Imperata brasiliensis* fibers, using sulfuric acid hydrolysis, were reinforced with poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) through electrospinning and solution casting methods. The purpose of the study was to prove the capability of using grass to improve certain properties of PHBV. The characterization of the PHBV/cellulose nanofibrils composites was done through Scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and Differential Scanning Calorimetry. A major increase in the crystallization rate was observed for solution casting, because CNFs act as a nucleating agent; while an improvement in process efficiency, and improved mats quality, were observed with electrospinning. The improved mats quality was due to the addition of CNFs in the polymer solution which improved the electrospinning process, since an increase in the viscosity of the solution is usually observed with the addition of CNFs. Likewise, the addition of CNFs increases the electrospinning time by 10 minutes. This study indicated the suitability of reinforced PHBV/cellulose composites, with the usage of the cellulose giving possibilities of feedstock [30].

Elsewhere in the literature, cellulose nanocrystals (CNC) were extracted from *Abelmoschus Esculentus* (okra bahmia) fibres and used to reinforce poly(vinyl alcohol) (PVA). The PVA was blended with cellulose nanocrystals (CNC) through solvent casting in water. The samples were analysed using FESEM, derivative thermogravimetric (DTG), (TGA), tensile test and FTIR. The results demonstrated compatibility between the hydrophilic crystalline nanocellulose and the polymer matrix. According to the study by Furnati *et al*. [32], the crystallinity of PVA film was enhanced by the CNC. The reason for this observation was the increased reaction time. The results revealed the potential for the usage of okra fibres for applications in the CNC in nanocomposite system [32].

Ng *et al*. [34] investigated the effect of treated cellulose with different bleaching conditions on the poly(vinyl alcohol) matrix. Cellulose extracted from oil palm empty fruit bunch fibre through chlorite bleaching, alkali treatment and acid hydrolysis was used for this investigation. A casting technique was used for the preparation of PVA/fibre composites. The composites were characterised by TGA and UV/Vis spectrometry. Higher loading of the treated cellulose into PVA composite resulted in a lower decomposition temperature, and a better transmission in the range of visible light. The lower decomposition temperature was reported to have been caused by the aggregation between cellulose which was getting higher, as the loading of cellulose increased. The interaction between the cellulose and PVA may be limited by the aggregates, which in turn may affect the thermal stability. PVA composites reinforced with cellulose offered slightly improved thermal stability, as it did not differ much from the pure PVA, which may result from the purer cellulose produced by the chlorite bleaching for a longer period [34].

In studies by Tawakkal *et al*. [36] and Siqueira *et al*. [37], cellulose derived from *Hibiscus cannabinus* (kenaf) was prepared with poly(lactic acid) (PLA) composites through melt blending and compression molding. The purpose of these studies was to improve the properties of PLA from plant-based kenaf bast fibres. The composites were analysed using tensile tests and SEM analysis. The flexural strength and modulus of the PLA/Kenaf composites were improved. The reason given for this observation is that the cellulose might curb the flexibility of the polymer chain; therefore, the deformation ability of the polymer may be hindered. Kenaf fibres

(core and bast) are usually used for biocomposites for the building industry, car components, furniture, and food packaging [36].

Zarina and Ahmad [38] conducted a study in which cellulose nanocrystals were extracted from kenaf fibres. The CNCs were extracted through alkali treatment, bleaching, and sulfuric acid hydrolysis. The extracted CNCs were used for reinforcement of κ-carrageenan biocomposites. The samples were prepared through solution casting. The characterization techniques used to analyse the samples were FTIR, FESEM, TEM, TGA and tensile tests. The results showed improved mechanical and thermal properties, which was attributed to the increased stiffness in the composites after the addition of CNC [38].

In a study conducted by Bitinis *et al*. [39], PLA, rubber (NR), and cellulose (PLA/NR/cellulose) composites were prepared using solvent casting and extrusion methods. The CNC were obtained through acid hydrolysis. The purpose of the study was to reinforce PLA with NR and cellulose. Analysis of the samples was conducted with the use of scanning electron microscope, FTIR, TGA, TEM, and XRD techniques. The study showed improved crystallinity, increased modulus due to the low cellulose nanowhiskers content in the modified CNC, and increased viscosity of the samples, which was due to the good dispersion of CNC whiskers [39].

In an investigation of cellulose nanocrystals extracted through sulfuric acid hydrolysis from sugarcane bagasse, the CNC was used to reinforce carboxymethyl cellulose (CMC)/starch (ST) polysaccharide through the solution casting method. The analytical techniques employed in this investigation were the Atomic Force Microscopy (AFM), FTIR, SEM, UV–Vis analysis, and tensile tests, respectively. The CNC reinforced CMC/ST polysaccharide sample showed increased elastic modulus and tensile strength. This is due to the fairly good dispersion of CNC within the polymeric blend matrix [40].

Wang *et al*. [41] studied cellulose nanowhiskers (CNWs) and poly(propylene carbonate) (PPC) bionanocomposites blended through a solution casting method. TEM, SEM, static and dynamic mechanical measurements (DMA), DSC, TGA and tensile testing were the methods used to investigate the morphological, thermo-mechanical, and thermal properties of the nanocomposites. The results showed that the addition of CNWs greatly increased the tensile strength and modulus of PPC. These results can be attributed to the dispersion, great modulus, 3-D network structure

of CNW and strong interfacial interactions between CNW and PPC matrix. This study proved that PPC/CNW nanocomposite films can be considered green materials, as the production process of PPC might reduce the emission of greenhouse gas, as PPC is produced by copolymerization of propylene oxide and carbon dioxide, and can therefore be applied in food packaging and medical applications, because CNW is renewable, biodegradable, and non-toxic, and due to the low cost, outstanding barrier properties, and ductility of PPC [41].

In summary, the results presented in Table 2.2 show that most of the properties of the biopolymers reinforced with cellulose nanoparticles have been improved, including crystallinity, compatibility, thermal stability, modulus, flexural strength, and tensile strength. The inherent properties of cellulose such as its extraordinary mechanical properties (high specific strength and modulus), large specific surface area, high aspect ratio, and environmental benefits, are some of the reasons for the improvement in the properties of the cellulose reinforced polymers [14,30,32,36,37,39]. These results, therefore, show that cellulose extracted from medicinal/non-medicinal plants has the potential to reinforce biopolymers and to be used in packaging, building industry, car components, furniture, and medicinal applications.

Table 2.1 Summary of medicinal use, methods of extraction and percentage yield and crystallinity of the cellulose extracted from medicinal plants

Medicinal plant	Traditional medicinal use	Biological properties	Methods of cellulose extraction	Cellulose extraction (Yes/No)	Type of cellulose	% yield of cellulose	% crystallinity	References
Citrullus colocynthis	Diabetes, cancer, common cold, asthma, bronchitis, joint pain, jaundice, toothache, and gastrointestinal disorders	Antidiabetic, antilipidemic, antioxidant, insecticide, cytotoxic, antimicrobial and anti-inflammatory.	Chemical extraction processes: Deproteinization lipid extraction acid hydrolysis	Yes	Nanofibers	40	50-49	[4-6]
Kigelia Africana	Treating of syphilis and rheumatism, cure wounds, abscesses, and ulcers	Anti-microbial, anti-bacterial, antifungal, anti-oxidant, and anti-inflammatory.	Alkali treatment, bleaching treatment.	Yes	Fibres	71	Not specified	[7-8]
Helicteres Isora	Treating of diarrhoeal infections	Not specified	Alkaline treatment, bleaching, acidic steam treatment and homogenization.	Yes	Nanofibrils	71	90	[2,3,9,10]
Retama raetam	Treating wounds, skin rash, kidney disorders, and microbiological infections	Not specified	Alkali and bleaching treatments.	Yes	Microfibers	52	78	[11-12]
Syngonanthus nitens	Dried ornamental flowers	Not specified	Alkali and bleaching treatments	Yes	Fibres	67	70-91	[13]

Phormium tenax	Production of woven mats and ropes	Not specified	Acid hydrolysis	Yes	Nanocrystals	61	Not specified	[14,27]
Leacaena leucocephala	Used to cure skin ailments and eliminate body hair	Anti-inflammatory and anti-diabetic.	Not specified	Yes	Not specified	Not specified	Not specified	[16-19]
Eucomis autumnalis	Cancer, wounds, and sexually transmitted infections	Anti-microbial, anti-carcinogenic, anti-oxidative, anti-inflammatory, and anti-histaminic.	Not specified	No	Not specified	Not specified	Not specified	[56,21-26]
Imperata brasiliensis	Diuretic, analgesic	Not specified	Sulfuric acid hydrolysis	Yes	Nanofibrils	44	56-64	[28-30]
Abelmoschus Esculentus (okra bahmia)	Treat cuts, wounds, boils, catarrhal infections, ardor urinae, dysuria, gonorrhoea, plasma replacement, blood-volume expander	Anti-spasmodic	Sulphuric acid hydrolysis	Yes	Nanocrystals	60-70	Not specified	[31-32]
Oil palm empty fruit bunch	Sweetener in foods for diabetic patients	Anti-cariogenic agent in tooth paste formulations, as thin coatings on chewing vitamin tablets, in mouth washes, in beverages and in bakery products	Chlorite bleaching, alkali treatment and acid hydrolysis	Yes	Not specified	Not specified	Not specified	[33-34]

