Preparation and characterisation of polylactic acid composites/nanocellulose extracted from *Eucomis autumnalis* for various applications

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DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO INDEPENDENT WORK

I, Sylvia Tlholohelo Sikhosana, 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 Sciences: Environmental Health, 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 or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

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Abstract

Polymers produced in living cells have attracted exceptional attention due to their eco-friendliness and biodegradability. These polymers possess similar properties to conventional plastics, making them suitable potential plastic substitutes for sustainable development and conservation of finite oil resources. Polylactic acid (PLA) is comparable to commonly used plastics but is inferior due to poor impact resistance, hydrophobicity, a lack of reactive side chain groups, a slow degradation rate, and a relatively high cost. However, PLA can benefit from the incorporation of various polymer components with complementary properties to yield improved physical properties. Developing fully bio-based composites of PLA and cellulose is particularly interesting. They have mechanical and physiological properties that make them more suitable for a wider range of applications. Herbaceous plant cell walls comprise semicrystalline cellulose fibres embedded in a soft matrix. The crystalline part of the inherently stiff chains extracted from plants such as *E. autumnalis* is subject to research for potential roles as fillers. In this study, to fabricate a novel composite consisting of PLA/Cellulose (stem) (PLA/Cs), three characteristically different celluloses were extracted from various parts of *E. autumnalis* (bulbs, leaves, and stem). Based on a distinguished cellulose spectrum (showing all the relevant functional groups), increased crystallinity and thermal stability compared cellulose from the leaves, varying contents (99.5/0.5 and 97/3.0 PLA/Cs) of a mixture of fibre bundles and coarse aggregates of cellulose from the stems were incorporated in a PLA matrix. The surface morphology and structural properties of the novel composites were investigated using FTIR and SEM. In addition, the thermal and mechanical properties of the composites were investigated using standard techniques (i.e., TGA and Tensile). Generally, there was better distribution of Cs in PLA and improved interfacial adhesion at high filler content (97/3.0), resulting in a high Young's modulus. However, no significant improvement in thermal properties was observed.

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List of symbols and abbreviations

$	US Dollar
%	Percentage
Tg	Glass transition temperature
ε_b	Strain at break
@	At
®	Registered trademark symbol
°C	Degrees Celsius
C_B	Cellulose from the bulbs
CH_2	Methylene
CH_4	Methane
C_L	Cellulose from the leaves
CO_2	Carbon dioxide
COOH	Carboxylic acids
CP	Centipoise unit/viscosity
C_S	Cellulose from the stems
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DSC	Differential Scanning Calorimetry
DTG	Derivative Thermogravimetry
E	Young’s modulus
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared
GRAS	Generally Recognised as Safe
H_2O	Water
kN	Kilonewton
MPA	Mega Pascal Unit
MPa	Megapascals
PBS	Polybutylene succinate
PDLA	Poly(D-lactide)

PDLLA	Poly(D, L-lactide)
PET	Poly(ethylene terephthalate)
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHBV	Polyhydroxybutyrate-co-hydroxyvalerate
PLA	Polylactic acid
PLLA	Poly(L-Lactide)
PS	Polystyrene
RNA	Ribonucleic acid
ROP	Ring-Opening Polymerization
SEM	Scanning electron microscopy
TGA	Thermogravimetric analysis
TPS	Thermoplastic starch
XRD	X-Ray Diffraction
α	Alpha
β	Beta
ε_b	Strain at break
θ	Angle
σ_b	Stress at break
σ_y	Stress at yield

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CHAPTER 1

Introduction

1.1. Overview

1.1.1. Background

Plastics have been identified as one of the major environmental pollutants globally. The accumulation of plastic products in the environment resulting from ineffective waste management systems is known as plastic pollution (Hidayah & Syafrudin, 2018; Chamas *et al.*, 2020). From the total plastic solid waste generated, 58% gets disposed in landfills across the globe, 18% is recycled and, 24% is incinerated (Chamas *et al.*, 2020) (Figure 1.1). Plastic commodities do not only pose harm when discarded but, throughout their life cycle, they continuously release toxins in the ecosystem. Increased consumer demand and dependence on plastic consumables result in the continuous production of plastic, leading to the release of toxic compounds into the ecosystem during and after synthesis. Due to the lack of implementation of legislation on the consumption of plastic and improper disposal practices, particularly in developing countries such as South Africa, an increase in plastic waste continues to mount uncontrollably in agricultural land and water bodies, thus posing health and environmental concerns (Ahmed *et al*., 2018; Frost, 2019; MARPLASTICCs, 2020). Moreover, using non-renewable sources such as fossil fuels to produce plastics also threatens energy security (Ferronato & Torretta, 2019). An estimated 380 million tons of plastics are synthesised across the globe annually and, their consumption increases by 5% every year (Rajmohan *et al*., 2019; Ritchie & Roser, 2018).