Hibiscus cannabinus (kenaf)	Improve oxidative stability	Anti-oxidant, anti-microbial, anti-inflammatory and anti-thrombotic	Alkali treatment	Yes	Fibres	69	Not specified	([35-37]
Agave sisalana (sisal fibers)	Lowering blood pressure, antiseptic, and stops the growth of bacteria in the stomach and intestine	Anti-oxident, anti-microbial	Sulfuric acid hydrolysis	Yes	Microfibers	Not specified	51	[45-48]
Humulus Japonicus (HJ)	Heal pelvic inflammatory disease, relieve symptoms of heat, dampness, and toxicity	Anti-oxidative, anti-tumor, anti-tuberculosis, anti-bacterial	Sulfuric acid hydrolysis	Yes	Nanocrystals	Not specified	Not specified	[49-52]
Luffa cylindrical	Effects on hypersensitivity reactions, immunostimulant, glycosidase activity, inhibit protein synthesis, and induce uterine contraction to speed up child birth (Oxytocics)	Anti-inflammatory, anti-tumour, and anti-viral	Bleaching process, acid hydrolysis	Yes	Nanocrystals	Not specified	64	[53-56]

Table 2.2: Biopolimer/cellulose extracted from non-medicinal plants

Composite	Plant	Preparation method	Characterization techniques	Improved properties	Applications	References
PLA/cellulose	*Phormium tenax*	Melt blending	FESEM, DSC, tensile tests, TGA, FTIR	The crystallinity and thermal properties of PLA were improved	Food packaging	[13]
PHBV/cellulose	*Imperata brasiliensis* fibers	Electrospinning and solution casting	SEM, XRD, DSC	For solution mixing: increase in the crystallization rate. For electrospinning: improvement in process efficiency, and improved mats quality	Feedstock	[30]
PVA/cellulose	*Abelmoschus Esculentus*	Solvent casting	FESEM, TGA, FTIR, tensile tester	Improvement in compatibility and crystallinity	CNC in nanocomposite system	[27]
PVA/cellulose	*Elaeisguineensis*	Casting technique	TGA, UV-Vis spectrometry	Improved thermal stability and better transmission in the range of visible light	Production and processing of polymeric materials	[34]

PLA/cellulose	*Hibiscus cannabinus (Kenaf)*	Melt blending and compression molding	Tensile tests, SEM analysis	Flexural strength and modulus were improved	Building industry, car components, furniture, and food packaging	[36,37]
κ-carrageenan/CNC	κ-carrageenan	Solution casting	FTIR, FESEM, TEM, TGA and tensile tests	Improved mechanical and thermal properties	Packaging	[38]
PLA/NR/cellulose	Not specified	Solvent casting and extrusion methods	ESEM, FTIR, TGA, TEM, and XRD	Crystallinity properties and storage modulus improved	Bioplastics	[39]
CMC/starch/CNC	Sugarcane bagasse	Solution casting	Atomic Force Microscopy (AFM), FTIR, SEM, UV–vis spectroscopy analysis, and tensile tests	Elastic modulus and tensile strength	Packaging applications	[40]
PPC/CNWs	Not specified	Solution casting	TEM, SEM, static and dynamic mechanical measurements, DSC and TGA	Tensile strength and modulus were improved	Food packaging and medical applications	[41]

2.4 Polycaprolactone/cellulose extracted from non-medicinal plants

To improve the mechanical properties of PCL, [42] investigated the reinforcement of PCL with microfibrillated cellulose (MFC). The MFC was extracted from sugarcane bagasse (SCB). The PCL/MFC composites were prepared through solvent casting, and the analytical techniques that were used to characterize the morphology, thermal, and mechanical properties of the samples were FESEM, DSC and tensile tests, respectively. Reinforcing PCL with cellulose improved the thermal degradation of PCL. The reason for this increase was attributed to the increase in the cellulose loading [42].

Cellulose nanofibers extracted from rice husks, through acid hydrolysis, were blended with PCL in a study conducted by Dutta *et al.* [43]. The PCL/CNC nanofibers were prepared through an electrospinning method. The mechanical, morphological, and thermal characteristics of the PCL/CNC samples were analysed through tensile testing, SEM, TEM, FTIR, and TGA techniques, respectively. The results obtained showed improved mechanical and thermal properties in the composites. Moreover, no cytotoxicity was demonstrated by the CNCs [43]. The reasons for the results obtained include the high aspect ratio of CNC which permitted an enhanced platform for interactions, subsequently improving the mechanical and thermal properties of the polymer matrix as well as the addition of the CNC to the matrix which improved the cell viability. Furthermore, the results obtained showed that PCL/CNC composites have the potential to be used in tissue engineering applications [43].

In a study conducted by Hong *et al*. [44] surface-oxidized cellulose nanocrystals (SO-CNCs) were blended with PCL, through melt compounding extrusion and solvent casting to enhance the mechanical properties of PCL and encourage biomineral formation upon PCL resorption. AFM, DSC, and tensile tests were used to characterise the composites in terms of Young's modulus, tensile strength, crystallinity, and thermal transitions. The addition of the sulfuric acid-hydrolyzed CNCs (SH-CNCs) or SO-CNCs showed no toxicity to polymer matrix; there was an increase in Young's modulus (stiffness), tensile strength and crystallinity. Therefore, the ability of SO-CNCs to induce calcium phosphate mineral formation and increase the Young's modulus, tensile strength and crystallinity of PCL makes them effective for applications in PCL-based bone scaffolds [44].

2.5 Polycaprolactone/cellulose extracted from medicinal plants

Siqueira *et al.* [47] investigated microfibrillated cellulose (MFC) and cellulose nanowhiskers (CNW) extracted from sisal fibres (*Agave sisalana*) to reinforce polycaprolactone (PCL). The aim of this study was to investigate the mechanical and thermal properties of the reinforced nanocomposites. The composites were prepared through solution casting. Differential scanning calorimetry, dynamical mechanical analysis, and tensile tests were used to analyse the MFC/PCL and CNW/PCL samples. This investigation resulted in improved modulus and tensile strength for the CNW/PCL sample, due to the nucleating effect of the filler, which impacted the crystallisation rate [47-48].

Cellulose nanocrystal (CNC) extracts from *Humulus Japonicus* (HJS) were blended with poly(ε-caprolactone) (PCL) to prepare CNC/PCL composites through solution mixing for of 3D scaffold printing [50]. TEM, SEM and DMA were the characterization techniques used to investigate the characteristics of the composites. The CNC/PCL scaffold demonstrated enhanced thermo-mechanical properties and suitability for transportation of cells and nutrients. The above-mentioned properties are essential in 3D printing and scaffolds. The results in this study demonstrate that the CNC composites possess biological, mechanical properties and biocompatibility. The results are due to the porosity of the concave hexagon filled pattern for 3D printing, which enhanced the compression resistance in tensile properties for mechanical properties [50-52].

Cellulose nanocrystals extracted by acid hydrolysis from *Luffa cylindrical* were blended with polycarprolactone (PCL) composites using the casting/solvent evaporation method [54]. The CNC were used as reinforcing materials for the processing of bio-nanocomposites. The analytical techniques used were the transmission electron microscopy (TEM), infrared spectroscopy, X-ray diffraction analysis, tensile test, differential scanning calorimetry, as well as the dynamical mechanical analysis. In the study, tensile strength and crystallinity of the prepared PCL/CNC composites were enhanced due to the high aspect ratio of *Luffa cylindrical* and the addition of nanocrystals [54-56].

Luduena *et al.* [57] investigated the morphological, crystallinity, thermal, and mechanical properties of cotton (CO), cellulose (CE) and hydrolyzed-cellulose (HCE)

blended with PCL through melt-mixing method. The study was conducted in order to investigate the properties of the samples for packaging applications. Characterization of the samples was carried out through XRD, SEM, FESEM and tensile tests. The results obtained showed that CE and CO fillers lowered the crystallinity degree of PCL, while HCE increased it. CE composites, however, showed the highest interfacial strength, dispersion of the filler, and mechanical properties. Another attribute was the accelerated biodegradation process caused by the presence of CE in PCL. As a result, the properties of CE proved it as potential material for packaging applications [57].

The information presented in Table 2.3 shows PCL/cellulose composites obtained from various medicinal and non-medicinal plants. The mechanical and thermal properties of PCL were improved by the cellulose extracted from non-medicinal plants. For PCL/cellulose extracted from medicinal plants the following properties were enhanced: biocompatibility, crystallinity, tensile strength, and biodegradability. The difference between the non-medicinal and medical plants can be attributed to the biological properties attributed to the medicinal plants, as shown in Table 2.3. The applications attributed to the PCL/cellulose composite from medicinal plants include biomedical applications, biodegradable packaging materials, 3D-printing, and reinforcing materials for the processing of bio-nanocomposites [47-48,50-52,54-57]. However, for both PCL/cellulose extracted from non-medicinal and medicinal plants common applications include packaging and various biomedical applications [42-43,47-48, 51-52, 54-57].

Table 2.3: PCL/cellulose composites from various medicinal and non-medicinal plants

Composite name	Type of plant	Preparation method of the composites	Characterization techniques	Improved properties	Applications	References
PCL/cellulose (Sugarcane bagasse)	*Non-medicinal*	Solvent casting	FESEM, DSC, FTIR, tensile tests	Improved the mechanical properties	Food packaging and tissue engineering	[42]
PCL/cellulose (Rice husk)	*Non-medicinal*	Electrospinning	SEM, tensile testing, FTIR, TGA, TEM	Improved mechanical and thermal properties	Tissue engineering applications	[43]
PCL/cellulose (softwood pulp)	*Non-medicinal*	Solvent casting	AFM, DSC, tensile tests	Crystallinity and young modulus	Bone scaffolds	[44]
PCL/cellulose (*Agave sisalana*)	*Medicinal*	Solution casting	DSC, DMA, tensile tests	Improved modulus and tensile properties	Biomedical and biodegradable packaging materials	[47-48]
PCL/cellulose (*Humulus Japonicus*)	*Medicinal*	Solution mixing	TEM, SEM, DMA	Enhanced biological, biocompatibility and mechanical properties	3D-printing	[50-51,53]
PCL/cellulose (*Luffa cylindrical*)	*Medicinal*	Casting/solvent evaporation	TEM, FTIR, XRD, tensile tests, DSC, DMA	Tensile strength and crystallinity were enhanced	Reinforcing materials for the processing of bio-nanocomposites	[54-56]
PCL/cotton/cellulose/hydrolyzed-cellulose (cotton)	*Medicinal*	Melt mixing	XRD, SEM, FESEM and tensile tests.	Improved interfacial strength, dispersion,	Packaging applications	[47]

				mechanical properties, and biodegradation		

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CHAPTER 3: Methodology

3.1 Materials

3.1.1 Eucomis autumnalis

The *Eucomis autumnalis* medicinal plant (Figure 3.1) was collected from Ladybrand, in the Free State province, South Africa, and it was used as a source of cellulose in this study. To prepare the plant material for all the experiments, the leaves of *E. autumnalis* were stripped, followed by washing with distilled water, and oven drying at 36 ºC for 3 weeks. The dried leaves were then ground into fine powder using a blender (Figure 3.2).

Figure 3.1: Image of *Eucomis Autumnalis* (EA) plant leaves

Figure 3.2: Image of dried EA leaves ground into fine powder

3.1.1.1 Delignification and removal of hemicellulose [1]

The dried and finely grounded plant tissue was bleached in 0.7% (w/v) sodium chloride solution (fiber to liquor ratio of 1:50). The obtained solution was adjusted to a pH of 4 with 5% acetic acid. The mixture was then boiled for 5 hours to remove lignin, and the residue was washed with distilled water. Furthermore, the neutral residue was boiled with 250 ml of 5% (w/v) sodium sulphite solution for 5 hours, followed by washing it with distilled water to further remove lignin and hemicellulose.

3.1.1.2 EA cellulose isolation [1]

The obtained product was boiled with 250 ml of 18% (w/v) sodium hydroxide solution for 5 hours to remove hemicellulose completely. The resultant product (cellulose) was collected by filtration and then washed with distilled water until the filtrate became neutral. The obtained cellulose was treated with 50 ml dimethylsulfoxide (DMSO) for an hour to remove any other impurities. This step was followed by filtering the product, washing it with distilled water and oven-drying at 36 ^{o}C overnight (Figure 3.3).

Figure 3.3: Image of cellulose powder after isolation from *E. automnalis* plant

3.1.1.3 Preparation of EA nanocellulose [*1*]

The cellulose powder shown in Figure 3.3 was treated for 4 hours (in 1-hour intervals) with 1.5 wt.% sodium hydroxide solution at 80°C under mechanical stirring to remove any constituents other than cellulose that might be present. After the 4-hour treatment, the powder was filtered and rinsed with deionized water until a neutral pH was achieved. Subsequent bleaching treatment (containing active 1.5 ml sodium

hypochlorite) was carried out twice at a 3-hour interval, for 6 hours at 80°C. After the treatment, the powder was filtered and washed with deionized water until a neutral pH was achieved. The obtained suspension was then treated for 4 hours (1-hour intervals) with 1.5 wt.% potassium hydroxide solution at 80°C under mechanical stirring. After the treatment, the powder was filtered and washed with deionized water until a neutral pH was achieved. The recovered sample was then subjected to an overnight oven-drying at 36^{o}C, followed by grinding with a blender to form a fine powder nanocellulose sample (Figure 3.4).

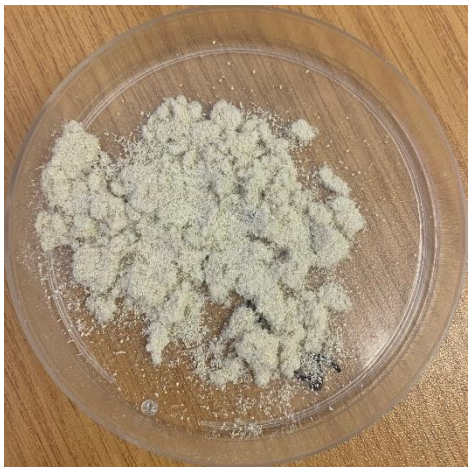

Figure 3.4: Image of nanocellulose obtained from the *E.autumnalis* plant

3.1.2 Polycaprolactone (PCL)

A commercial PCL (CAPA 6500, Johannesburg, South Africa) was purchased from Southern Chemicals. It has a density of 1.1 g cm^{-3}, a melting temperature of 58–60 ◦C, and a degree of crystallinity of ~35%. Its average molecular weight (M_w), number-average molecular weight (M_n) and polydispersity index (M_w/M_n) are 113,400 g mol^{-1}, 73,620 g mol^{-1}, and 1.54, respectively.

3.2 Sample preparation

3.2.1 Preparation of PCL/EA nanocellulose w/w 97/3 composite

In a study conducted by Sikhosana *et al*. (2023) [2], blending PLA with 3 wt.% EA cellulose resulted in improved physical properties [2]. This is the reason why the 3 wt.% EA cellulose was selected for this study.

3.2.1.1 Melt blending:

Prior to melt blending, the EA nanocellulose was grinded using a sieve size of 0.5 mm. The PCL and the grinded EA cellulose were dried using the conditions shown in Table 3.1.

Table 3.1: Conditions used to dry the PCL and the ground EA nanocellulose

Material	Drying conditions
PCL	Overnight at 40 ºC in an oven
EA nanocellulose	Overnight at 50 ºC in a vacuum oven

Melt blending of the PCL and the EA nanocellulose at a 97/3 wt.% composition was carried out in a DSM Micro 5cc Twin Screw Compounder. The conditions used were:

- Temperature profile: 60-90-90ºC.
- Screw rate: 100 rpm.
- Residence time: 35 s.

The same protocol was followed with neat PCL before injection moulding of the tensile specimens.

3.2.1.2 Tensile test specimens:

Tensile specimens of the neat PCL and the 97/3 w/w PCL/EA nanocellulose composite were obtained according to standard ISO 527-2 (type 1BA) with nominal dimensions of 75 mm length, 5 mm width and 2 mm thickness, as shown in Figure 3.5, using an Xplore Pro-face Micro Injection Moulder. The conditions used were:

- Injection temperature: 90ºC.
- Mold temperature: 20ºC.
- Cooling time: 1 min.

Figure 3.5: Image of tensile specimens of the (a) neat PLC and (b) 97/3 w/w PCL/EA nanocellulose composite

3.3 Sample characterization

3.2.1 Chemical structure analysis: Fourier-transform infrared (FTIR) spectroscopy

The powdered EA nanocellulose, neat PCL, as well as the 97/3 w/w PCL/EA nanocellulose composite were placed on the microscope stage and clamped. The light emerging from each sample was channelled into a detector and then analysed on the screen using an attenuated total reflectance (ATR) fourier-transform infrared (FTIR) spectroscope (Platinum-ATR Bruner Alpha II) from the wavelength range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}.

3.3.2 Surface morphology analysis: Scanning electron microscopy (SEM)

To analyse the surface morphology of the investigated samples, the samples were mounted on a metal stub using a sticky carbon tape. The mounted samples were sputter coated with a gold coater for conductivity before SEM analysis. The surface morphology of the specimens was imaged using a JEOL Scanning electron microscopy at an accelerating voltage of 5.0 kV.

3.3.3 Thermal stability: Thermo-gravimetric analysis (TGA)

TGA analyses were done using a PerkinElmer Pyris 1 TGA Q500 instrument to determine the purity and decomposition temperature of the investigated samples. Three samples of approximately 10 mg were heated from 25 to 600 °C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere (flow rate of 20 mL min^{-1}). The TGA instrument was computer-controlled, and the measurements were presented as a curve in which mass or mass percentage is plotted against temperature and/or time. The calculations performed on the curves were done using the Pyris software.

3.3.4 Thermal analysis: Differential scanning calorimetry (DSC)

Thermal transitions that occurred in the prepared samples were analysed using a Perkin Elmer DSC-7 calorimeter to determine the melting temperature (T_m) and the melting enthalpy (ΔH_m) of the neat PCL and the 97/3 w/w PCL/EA nanocellulose composite. A heating scan at a rate of 20 ºC min^{-1} was applied from 30 ºC to 160 ºC.

3.3.5 Structural properties: X-ray diffraction (XRD)

The structural analysis of the samples was carried out using a computer-controlled PAnalytical X'PERT PRO PW3040/60 X-ray diffractometer using a Cu-Kα (λ = 1.5405 Å) radiation.