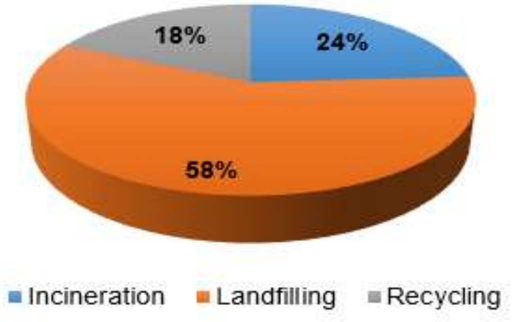


Figure 1.1. Global figures of waste management systems

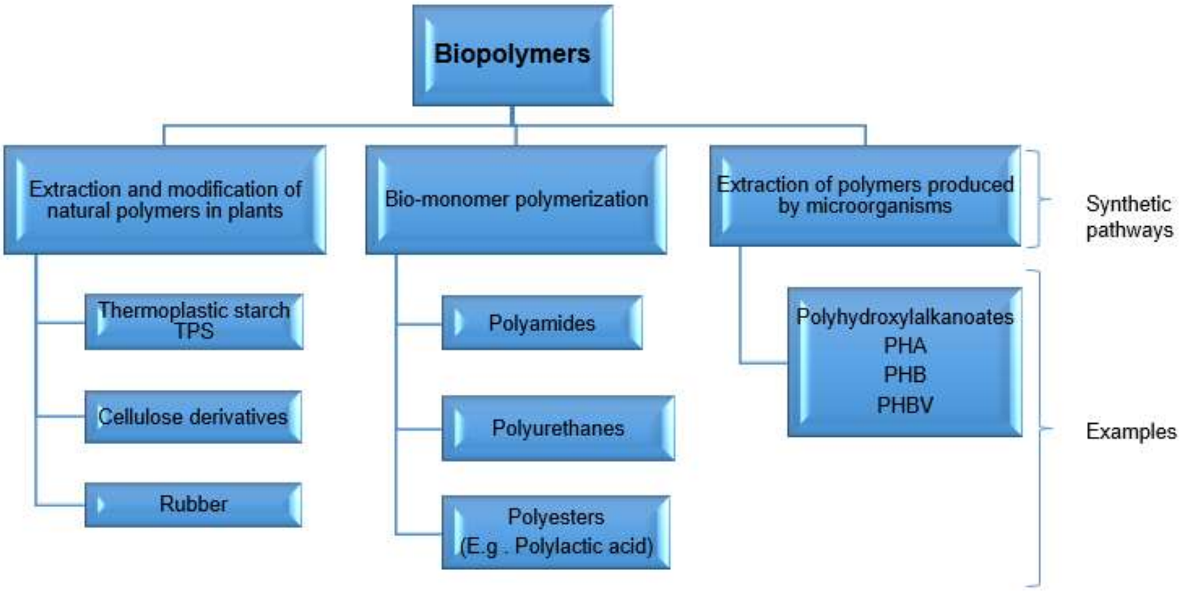
The word “plastic,” which is derived from the Greek word *plastikos*, is a general term used to describe a variety of high molecular weight and chemically synthesized polymers derived from different hydrocarbon and petroleum-derived compounds (Ahmed *et al*., 2018; Rajmohan *et al*., 2019). Thus, natural gas, coal, and petroleum are the primary raw materials used to make plastic polymers. Due to its unique properties, such as durability, lightweight, low cost, robust strength, corrosion resistance, thermal and electrical insulation, and numerous fabrication and design possibilities, plastics may be easily moulded into a range of consumables (Grigore, 2017; Thompson *et al*., 2009). Plastics have a broad application spectrum, and common applications include packaging, medical, construction, electronics, electrical goods, furniture, automobiles, households, agriculture, and many other industrial uses (Hidayah & Syafrudin, 2018; Singh *et al*., 2017). Since plastics are designed in such a way that they resist any form of degradation, the same durability that makes them ideal commodities for different applications results in waste disposal challenges. Most commercial plastics are very stable in the environment and do not break down into simple compounds by microbial action, which then leads to their accumulation when disposed of. As a result, the effects of plastics persist for decades, long after their disposal (Ahmed *et al*., 2018; Chamas *et al.*, 2020; Ilyas *et al.*, 2018).

Managing plastic pollution is a pervasive and persistent challenge for everyone, hence the adoption of numerous plastic waste handling strategies by local and international governments (Prata *et al*., 2019; Rajmohan *et al*., 2019). These strategies include decomposing in landfills, recycling, incineration, microbial degradations and conversion into useful materials. However, the same strategies meant to remediate the undesirable effects of plastics are in most instances costly, labour-intensive, time-consuming, and less effective and they tend to leave footprints in the environment through the emission of toxins during processing. Thus far, mitigation strategies have only been able to reduce the pressure exerted by plastic waste on the environment but are unable to handle the amount of plastic that is being disposed of or reverse its detrimental effects (Alabi *et al*., 2019; Ferronato & Torretta, 2019; Prata *et al*., 2019; Rajmohan *et al*., 2019; Singh *et al*., 2017). This has prompted various stakeholders to revise existing policies and innovate technologies that will be both economically feasible and environmentally friendly for plastic waste management (Ahmed *et al.*, 2018; Hidayah & Syafrudin, 2018; Ilyas *et al.*, 2018).

According to Seltenrich *et al.*, 2015, the next generation of plastic materials should be designed in such a way that they are readily biodegradable commodities to reduce their potential accumulation in the environment. However, while the terms 'biodegradable' and 'biopolymers' are often used interchangeably, 'biodegradable' does not necessarily mean a material is green or eco-friendly. Biodegradability of a polymer material signifies whether the chemical structure of the material can be metabolized into shorter polymer fragments through the action of microorganisms, that is, bacteria, fungi and algae. As such, biodegradable polymers are susceptible to microbial action but can be derived from either natural or petrochemical sources (Asimov, 2013; Kaushik *et al*., 2016; Kiruthika, 2020). Cleavage of these macromolecules into smaller fragments leads to larger surface areas and increased dispersion of the polymer molecules across various ecosystems, which can result in more contamination, more especially with biodegradable oil-based polymers that contain additives to enhance their properties. When degraded, a blend of macro-, meso-, and micro-sized polymer segments, leached additives, and byproducts of degradation may be released into the receiving environment. These compounds are usually absorbed into the food chain and pose danger to all living organisms (Ferronato & Torretta, 2019; Lambert, 2014). On the other hand, biopolymers, also known as bioplastics, are organic molecules that are composed of repeating monomer units and are produced by living organisms. As a result, they are important to all living matter and found everywhere. Therefore, biopolymers are a class of biomaterials that represent a suitable alternative for maintaining economically and environmentally sustainable development of attractive technologies (Asimov, 2013; Kaushik *et al*., 2016; Kiruthika, 2020).

1.1.2. Justification

Nature constitutes a great source of organic materials, and it is the only source of renewable materials and energy available to humans. Biopolymers are abundant in nature and their sources include plants, animals, and microorganisms (Asimov, 2013; Kaushik *et al*., 2016). Thus, biopolymers can be isolated from these sources and developed into commercial plastics using different techniques, depending on the type of the raw material (Scheme 1). Typically, the processes include: (i) the extraction of polymers produced in microorganisms; (ii) the extraction and modification of natural polymers from plant material; and (iii) polymerization of bio-based monomers. Converse to petrochemical polymers, polymers that are fully derived from natural sources degrade completely and their degradation products are simple compounds like water and carbon dioxide that can be reused by other organisms. Again, most polymers from natural sources are common in all living matter and therefore not harmful to either humans or the environment. Cellulose, lignin, hemicellulose, starch, protein, and modified compounds, as natural polymers, have been used extensively for the fabrication of biopolymers and composites, and for other applications including food, cosmetics, and medicine (Asimov, 2013; Jaiswal *et al.*, 2019; Kaushik *et al.*, 2016; Zhong *et al*., 2019).



Scheme 1.1. Polymers of natural origin

Amongst the most widely used natural polymers, polylactic acid (PLA) has received considerable attention because it is usually derived from starch-based plants like corn, potatoes, and cane through fermentation (Farah *et al*., 2016; Vink *et al*., 2003). PLA possesses high mechanical strength, thermoplastic characteristics, good processibility and biocompatibility. However, properties of PLA such as poor impact resistance, hydrophobicity, lack of reactive side chain groups, slow degradation rate and relatively high cost make it inferior to those of conventional petroleum-based polymers and limit its use to certain medical and packaging applications (Jaiswal *et al*., 2019; Kiruthika, 2020; Orellana *et al.*, 2018). To overcome these limitations and expand its scope of application, current advances in polymer science include the deployment of cellulose in PLA infusions as complementary materials. A composite of PLA and cellulose nanocrystals may exhibit a synergistic effect compared to individual components. Cellulose is another naturally occurring polymer widely used as a filler to address limitations suffered by other polymers because of its superior mechanical properties, tunability, abundance, and eco-friendliness. Cellulose is the major constituent of plant cell walls, and it is potentially attractive in terms of cost effectiveness and non-toxicity (Alaribe *et al*., 2018; Jaiswal *et al*., 2019; Kiruthika, 2020; Mishra *et al*., 2017).

Several studies have been conducted to investigate the potential reinforcement properties in PLA by using cellulose extracted from various medicinal (Zhang *et al*., 2018; Zhou *et al*., 2018) and non-medicinal plants (Costa *et al*., 2015; Sun, 2004; Szymańska-Chargot *et al*., 2020). Although tremendous research has been conducted on cellulose-based materials derived from non-medicinal plants (i.e., sugar cane bagasse, maize stalk, etc) as potential materials for fabricating PLA composites, cellulose extracted from medicinal plants is also interesting. This is because it is used as an additive in pharmaceutical, neutraceutical, toxicology and other chemical industries, not only for the treatment of diseases but also as potential material for maintaining good health and environmental conditions (Oladeji, 2016; Alaribe *et al.*, 2018; Bamola *et al.*, 2018). Consequently, current trends in biotechnology and polymer science include the employment of indigenous knowledge acquired from the traditional use of medicinal plants and their functional activities, as a guide to discovering polymeric material with potentially new characteristics, unique to the source material (Xie *et al*., 2016). *Eucomis autumnalis* is one of the most valuable African traditional medicinal plants that are commonly used in herbalism for treatment of various ailments including wounds, pain, inflammation, and other diseases. This bulbous plant is known to exhibit versatile biological properties such as anti-microbial, anti-inflammatory, anti-cancer, anti-oxidative and anti-histaminic. Despite the widely acclaimed therapeutic values, proper identification and understanding of *E. autumnalis* and its morphogenetic factors are yet to be efficiently evaluated (Alaribe *et al*., 2018;

Masondo *et al*., 2014). To date, little or no information is available on the use of this plant species as a potential source of cellulose for incorporation in PLA matrices. Therefore, this study explores the possibility of using cellulose obtained from an unusual source; namely, *E. autumnalis,* and blending it with polylactic acid (PLA) for various industrial applications.

1.2. Problem statement

The continued production of petroleum-based plastics and the accumulation of plastic products in the environment has long-term detrimental effects on the ecosystem and its habitats (Hidayah & Syafrudin, 2018; Chamas *et al.*, 2020).

1.2.1. The sub problems in relation to the problem statement include:

- Increased plastic production renders current remediation strategies ineffective or less effective, resulting in increased pollution.
- Potential plastic substitutes, such as biopolymers like polylactic acid and cellulose, are inferior to commercial plastics, and their applications are limited, necessitating additional research to broaden this aspect.