3.3.6 Mechanical properties: Tensile testing

Tensile tests were performed using test specimens obtained as indicated in Figure 3.5 to determine the Young's modulus, the tensile strength, and the elongation at break. The following test conditions were applied:

- Initial grip separation: 55 mm.
- Test rate: 10 mm min^{-1}.
- Grips: pneumatic, with a pressure of 6 bars.

5 measurements were performed for each sample and the average values, as well as their standard deviations, are provided.

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CHAPTER 4: Results and Discussion

4.1 Extraction of cellulose and nanocellulose from the *Eucomis autumnalis* plant leaves

4.1.1 Chemical structure analysis: Fourier-transform infrared (FTIR) spectroscopy

The FTIR spectra of the powdered leaves, cellulose, and nanocellulose extracted from EA plant are shown in Figure 4.1. The EA leaves show a broad absorption band at 3316 cm-1, two double sharp absorption bands at 2922 cm-1 and 2853 cm-1, two characteristic bands at 1741 cm-1 and 1600 cm-1 and an intense band around 1161–1023 cm-1. The band at 3316 cm-1corresponds to O-H groups stretching. The double absorption bands at 2922 cm-1 and 2853 cm-1 are due to the presence of long alkyl chains. These alkyl bands are attributed to –CH3 and –CH2 asymmetrical stretching vibrations and symmetrical stretching vibrations, respectively, which are commonly associated with methylcellulose [1-2]. Moreover, the double bands at 1741 cm-1 and 1600 cm-1 are due to CH3COO and COOH functionality of oleanolic acid associated with lignin and hemicellulose [1,3]. At around 1161–1023 cm-1, the intense peak is associated to the C-O ether stretch. For the EA cellulose and EA nanocellulose, both their spectra show similar bands and the position of the bands in the samples remain unchanged. EA cellulose and EA nanocellulose are characterized by an absorption band at around 3316 cm-1, possibly due to hydroxyl (O-H) stretching vibrations in cellulose [4]. Bands at 2900 cm-1 and 1700 cm-1 indicate C-H stretching vibrations of cellulose [5], as well as C=O stretching vibrations, respectively. Peaks around 1400 and 1300 cm-1 indicate C-O vibration of the syringyl ring units showing cellulose peaks [6]. In all the investigated samples, there is a common intense peak at 1161–1023 cm-1 which is related to the C-O ether stretching vibrations which may indicate the high presence of cellulose [7]. EA nanocellulose shows a broad absorption band at 3316 cm-1 which corresponds to O-H stretching. The peak at 2826 cm-1 is attributed to the C-H stretch, while the absorption band at 1600 cm-1 is ascribed to the C=O stretching vibrations [3,8-9]. The absence of some bands in the EA cellulose and EA nanocellulose sample in comparison to the EA leave sample is a confirmation of the successful extraction of cellulose from the leaves of the Eucomis autumnalis plant.

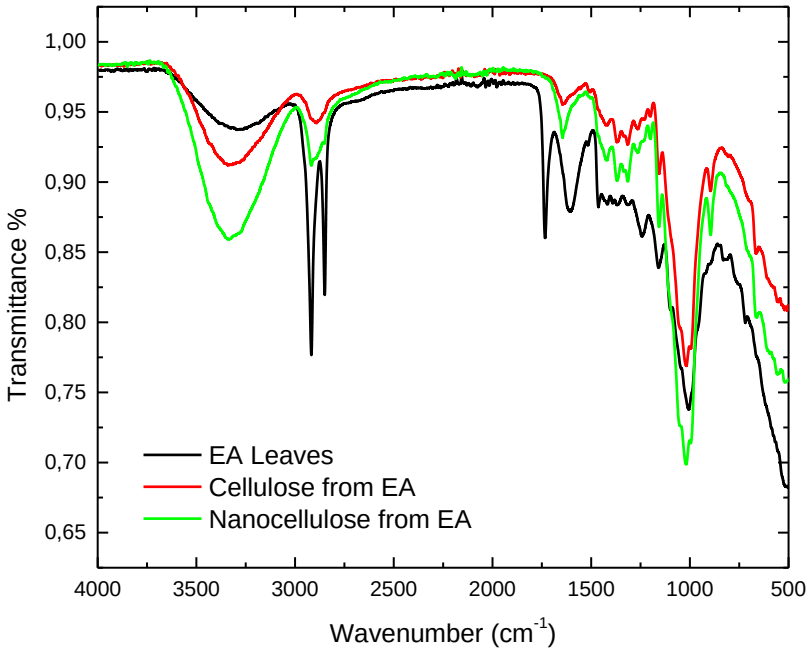

Figure 4.1: FTIR spectra of the powdered leaves, cellulose and nanocellulose extracted from the leaves of the *Eucomnis autumnalis* (EA) plant

4.1.2 Surface morphology analysis: Scanning electron microscopy (SEM)

Figure 4.2 shows the SEM images of (a) EA leaves, (b) EA cellulose and (c) EA nanocellulose. The EA leaves (Figure 4.2a) show a smooth surface of cellulose as well as coarse aggregates of smaller dimensions which may indicate the availability of some non-cellulose constituents such as lignin and hemicellulose on the surface of the powdered leaves as depicted by Khandanlou *et al*., 2016; Abiaziem *et al*., 2019 and Qasim *et al*., 2020 [10-13]. Figure 4.2b demonstrates the cellulose extracted from the EA leaves. The EA cellulose shows some separation of fibrils, indicating that some components such as lignin and hemicellulose have been removed [11-12]. Figure 4.2c shows the SEM micrograph of the nanocellulose extracted from EA cellulose. The smooth surface of nanocellulose may be a result of the chemical treatments, which promote the prevention of agglomeration in the fibers, as suggested by Gadzama *et al*., 2020 [13].

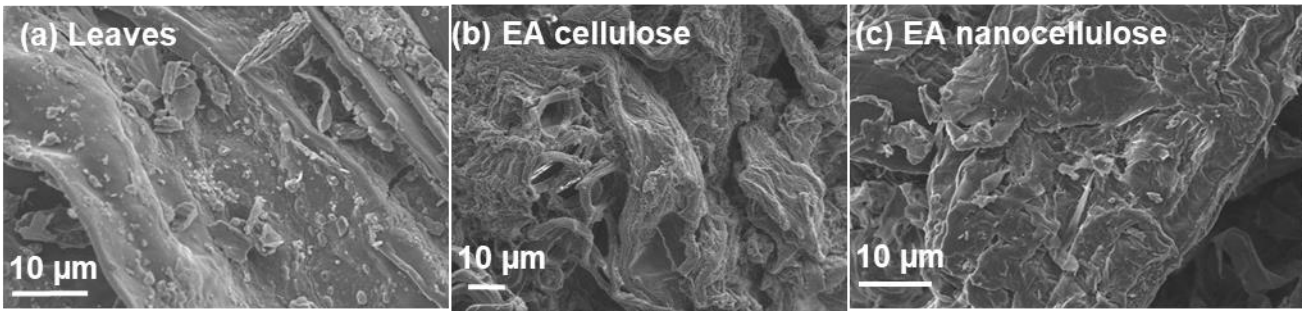

Figure 4.2: SEM images of (a) leaves, (b) cellulose and (c) nanocellulose extracted from the Eucomis autumnalist (EA) plant

4.1.3 Crystalline structure: X-ray diffraction analysis (XRD)

The X-ray diffraction patterns for the EA leaves, EA cellulose and EA nanocellulose are shown in Figure 4.3. The EA leaves show characteristic peaks at 2θ=16°, 2θ=21°, 2θ=25°, 2θ=28° and 2θ=40°, which are associated with the presence of lignin, hemicellulose, and cellulose. The diffractograms of EA cellulose and EA nanocellulose show well-defined peaks that confirm the co-existence of cellulose I and cellulose II. The presence of the crystal structure of cellulose type I can be observed at 2θ=12° (110) and 35° (004) as well as cellulose II at 2θ=12° (110), 20° (210), and 22° (200) [14-15]. Both EA cellulose and EA nanocellulose show higher crystallinity compared to EA leaves. The presence of the main peaks around 20° and 22º show the characteristics of crystal polymorph I of cellulose, which may indicate that the chemical treatments had no impact on the crystal polymorphism of cellulose but may have successfully removed lignin and hemicellulose [12,16]. However, when comparing EA cellulose and EA nanocellulose, it can be seen that at 20° (210), and 22° (200), the intensity of the peaks is not the same. For EA cellulose, both peaks are more or less the same height, while for EA nanocellulose the peak height differs. This may be due to the chemical treatment that influenced the microcrystalline structure of the EA nanocellulose [17-19].

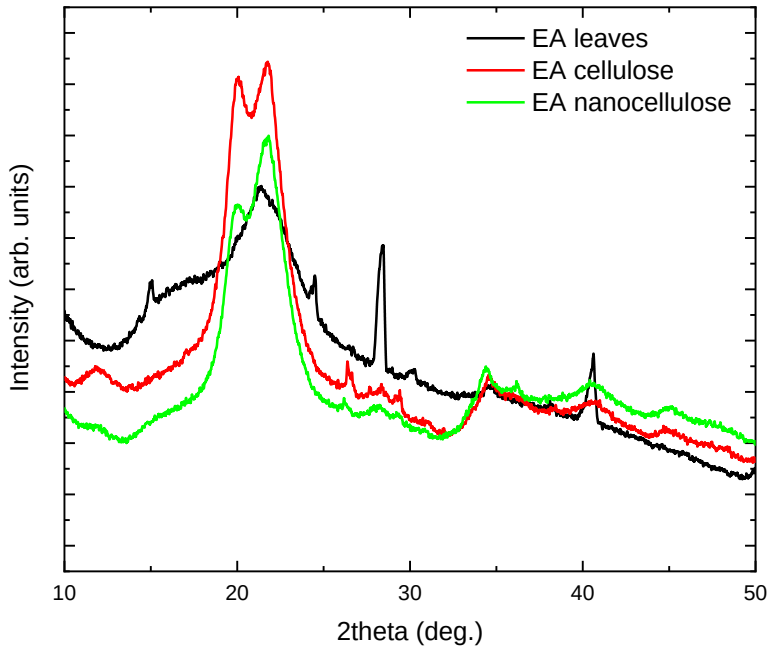

Figure 4.3: XRD graphs of powdered leaves, cellulose and nanocellulose extracted from the Eucomis autumnalis (EA) plant

4.1.4 Thermal stability: Thermogravimetric analysis (TGA)

Figure 4.4 shows the TGA curves for all the investigated samples. In the EA leaves sample, the first stage weight loss from 25 to 62.0 ^{o}C may be due to the absorbed moisture in the sample. The second weight loss around 185.4 ^{o}C may be due to the loss of absorbed water molecules from the polysaccharide structures through evaporation [20-21]. The third and fourth weight losses are observed between 238.6 - 290.0 °C and they are attributed to the breakdown of non-cellulosic and cellulosic materials. The mass loss for EA nanocellulose occurs in two steps. The first is from room temperature to about 100 °C and can be attributed to the removal of absorbed water and moisture present in the sample. The second step between 200 and 500 °C correspond to the decomposition of cellulosic and non-cellulosic compounds [22]. The higher char yield of the EA leaves as compared to the EA cellulose as well as the EA nanocellulose could be attributed to the presence of highly thermal stable lignin residues. Lignin is considered comparatively stable at elevated temperatures, and its decomposition occurs over a broader range that initiates earlier but extends to higher

temperatures than hemicellulose and cellulose [23-24]. This is because lignin contains oxygenated functional groups with varied thermal stability values depending on the chain scission [25]. Considering the cellulose and nanocellulose extracted from the EA plant, the degradation takes places in two steps. The first step around 110 ^{o}C is the loss of moisture, and the final step around 345.2 ^{o}C is the breakdown of cellulose [7]. The low residual mass of EA cellulose and EA nanocellulose as compared to EA leaves may be due to small residues of lignin. This can be further confirmed by the FTIR results, which showed the absence of some bands in the EA cellulose and EA nanocellulose sample in comparison to the EA leave sample, which is a confirmation of the successful extraction of cellulose from the EA leaves. Furthermore, it is worth noting that both the cellulose and nanocellulose degrade at higher temperatures compared to the leaves (especially the EA cellulose). However, the EA cellulose is more thermally stable than the EA nanocellulose.

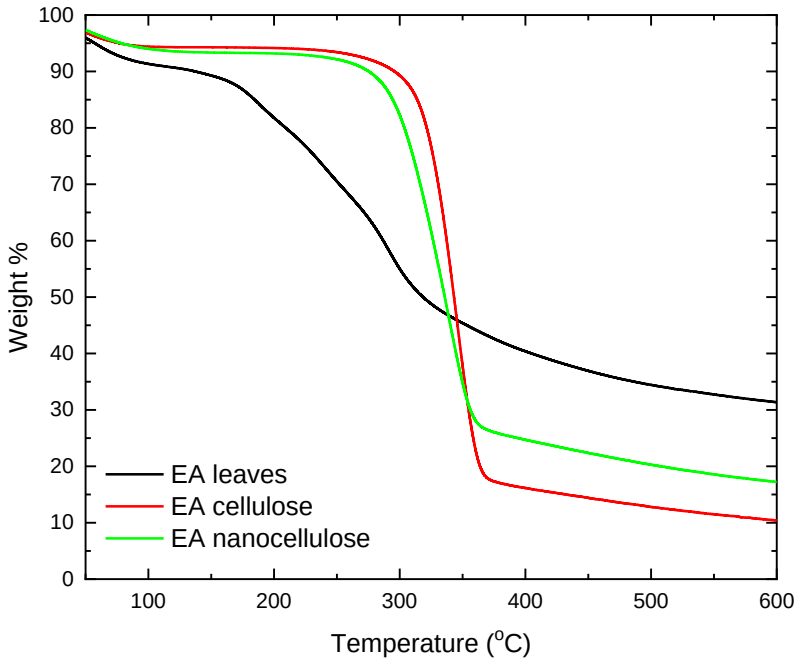

Figure 4.4: TGA curves of the powdered leaves, cellulose and nanocellulose extracted from the Eucomis autumnalis (EA) plant

4.2 PCL/nanocellulose composites

4.2.1 Chemical structure analysis: Fourier-transform infrared (FTIR) spectroscopy

Figure 4.5 presents FTIR spectra of neat PCL, EA nanocellulose and 97/3 w/w PCL/EA nanocellulose composites. Neat PCL is characterized by peaks at around 2900 and 1750 cm^{-1}, and these correspond to C-H and C=O signals, respectively [26]. Refer to section 4.1.1. The spectrum of PCL/EA nanocellulose composites is dominated by absorption peaks characteristic PCL due to a high content of the polymer in the composites. However, the presence of the C-H stretch in the spectrum is proof of successful fabrication of the composites. These FTIR results suggest that no chemical reactions occurred during melt-compounding as there is no change in the shape or intensity of the observed peaks [27].

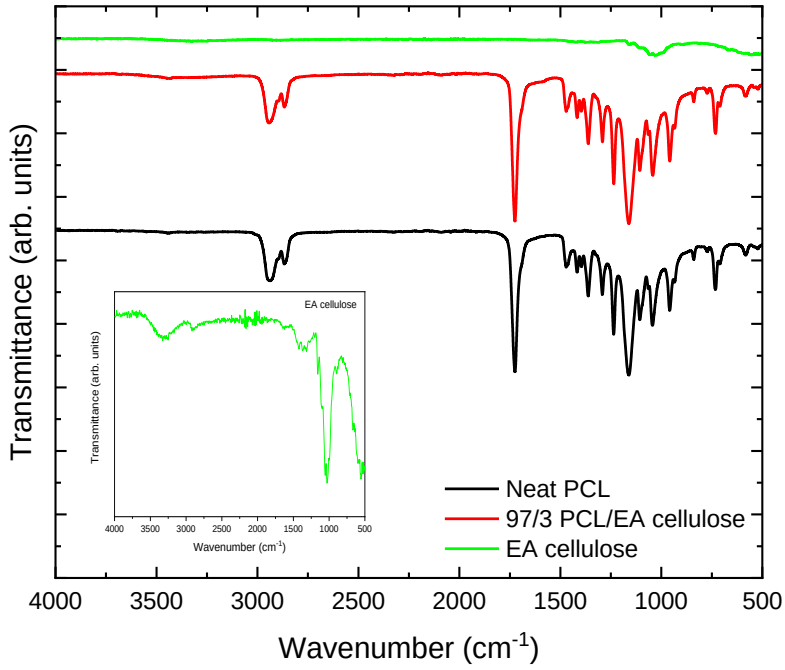

Figure 4.5: FTIR spectra of neat PCL, EA nanocellulose and 97/3 w/w PCL/EA nanocellulose composite

4.2.2 Surface morphology analysis: Scanning electron microscopy (SEM)

The surface morphology of the samples was analysed using scanning electron microscopy (SEM). Figure 4.6 shows SEM micrographs of neat PCL, EA nanocellulose and 97/3 w/w PCL/EA nanocellulose composite.

PCL forms a continuous phase (Figure 4.6(a)) and shows a relatively smooth fracture surface (Figure 4.6(b)) [27-28]. In contrast, EA nanocellulose takes a fibrous morphology and forms a three-dimensional network (Figure 4.6(c)). In addition, the fibre surface appears to be relatively rough compared to the PCL surface (Figure 4.6(d)). Upon the successful fabrication of the composites, the EA nanocellulose fibres can be seen on the composite surface embedded in the PCL matrix (Figure 4.6(e and f)). However, the fractured surface of the composites clearly shows empty cavities, which might suggest fibre pull out [29]. This can be explained in terms of poor interaction between the hydrophilic fibres and the hydrophobic matrix, which is in line with what was observed from FTIR results. It has also previously been reported that cellulose fibres are incompatible with hydrophobic matrices [30-32].