1.3. Research aims

The aim of the study is to improve the properties of PLA by blending it with cellulose extracted from the *E. autumnalis* medicinal plant and assessing its potential for various industrial applications.

1.4. Research objectives

- To collect and process *E. autumnalis* (drying and milling);
- To extract cellulose from the plant parts (leaves, stem, and bulbs) with potentially new features using the acid and alkaline treatment;
- To prepare composites by melt-blending the extracted cellulose with polylactic acid (PLA); and

- To characterise the PLA/cellulose composites using different characterization techniques and study the thermal, thermo-mechanical, structural, and mechanical properties, as well as the morphology of cellulose and its dispersion within the PLA matrix.

1.5. Structure of the dissertation

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

1.6. References

Ahmed, T., Shahid, M., Azeem, F., Rasul, I., Ali, S.A., Noman, M., Hameed, A., Manzoor, N., Manzoor, I. & Muhammad S. **2018**. Biodegradation of plastics: Current scenario and future prospects for environmental safety. *Environmental Science and Pollution Research*. 25(8): 7287–7298. https://doi.org/10.1007/s11356-018-1234-9

Alabi, O.A., Ologbonjaye, K.I., Awosolu, O. & Alalade, O.E. **2019**. Public and environmental health effects of plastic wastes disposal: A review. *Journal of Toxicology and Risk Assessment*. 5(1): 1–13. https://doi.org/10.23937/2572-4061.1510021

Alaribe, F.N., Maepa, M.J., Mkhumbeni, N. & Motaung, S.C.M.K. **2018.** Possible roles of *Eucomis autumnalis* in bone and cartilage regeneration: A review. *Tropical Journal of Pharmaceutical Research.* 17(4): 741–747. https://doi.org/10.4314/tjpr.v17i4.25

Bamola, N., Verma, P. & Negi, C. **2018**. A review on traditional medicinal plants. *Institute of Training and Research*. India. 4(1): 1550–1556. https://doi.org/10.21276/ijlssr.2018.4.1.7

Asimov, I. **2013**. Biopolymers. In Rudin, A. & Choi, P. (Ed.). *Biopolymers in the elements of polymer science and engineering*. Cambridge: Academic Press. pp 512–535.

https://doi.org/10.1016/B978-0-12-382178-2.00013-4

Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J.H., Abu-Omar, M., Scott, S. L. & Suh S. **2020**. Degradation rates of plastics in the environment. *ACS Sustainable Chemical and Engineering*. 8(1): 3494−3511. https://dx.doi.org/10.1021/acssuschemeng.9b06635

Costa, L.A.S., Assis, D. de J., Gomes, G.V.P., Silva, J.B.A. da, Fonsêca, A. F. & Druzian, J. I. **2015**. Extraction and characterization of nanocellulose from corn stover. *Materials Today: Proceedings*. 2(1): 287–294. https://doi.org/10.1016/j.matpr.2015.04.045

Farah, S., Anderson, D.G. & Langer, R. **2016**. Physical and mechanical properties of PLA, and their functions in widespread applications — A comprehensive review. *Advanced Drug Delivery Reviews*. 107(2016): 367–392. https://doi.org/10.1016/j.addr.2016.06.012

Ferronato, N. & Torretta, V. **2019**. Waste mismanagement in developing countries: A review of global issues. *International Journal of Environmental Research and Public Health*. 16(6): 1–28. https://doi.org/10.3390/ijerph16061060

Frost, F.H. **2019**. “Is the regulation of single-use plastic in South Africa a waste of time?” (Masters Dissertation.) University of KwaZulu-Natal, KwaZulu-Natal, South Africa.

Grigore, M. **2017**. Methods of recycling, properties, and applications of recycled thermoplastic polymers. *Recycling*. 2(4): 1–11. https://doi.org/10.3390/recycling2040024

Hidayah, N. & Syafrudin. **2018**. A Review on landfill management in the utilization of plastic waste as an alternative fuel. (“E3S Web of Conferences: EDP Sciences – 05013” held in Diponegoro). School of Postgraduate Studies, Indonesia. https://doi.org/10.1051/e3sconf/20183105013

Ilyas, M., Ahmad, W., Khan, H., Yousaf, S., Khan, K. & Nazir, S. **2018**. Plastic waste as a significant threat to environment – A systematic literature review*. Reviews on Environmental health*. 33(4): 1–24. https://doi.org/10.1515/reveh-2017-0035

Jaiswal, L., Shankar S. & Rhim J.-W. **2019**. Applications of nanotechnology in food microbiology. In Gurtler, V., Ball, A.S. & Soni, S. (Eds.). *Methods in Microbiology.* Netherlands: Elsevier. pp 43–60.

https://doi.org/10.1016/bs.mim.2019.03.002

Kaushik, K., Sharma, R.B. & Agarwal, S. **2016**. Natural polymers and their applications. *International Journal of Pharmaceutical Sciences Review and Research.* 37(2): 30–36.