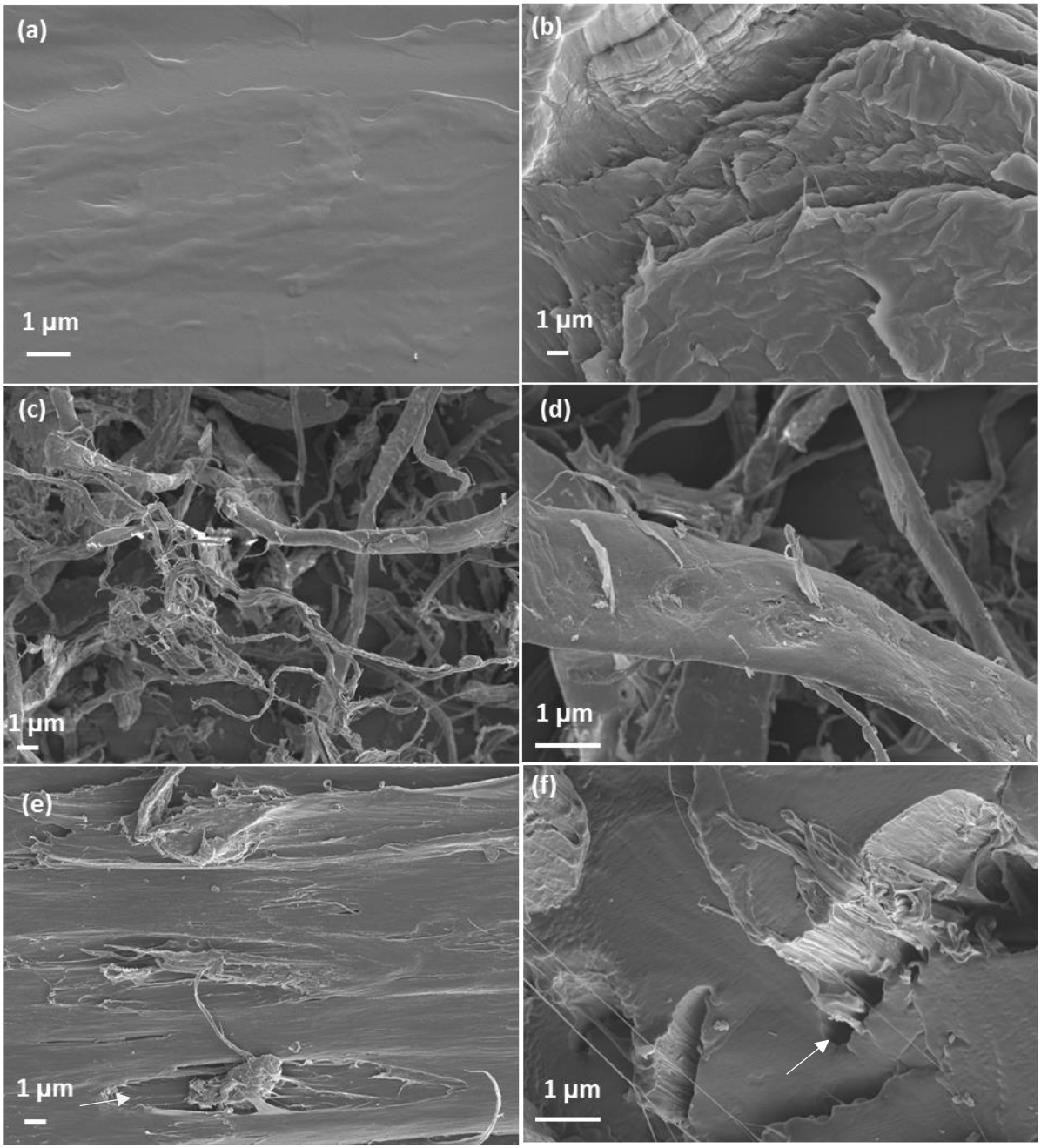

Figure 4.6: SEM micrographs of (a) neat PLC (low magnification), (b) neat PCL (high magnification), (c) EA nanocellulose (low magnification), (d) EA nanocellulose (high magnification), (e) 97/3 w/w PCL/EA nanocellulose composite (low magnification), and (f) 97/3 w/w PCL/EA nanocellulose composite (high magnification)

4.2.3 Thermal analysis: Thermogravimetric analysis (TGA)

Figure 4.7 shows the TGA curves for neat PCL, EA nanocellulose as well as the 97/3 w/w PCL/EA nanocellulose composites. Refer to 4.1.4 Neat PCL degrades in one step around 420 °C, in line with previous studies [33-34]. Interestingly, the PCL/EA

nanocellulose composites also degrade in one step, and a clear improvement in thermal stability is evident. It looks as if PCL shielded EA nanocellulose from low temperature degradation, and at the same time, EA nanocellulose delayed the degradation of PCL, causing it to degrade at higher temperatures. Similar behaviour was observed when other matrices were reinforced with natural fibres [35].

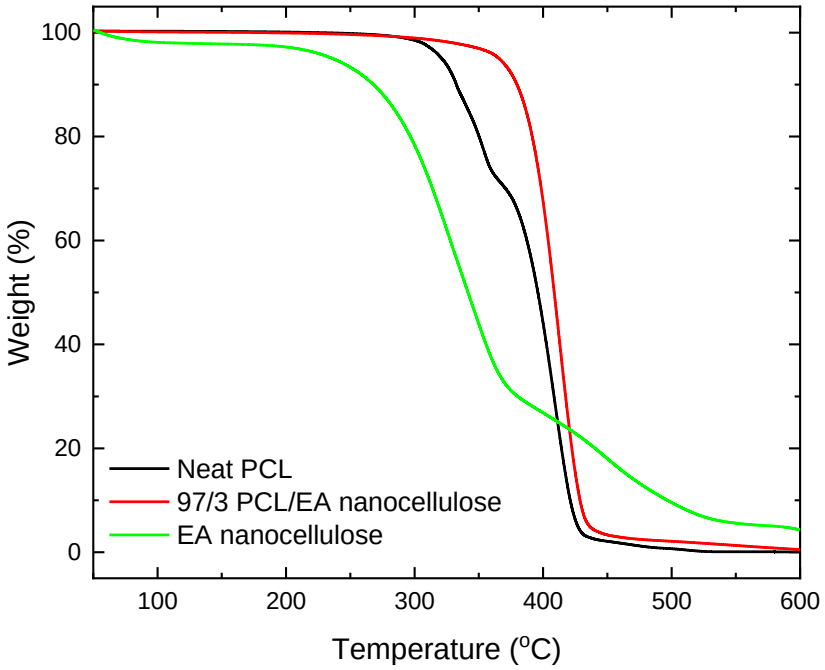

Figure 4.7: TGA curves of neat PCL, EA nanocollulose and 97/3 w/w PCL/EA nanocellulose composite

4.2.4 Thermal transitions: Differential scanning calorimetry (DSC)

The influence of EA nanocellulose on the melting temperature (T_m) of PCL was investigated using DSC. The measurements were performed at a heating and cooling rate of 20 ^oC min^{-1}. The data is summarized in Table 4.1. The normalised melting enthalpy values shown in Table 4.2 were determined using Equation 1.

$$\Delta H_m^n = \Delta H_m / w \quad (1)$$

where ΔH_m^n is the melting enthalpy normalised to the amount of the pure component in the sample (i.e., PCL). ΔH_m is the melting enthalpy of the PCL in the nanocomposite, and *w* is the weight fraction of the PCL in the nanocomposite.

Table 4.1: T_m and ΔH_m of neat PCL and the PCL/EA nanocellulose composite

Material	T_m (°C)	ΔH_m (J g^{-1})	ΔH_m^n (J g^{-1})
Neat PCL	65.9 ± 1.1	52 ± 2	52 ± 2
97/3 w/w PCL/EA nanocellulose	66.6 ± 1.2	50 ± 3	52 ± 3

Figure 4.8 shows the DSC melting curves of neat PCL and the 97/3 w/w PCL/EA nanocomposite. Neat PCL shows a melting temperature peak at 65.9 °C and a normalized enthalpy value at 52 J g^{-1}, while the nanocomposite shows a melting temperature peak at 66.6 °C and a normalized melting enthalpy value at 52 J g^{-1}. According to these results, the values are within experimental error and do not change significantly in the absence and presence of EA nanocellulose. These results are consistent with the study conducted by Zoppe *et al*., (2009) [36]; where the cellulose was extracted from a non-medicinal plant (ramie) through acid hydrolysis [36]. The electrospun cellulose fibres obtained from the ramie plant showed minimal changes in the thermal characteristics of PCL/CNC composites.

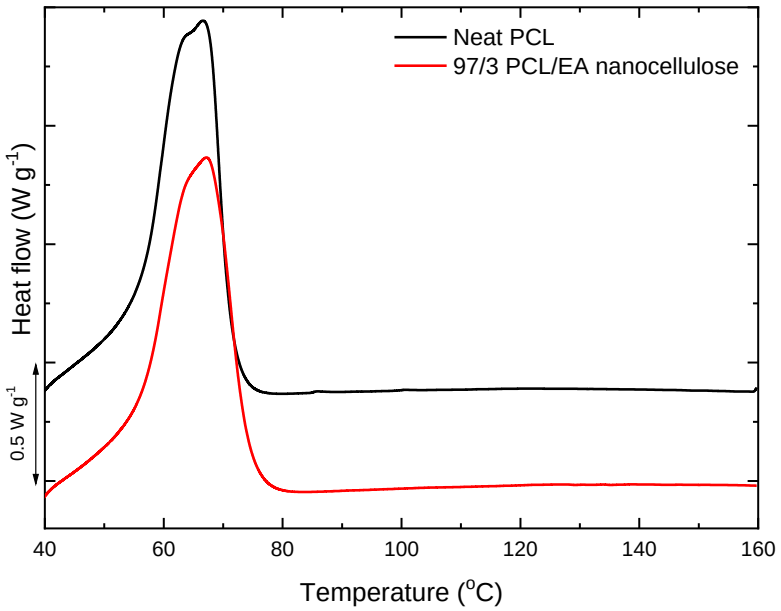

Figure 4.8: DSC second heating curves for neat PCL and the 97/3 w/w PCL/EA nanocellulose composite

4.2.5 Crystalline structure: X-ray diffraction analysis (XRD)

To determine the phase purity and crystalline nature of the samples, XRD investigation was carried out and the resulting diffraction patterns are shown in Figure 4.9. Neat PCL revealed diffraction peaks at 21.8º and 24.2º corresponding to the (110) and (200) planes of the orthorhombic crystal structure; whereas the EA nanocellulose demonstrated a diffraction peak at 22º which corresponds to the (110) plane. Furthermore, EA nanocellulose showed a (broad, short) diffraction peak which corresponds to small crystalline size, which can confirm that the crystals of EA nanocellulose are in nanoscale; whereas neat PCL showed (sharp, intense) diffraction peaks compared to the diffraction peaks of EA nanocellulose. Sharp, intense peaks correspond to large crystalline size of neat PCL, and they show that the crystals of neat PCL are well ordered compared to the crystals of EA nanocellulose. According to the DSC melting curves, PCL shows a melting temperature peak at 65.9 ºC and a normalized enthalpy value at 52 J g^{-1}, while the nanocomposite shows a melting temperature peak at 66.6 ºC and a normalized melting enthalpy value at 52 J g^{-1}.