Kiruthika, A.V. **2020**. Properties and end-of-life of polymers from renewable resources. In Hashmi, S. & Choudhury, I.A. (Eds.) *Encyclopedia of Renewable and Sustainable Materials*. Amsterdam: Elsevier. pp 253–262. https://doi.org/10.1016/B978-0-12-803581-8.10603-4

Lambert, S., Sinclair C.J. & Boxall A.B. **2014**. Occurrence, degradation and effect of polymer-based materials in the environment. *Reviews of Environmental Contamination and Toxicology*. 227(1): 1–53. https://doi.org/10.1007/978-3-319-01327-5_1

MARPLASTICCs. **2020**. The legal, policy and institutional frameworks governing marine plastics in South Africa: Exchange of perspectives to define priorities. *Published online at www.iucn.org*.
Accessed on: 19 November 2021
https://www.iucn.org/sites/dev/files/content/documents/webinar_report_south_africa_05112020

Masondo, N.A., Finnie, F.F. & Van Staden, J. **2014**. Pharmacological potential and conservation prospects of the genus *Eucomis* (Hyacinthaceae) endemic to South Africa. *Journal of Ethnopharmacology*. 151(1): 44–53. https://doi.org/10.1016/j.jep.2013.11.002

Mishra, R.K., Sabu, A.B. & Tiwari, S.K. **2017**. Materials chemistry and the futurist eco-friendly applications of nanocellulose: Status and prospect. *Journal of Saudi Chemical Society*. 22(8): 949–978. https://doi.org/10.1016/j.jscs.2018.02.005

Oladeji, O. **2016**. The characteristics and role of medicinal plants: Some important medicinal plants in Nigeria. *Natural Products: An Indian Journal*. 12(1):1–8.

Pawar, R.P., Tekalea, S.U., Shisodiaa, S.U., Totrea, J.T. & Dombb, A.J. **2014**. Biomedical applications of poly (lactic acid). *Recent Patents on Regenerative Medicine*. 4(1): 40–51. https://doi.org/10.2174/2210296504666140402235024

Orellana, J.L., Wichhart, D. & Kitchens, C.L. **2018**. Mechanical and optical properties of poly(lactic acid) films containing surfactant-modified nanocrystals. *Hindawi Journal of Nanoparticles*. 2018(1): 1–12.
https://doi.org/10.1155/2018/7124260

Prata, J.C., Silva, A.L.P., da Costa, J.P., Mouneyrac, C., Walker, T.R., Duarte, A.C. & Rocha-Santos, T. **2019**. Solutions and integrated strategies for the control and mitigation of plastic and microplastic pollution. *International Journal of Environmental Research and Public Health*. 16(13): 1–19.
http://doi.org/10.3390/ijerph16132411

Rajmohan, K.V.S., Ramya, C., Viswanathan, M.R. & Varjani, S. **2019**. Plastic pollutants: Effective waste management for pollution control and abatement. *Current Opinion in Environmental Science and Health*. 12(12):72–84.
https://doi.org/10.1016/j.coesh.2019.08.006

Ritchie, H. & Roser, M. **2018**. "Plastic Pollution". *Published online at OurWorldInData.org.*
Accessed on: 07 January 2022
https://ourworldindata.org/plastic-pollution

Seltenrich, N. **2015**. Newlink in the food-chain? Marine plastic pollution and seafood safety. *Environmental Health Perspectives*. 123(2): 34–41.
https://doi.org/10.1289/ehp.123-A34

Singh, N., Hui, D., Singh, R., Ahuja, I.P.S., Feo, L. & Fraternali, F. **2017**. Recycling of plastic solid waste: A state of art review and future applications. *Composites Part B: Engineering*. 115(2017): 409–422.
https://doi.org.10.1016/j.compositesb.2016.09.013

Sun, J. **2004**. Isolation and characterization of cellulose from sugarcane bagasse. *Polymer Degradation and Stability*. 84(2): 331–339.
https://doi.org/10.1016/j.polymdegradstab.2004.02.008

Szymańska-Chargot, M., Chylińska, M., Pieczywek, P.M., Walkiewicz, A., Pertile, G., Frąc, M. & Zdunek, A. **2020**. Evaluation of nanocomposite made of poly(lactic) acid and nanocellulose from carrot pomace modified with silver nanoparticles. *Polymers*. 12(4): 1–14.
https://doi.org/10.3390/polym12040812

Thompson, R.C., Moore, C.J., vom Saal, F.S. & Swan, S.H. **2009**. Plastics, the environment and human health: current consensus and future trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 364(1526): 2153–2166. https://doi.org/10.1098/rstb.2009.0053

Vink, E.T.H., Ra´bago, K.R., Glassner, D.A. & Gruber, P.R. **2003**. Applications of life cycle assessment to NatureWorks^{TM} polylactide (PLA) production. *Polymer Degradation and Stability*. 80(3): 403–419. http://doi.org/10.1016/S0141-3910(02)00372-5

Xie, J, Jin, M., Morris, G.A., Zha, X., Chen, H., Yi, Y., Li, J., Wang, Z., Gao, J., Nie, S., Shang, P. & Xie, M. **2016**. Advances on bioactive polysaccharides from medicinal plants. *Critical Reviews in Food Science and Nutrition*. 56(1): S60–S84. https://doi.org/10.1080/10408398.2015.1069255

Zhang, L., Li, Z., Pan, Y.-T., Yáñez, A. P., Hu, S., Zhang, X.-Q., Wang, R. & Wang, D.-Y. **2018**. Polydopamine induced natural fiber surface functionalization: a way towards flame retardancy of flax/poly(lactic acid) biocomposites. *Composites Part B: Engineering*. 154: 56–63. https://doi.org/10.1016/j.compositesb.2018.07

Zhong, Y., Godwin, P., Jin, Y. & Xiao, H. **2019**. Biodegradable polymers and green-based antimicrobial packaging materials: A mini-review. *Advanced Industrial and Engineering Polymer Research.* 3(1): 27–35. https://doi.org/10.1016/j.aiepr.2019.11.002

Zhou, L., He, H., Li, M., Huang, S., Mei, C. & Wu, Q. **2018**. Enhancing mechanical properties of poly (lactic acid) through its in-situ crosslinking with maleic anhydride-modified cellulose nanocrystals from cottonseed hulls*. Industrial Crops and Products*. 112: 449–459. https://doi.org/10.1016/j.indcrop.2017.12.044

CHAPTER 2

Literature Review

2.1. Biopolymers: origin, uses, markets

Biopolymers are the building blocks of nature found in all living matter either of plant, animal, or microbial origin (Jaiswal *et al.*, 2019; Wankhade, 2020). These biomacromolecules play pivotal roles in the growth and development of all living organisms (Sampath *et al*., 2016; Swain *et al.*, 2018). The use of biopolymers can be traced back to pre-historic times when the Egyptians used natural fibres as reinforcing materials in bricks, while the Babylonians used them for burial purposes. Cellulosic materials such as paper reed, cotton and linen fibres were used for making garments, paper, and pottery, among others (Mwaikambo, 2006). Thus, by the time the Americans started fabricating and commercializing water-proof rubber balls, garments and shoes, the Mayans had long been extracting and using rubber from a *Hevea* tree for the same purpose (Marturano *et al*., 2017). Natural polymers were introduced into the industry much earlier than synthetic polymers. However, in the second half of the twentieth century, global oil exploration changed everything, and they were mostly replaced by petroleum-based synthetic polymers (Bao, 2015).

In the natural environment, biopolymers can be found in almost all living organisms, including in seed tissues, stems and leaves of medicinal plants, body fluids of animals, cell walls and extracellular fluids of bacteria, yeast, and fungi (Jaiswal *et al.*, 2019; Wankhade, 2020; Xie *et al.*, 2012). Thus, the prefix 'bio' suggests that the sources of biopolymers are natural and therefore can be consumed by all living organisms. Biopolymers are synthesized from carbon-neutral sources and comprise mainly of polymeric material such as proteins and carbohydrates that impart improved biodegradability relative to synthetic polymers. They are the most organic compounds in the ecosphere synthesized during the growth of living organisms (Barkhad *et al*., 2020; Kabir *et al*., 2020; Prodanović & Milutinović, 2017; Shamsuddin *et al*., 2017).

More recently, the growing interest in a sustainable environment and improved quality of life has prompted researchers across the globe to study biopolymers for potential roles in the development of "green" or environmentally friendly sustainable substitutes. The use of green material is crucial in modern societies to address issues concerning the finite fossil fuel resources, health hazards, environmental threats, and solid waste management (Prodanović & Milutinović, 2017; Wankhade, 2020; Barkhad *et al*., 2020). Although their development is still at a rudimentary stage, biopolymers present more advantages than disadvantages as described in Table 2.1. Moreover, they comply with most of the requirements for material design and development, all while meeting consumer demands for material supplements, optimized land use, increased plant biodiversity, reduced pollution, and increased energy efficiency (Ibrahim *et al*., 2019; Prodanović & Milutinović, 2017; Swain *et al*., 2018).

Table 2.1. Advantages and Disadvantages of biopolymers across sectors

Advantages	**References**
• Biopolymers are fully based on biological compounds (Bio-based).	(Choudhury, 2019; Ibrahim *et al*., 2019)
• Their synthesis consumes less 'oil (petroleum)'.	(Choudhury, 2019; Ibrahim *et al*., 2019)
• Reduced emission of greenhouse gasses during production, thereby protecting the environment. ○ Other polymers require 60% more greenhouse gases and 50% more non-renewable energy than Ingeo® (PLA from Nature Works).	(Choudhury, 2019; Farah *et al*., 2016; Ibrahim *et al*., 2019; Vink, 2003)
• Natural polymers proliferate water and nutrient retention and increase soil organic content while suppressing plant disease and reducing chemical inputs.	(Ibrahim *et al*., 2019)
• Specific characteristics, such as biodegradability, reduced weight, etc.	(Ibrahim *et al*., 2019; Jaiswal *et al*., 2019)
• Development of rural areas through an increase in the number of workplaces (Nurseries, forestry) and saving of resources (fossil fuels).	(Prodanović & Milutinović, 2017)
Disadvantages	**References**

• competition over natural resources that can be used as food and energy sources.	(Choudhury, 2019)
• Before recycling, additional sorting is required to avoid contamination.	(Choudhury, 2019; Hottle, 2015; Ibrahim *et al*., 2019)
• Inferior performance to oil-based polymers in durable applications due to mechanical problems—poor heat and moisture resistance.	(Choudhury, 2019; Jaiswal *et al*., 2019; Kiruthika, 2020; Orellana *et al*., 2018)

Biopolymers are gradually migrating into mainstream use and may soon be competing with common petroleum-based synthetic polymers in the market. The production of chemicals and materials from bio-based raw material is expected to increase by 25% of the global market by 2030. Renewable resources are predicted to eventually account for two-thirds of the $1.5 trillion global chemical industry (Mohanty *et al*., 2005). Different market segments are gradually adopting biopolymers for numerous applications, as shown in Figure 2.1. As such, biopolymers remain a reasonable option to plastic waste generated from a variety of sources linked with traditional petroleum-derived plastics.