Therefore, the results do not change significantly in the absence and presence of EA nanocellulose.

The PCL/EA nanocellulose composite showed enhanced diffraction peaks, which demonstrated enhanced crystallinity upon combination of the PCL and EA nanocellulose. The intensity of PCL/EA nanocellulose composite is very high compared to that of neat PCL. This is an indication that the presence of EA nanocellulose in the PCL matrix increases the order of the PCL crystals, and therefore the mechanical properties. In addition, there is a peak shift in the PCL/EA nanocellulose composite to lower 2θ. This may be an indication of the changes in lattice parameters induced by the addition of EA nanocellulose into the PCL lattice [37].

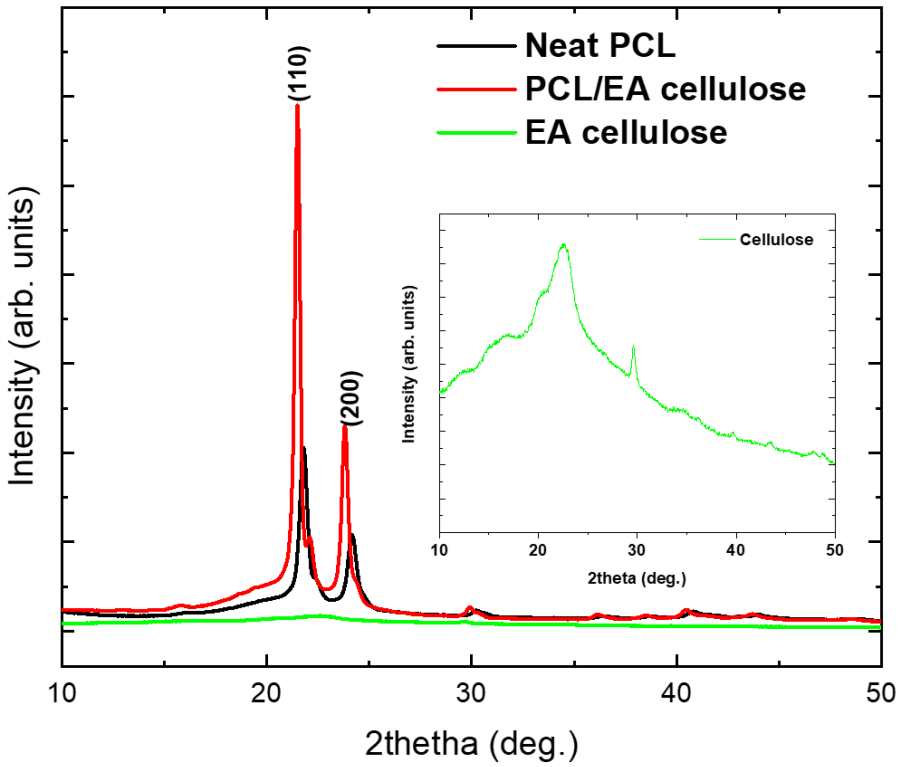

Figure 4.9: (a) XRD diffractograms of neat PCL, EA nanocellulose, and 97/3 PCL/EA nanocellulose composite

4.2.6 Mechanical properties: Tensile testing

Figure 4.10 presents the stress-strain curves for neat PCL and the 97/3 w/w PCL/EA nanocellulose composite. The Young's modulus, the yield strength, the tensile

strength, and the elongation at break of the PCL and the PCL/cellulose composites are summarized in Table 4.2.

PCL is a ductile polymer and is characterized by low stiffness [38,39]. Upon the incorporation of EA nanocellulose, the stiffness is slightly improved, as evident from higher Young's modulus values (Table 4.2. This can be attributed to the presence of rigid EA nanocellulose fibres within the PCL matrix. However, all of yield strength, tensile strength, and elongation at break slightly decreased after the addition of EA nanocellulose. This suggests that the presence of EA nanocellulose led to the reduction in ductility in the composites compared to neat PCL. In addition, the inferior mechanical properties were a result of poor interfacial interaction between PCL and EA nanocellulose, as observed from SEM results (Figure 4.6(e and f)). Similar trends were reported in other studies, where PCL was reinforced with bacterial cellulose [40] In another study, it was shown that Young's modulus has a strong dependence on cellulose fibre content [41]. The modulus increased with cellulose fibre content, suggesting that in our case, the small increment in the Young's modulus is due to the relatively low EA nanocellulose content (3 wt.%) in the composites. Concerning fracture mechanics, the same trend observed in the general mechanical properties is maintained. For example, the work of fracture was the highest in neat PCL, but was significantly reduced in the case of PCL/EA cellulose composites. This can be ascribed to poor compatibility between hydrophilic EA cellulose and the relatively hydrophobic PCL matrix. Although the EA cellulose fibres increased the Young's modulus of the composites due to their inherent stiffness, poor interfacial adhesion meant inhomogeneous stress transfer between PCL and EA cellulose. Consequently, increased local stress concentrations at the interface led to marked reduction in the strain at break, and thus fracture strength of the composites.

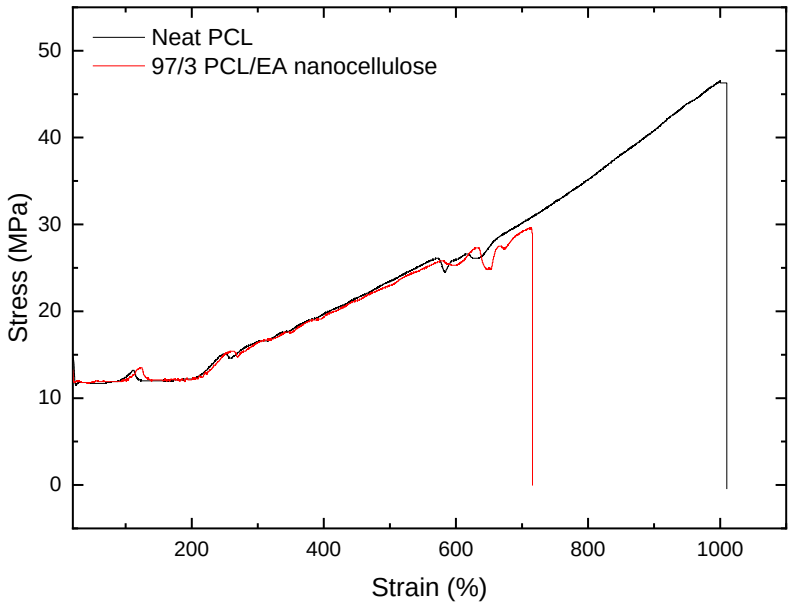

Figure 4.10: Stress-strain curves for neat PCL and the 97/3 w/w PCL/EA nanocellulose composite

Table 4.2: Young's modulus, yield strength, tensile strength and elongation at break of the PCL and the PCL/EA nanocollulose composites

Material	Young's modulus (MPa)	Yield Strength (MPa)	Tensile Strength (MPa)	Elongation at break (%)
Neat PCL	380 ± 10	15.7 ± 0.2	43.0 ± 3.1	956 ± 58
97/3 w/w PCL/EA nanocellulose	390 ± 10	14.6 ± 0.8	29.4 ± 1.0	731 ± 24

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CHAPTER 5: Conclusion

This study demonstrated the successful isolation and preparation of nanocellulose from *Eucomis autumnalis* plant leaves, through melt blending preparation method. The obtained nanocellulose was used as a filler material in a polycaprolactone matrix to create a composite material. The resulting PCL/EA nanocellulose composite showed improved mechanical properties, indicating that EA nanocellulose has great potential for use as a reinforcing agent in composites. The results demonstrate that the incorporation of EA nanocellulose into PCL can slightly enhance the stiffness of the composite, but the ductility is reduced. The FTIR spectroscopy results confirmed the successful fabrication of PCL/EA cellulose composites, indicating no chemical reactions during melt-compounding. The characteristic peaks of neat PCL and EA cellulose were evident in the spectra, with the dominance of PCL in the composite. . SEM analysis revealed that PCL formed a continuous phase with a smooth surface, while EA cellulose exhibited a fibrous morphology forming a three-dimensional network. Although the composite surface showed embedded EA cellulose fibers in the PCL matrix, fractured surfaces indicated poor interaction, potentially leading to fiber pull out. The thermal stability of PCL/EA nanocellulose composite did not change significantly in the absence and presence of EA nanocellulose. According to the DSC results EA nanocellulose showed a (broad, short) diffraction peak which corresponds to small crystalline size, which can confirm that the crystals of EA nanocellulose are in nanoscale; whereas neat PCL showed (sharp, intense) diffraction peaks; the sharp, intense peaks correspond to large crystalline size of neat PCL, and they show that the crystals of neat PCL are well ordered compared to the crystals of EA nanocellulose, this showed enhanced crystallinity. The XDR results confirm that the presence of EA nanocellulose in the PCL matrix increases the order of the PCL crystals, and therefore enhances the mechanical properties.