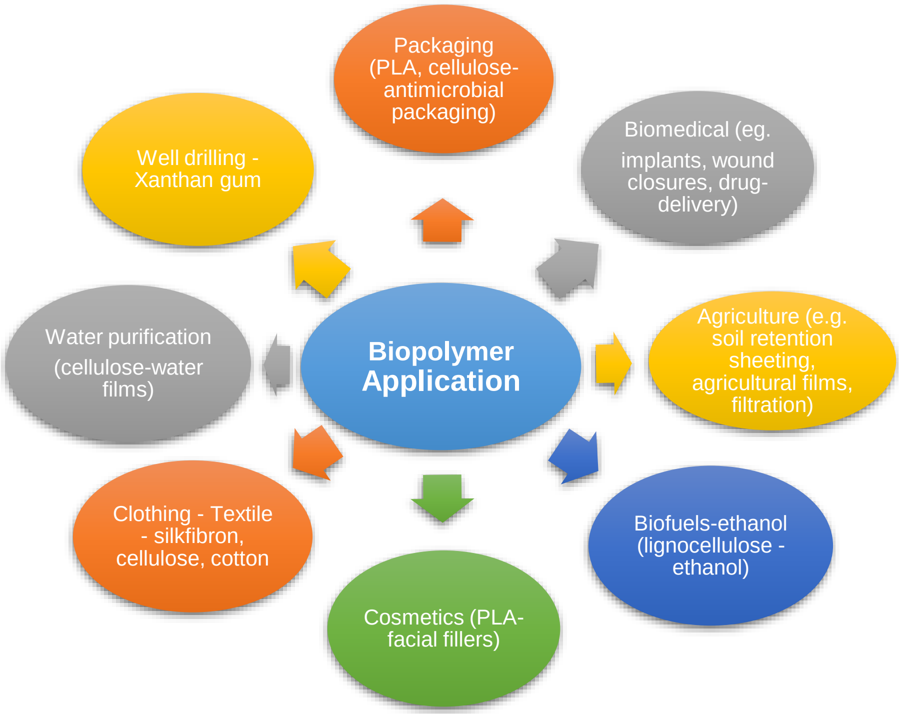


Figure 2.1. Different markets of biopolymers and their uses

Examples of commercial biopolymers include cellulose plastics, polylactides, starch plastics, soy-based plastics, and polyhydroxyalkanoate polymers (polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), polyhydroxybutyrate-co-hydroxyvalerate (PHBV)). Among these biopolymers, polylactic acid has received the most attention because it is as tough as polymers such as polyethylene, polypropylene, and polystyrene. PLA can be produced from renewable materials such as corn, wheat bran, rice bran and sugarcane bagasse. The United States Food and Drug Administration (FDA) awarded it the GRAS (Generally Recognized as Safe) status, and its feasibility in packaging and biomedical applications has been proven (Fairag *et al*., 2019; Kim *et al*., 2019; Pawar *et al*., 2014; Sikhosana *et al*., 2021). Again, its tuneable properties that permit ease of manipulation with various fabrication techniques have led to its broad evaluation as a potential material for tissue engineering and automobile interior parts among others (Fairag *et al*., 2019; Petinakis *et al*., 2013; Sawpan, 2011; Sikhosana *et al*., 2021).

2.2. Polylactic acid

Polylactic acid is a polymer of lactic acid with good biocompatibility and degradability, and its degradation products are not harmful. Due to the monomer lactic acid that can be acquired by fermentation of sugars derived from organic sources, PLA is thus a harmless, environmentally friendly polymer with properties that allow use in both humans and the environment. Commercial synthesis of PLA starts with the synthesis of lactic acid and finishes with its polymerization, with lactide generation as an intermediary step. Currently, polymers based on lactic acid are the most promising category of polymers made from renewable resources. Generally, PLA is synthesized in three different steps: (i) microbial fermentation of lactic acid, (ii) purification of lactic acid followed by production of its cyclic dimer (lactide) and (iii) polycondensation of lactic acid or ring-opening polymerization (ROP) of lactides. A summary of these steps is illustrated in Figure 2.2. The ROP is the most preferred method of PLA synthesis because when employed, it is possible to control the chemistry of polymerization accurately and thus, the properties of the resulting polymer can be varied to suit the application (Byun & Kim, 2014; Lopes *et al.*, 2012; Singhvi *et al*., 2019). In 2019 the total production volume of PLA was projected around 190,000 tons globally (Jem &

Tan, 2020). PLA synthesis consumes less fossil energy compared to petroleum-based polymers and has the potential to reduce air and water pollutant emissions significantly. Again, reduced consumption of energy makes PLA production potentially advantageous concerning cost as well (Byun & Kim, 2014; Lopes *et al.*, 2012; Singhvi *et al*., 2019; Jem & Tan, 2020).