The study provides comprehensive insights into the structural, thermal, and mechanical characteristics of PCL/EA cellulose composites, laying groundwork for potential applications in biomedical and packaging materials.

Valuable contributions are made to the field of biocomposites and nanocellulose materials and highlights the potential of *Eucomis autumnalis* as a source of cellulose for various applications.

Future prospects

Bone tissue engineering has emerged as a promising field in regenerative medicine, which aims to develop advanced strategies for the repairing and regeneration of damaged or lost bone tissue. In this context, the combination of PCL and EA nanocellulose has gathered substantial attention due to their potential for enhancing the mechanical properties of scaffolds, also offering a novel biomaterial with enhanced mechanical strength, improved biological performance. Further studies can be carried out to explore the potential of nanocellulose from *Eucomis autumnalis* in other fields, such as drug delivery and tissue engineering. The outcomes of this research hold immense potential for advancing the field of bone tissue engineering, it also paves the way for the translation of this composite into clinical applications for bone regeneration.

Appendices

Appendix 1: Published Conference proceeding

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A Brief Overview on the Extraction of Cellulose from Medicinal Plants for Advanced Applications

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Keywords: Cellulose, extraction, medicinal plants.

Abstract. For over a thousand years, cellulose has been known as a polysaccharide that is readily available in nature. It is branded as a main constituent of the cell wall. Since the cell wall is produced by all plants, it is probably the amplest organic compound on Earth. The extraction of cellulose from medicinal plants is becoming a topic of interest. This is because compounds extracted from medicinal plants including cellulose are used as additives in pharmaceutical, neutraceutical, toxicology, and other chemical industries, for treating syphilis, kidney disorders, wound healing, ulcers, skin rash, gonorrhoea, and piles. Also, cellulose has been identified as useful for reinforcement and load-bearing purposes in composite materials due to its intrinsic stiffness and a high degree of crystallinity. However, the process of extracting cellulose, as well as the extent of the needed purity strongly depends on the application of the used polymers. This contribution focuses on medicinal plants as potential sources of bioactive cellulose.

Introduction

Medicinal plants are plants that are used in herbalism and contain extractable compounds in their leaves, stems, roots, flowers, and fruits. These extracts are used in their natural form or as additives in various industries. Medicinal plants are traditionally used for treating toothaches, syphilis, kidney disorders, wound healing, ulcers, skin rash, gonorrhoea, piles, and others [1]. Furthermore, the usage of medicinal plants in industrialized cultures has shown trends in the production of various medicines, chemotherapeutics, as well as biotechnological uses, such as biosensors and drug delivery systems [2-4].

All plants contain three major components: cellulose, lignin, and hemicellulose [5]. Cellulose is the most abundant naturally occurring organic compound and a key component in the cell wall of trees and plants [6]. It is a complex carbohydrate, or polysaccharide, consisting of about 3 000 or more glucose units. Cellulose is the organic compound consisting of a linear chain of numerous glucose units and its chemical formula is represented as $(C_6H_{10}O_5)_n$. Furthermore, cellulose plays a major economic role, as it can be processed to produce paper and fibres [7, 8]. The following are the three fundamental requirements of biomaterials: (i) biocompatibility, (ii) bioactivity, and (iii) biomechanics. Owing to its tunable chemical, physical and mechanical properties, cellulose has been considered for bio-material manufacturing. Moreover, cellulose is readily available and has unlimited sources resulting in low/reduced costs for tissue engineering. Since cellulose is insoluble in water, it is effortlessly separated from the other constituents of the plant [8, 9]. Cellulose can be easily produced, a classic production process of cellulose includes acid hydrolysis, washing, centrifugation, dialysis, and sonication to form a suspension followed by drying. However, isolation methods are subjective to the tissue of the plant that is utilized as the source of cellulose [10].

Although tremendous research has been made in developing cellulose based materials from non-medicinal plants; to date, limited information is available on the extraction of cellulose from medicinal plants as potential materials in the fabrication of biopolymers and composites. Therefore, this contribution seeks to address the possibility of using cellulose obtained from medicinal plants for advanced applications in polymer science, medicine, and engineering fields.

Medicinal Plants as a Potential Source of Bioactive Cellulose

Medicinal plants are currently gaining a lot of attention because of their unique interesting chemical compounds in their different parts responsible for their various traits [11]. These chemical compounds provide extensive leads for the development of green products across sectors. Regular screening of plant species with the objective to discover new bioactive compounds is a routine activity for many laboratories. Thus, proper identification and understanding of medicinal plants and their morphogenetic features are essential for proper analysis and application. In this section various medicinal plants characteristics are discussed, including the methods of cellulose extraction, percentage yield and crystallinity as reported in the literature.

Table 1. Summary of the methods of extraction, percentage yield and crystallinity of the cellulose extracted from medicinal plants.

Medicinal plant	Methods of cellulose extraction	Cellulose extraction (Yes/No)	Type of cellulose	% yield of cellulose	% crystallinity	References
Citrullus colocynthis	Chemical extraction processes: Deproteinization lipid extraction acid hydrolysis	Yes	Nanofibers	40	49-50	[12-14]
Kigelia Africana	Alkali treatment, bleaching treatment.	Yes	Fibers	71	Not specified	[15, 16]
Helicteres Isora	Alkaline treatment, bleaching, acidic steam treatment and homogenization.	Yes	Nanofibrils	71	90	[17, 18]
Retama raetam	Alkali and bleaching treatments.	Yes	Microfibers	52	78	[19, 20]
Syngonanthus nitens	Alkali and bleaching treatments	Yes	Fibers	67	70-91	[21]
Phormium tenax	Acid hydrolysis	Yes	Nanocrystals	61	Not specified	[22]
Eucomis autumnalis	-	No	-	-	-	[23-30]
Leacaena leucocephala	Not specified	Yes	Not specified	Not specified	Not specified	[31-34]
Imperata brasiliensis	Sulfuric acid hydrolysis	Yes	Nanofibrils	44	56-64	[35, 36]
Abelmoschus Esculentus (okra bahmia)	Sulphuric acid hydrolysis	Yes	Nanocrystals	60-70	Not specified	[37]
Oil palm empty fruit bunch	Chlorite bleaching, alkali treatment and acid hydrolysis	Yes	Not specified	Not specified	Not specified	[38, 39]

Hibiscus cannabinus (kenaf)	Alkali treatment	Yes	Fibers	69	Not specified	[40-42]
Agave sisalana (sisal fibers)	Sulfuric acid hydrolysis	Yes	Microfibers	Not specified	51	[43-46]
Humulus Japonicus (HJ)	Sulfuric acid hydrolysis	Yes	Nanocrystals	Not specified	Not specified	[47]

The isolation methods of cellulose from plants depend on the needed dimensions of the fibers. Generally, a number of studies extracted cellulose fibers and nano/micro crystals from medicinal plants using alkali and bleaching treatments as well as acid hydrolysis [12-47]. The obtained cellulose was characterized using morphological investigations (optical, scanning electron and atomic force microscopy), as well as physico-chemical techniques (infrared spectroscopy, X-ray diffraction and thermogravimetric analysis). The results demonstrated the successful extraction of cellulose from medicinal plants with a percentage yield ranging between 40-71% [12-22, 35, 36, 37, 40-42]. The percentage yield depends on the raw material and the process of extraction. From the table it can be seen that the cellulose obtained using the alkali and bleaching treatments revealed the highest cellulose yield and percentage crystallinity [17-21]. The high cellulose content suggests the possibility to use the cellulose obtained from medicinal plants in the production of biodegradable nanocomposites with enhanced properties. Fortunati *et al.* [22] investigated nanocellulose obtained from *Phormium tenax* and incorporated it into a polylactic acid (PLA) matrix to fabricate green nanocomposites. The extracted cellulose has shown to be used as a reinforcement for composites, as well as suitable as active food packaging materials.

Even though the use of *E. Autumnalis* derived compounds have been limited to pharmaceutical products [23-30], there is a need to explore their full potential such as extracting cellulose to develop new green composites and broaden its practical applications.

Conclusions

This contribution focuses on medicinal plants as a potential source of bioactive cellulose. A number of studies have shown the potential of cellulose to be extracted from various medicinal plants and potential applications. The extracted cellulose has shown to be used as a reinforcement for composites, as well as functional polymer composites. However, there is a need to investigate some medicinal plants such as *E. autumnalis* which are normally used in pharmaceutical products to develop nanocomposites with enhanced properties.

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