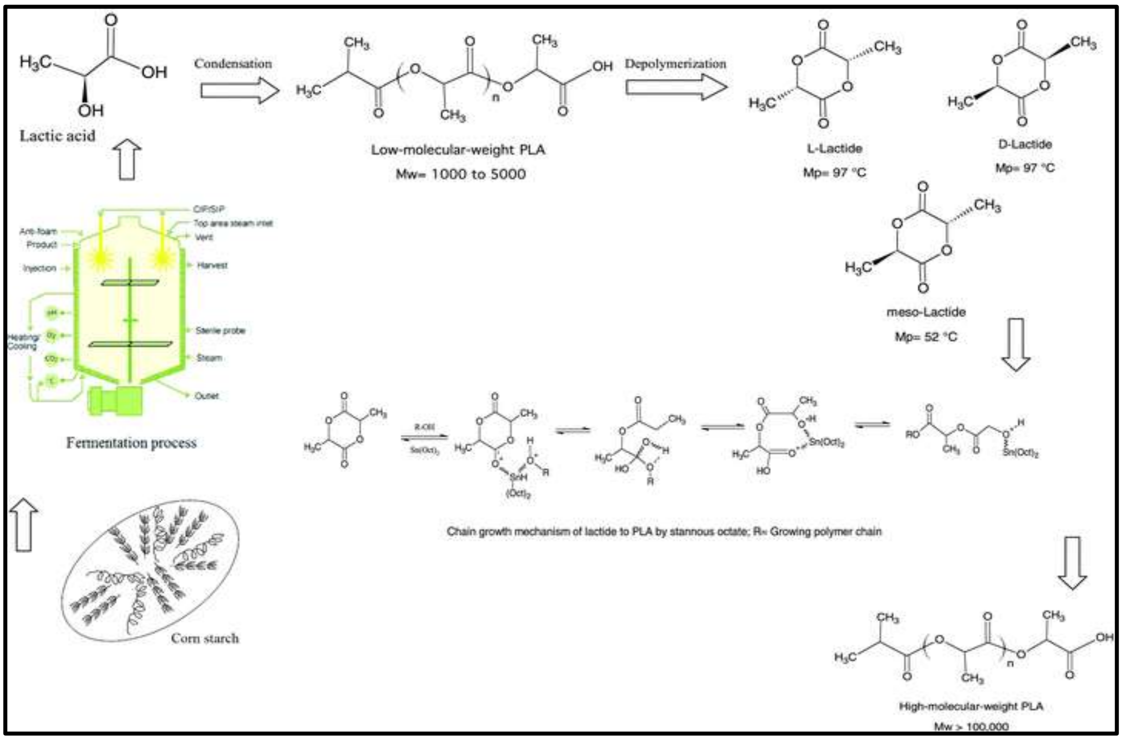


Figure 2.2. Current production steps for PLA. Reproduced with permission from (Jamshidian *et al*., 2010)

The employment of aliphatic thermoplastic polyesters such as PLA, presents a feasible alternative to petroleum-based plastics for commercial use, due to their exceptional properties. PLA possesses better transparency, durability and mechanical strength in comparison to other biopolymers such as PHA, PHB, PHBV, PBS and others (Farah *et al*., 2016; Jem & Tan, 2020; Naser *et al*., 2021). As a result, PLA has been widely adopted in the manufacturing of short life-time packaging products, such as food packaging and single-use items. Furthermore, due to the unique properties of PLA, such as eco-friendliness, biocompatibility, biodegradability, mechanical strength and processability, PLA has developed into a significant polymer for biological applications such as sutures, screws, and fracture fixation plates (Haider *et al*., 2020; Nagarajan *et al*., 2016; Peelman *et al*., 2015; Sikhosana *et al*., 2021).

Despite having tensile strength and elastic modulus comparable to poly(ethylene terephthalate) (PET) and polystyrene (PS), the use of PLA is limited in applications requiring plastic deformation at higher stress levels due to low heat resistance and poor toughness in comparison to conventional petroleum-based plastics. PLA has a low heat distortion temperature and is not resistant to long-term processing. Therefore, limiting its use in applications that involve extensive heat exposure, especially food undergoing heat treatment before or after packaging, automotive industries or housing electronic devices, as the polymer may degrade during processing (Farah *et al*., 2016; Haider *et al*., 2020; Peelman *et al*., 2015; Shankar & Rhim, 2018; Zhao *et al*., 2020).

Heat resistance of a material refers to the ability to retain the desired quality for a prolonged period at a predefined temperature (Nagarajan *et al*., 2016). One of the most important criteria for selecting materials is their ability to withstand heat. Numerous studies have investigated various techniques to improve the heat resistance of biopolymers (Barkhad *et al*., 2020; Jin *et al*., 2019). PLA heat resistance is strongly linked to its degree of crystallinity and crystallization behaviour. The crystallization model of PLA exists in three varying forms; that is, the crystalline, rigid amorphous and mobile amorphous sections. The molecular chains of the crystalline region are not likely to move because of strong intermolecular interactions when a polymer approaches its glass transition temperature (T_g), but chains of the amorphous phase move freely. However, inside the amorphous region, some chain segments are rigid and as a result hinder free movement of the entire long chain. PLA which has a very low degree of crystallinity has a large proportion of its chains in the mobile amorphous fraction with high mobility close to its T_g and therefore exhibits very low heat resistance, including distortion temperatures often occurring close to its T_g. Facilitation of PLA crystallization with enhancers such as nucleating agents and stereocomplex, heat resistant polymers, nano-reinforcing agents, can increase the proportion of the crystalline and rigid amorphous fraction, which obstructs chain mobility and resists heat-induced distortions (Jin *et al*., 2019; Nagarajan *et al*., 2016; Peelman *et al*., 2015).

Although numerous methods, including the use of nucleating agents, stereocomplexation, chain extenders have proven to be successful in overcoming the poor heat resistance of PLA. Similarly to other biopolymers, its toughness remains problematic due to the stiff chains of its backbone. As a result, this currently prevents it from being widely adopted for durable applications in many fields. Toughness is the ability of a material to absorb energy before failure during stress. It is generally classified as tensile and impact toughness or strength. Tensile toughness is the ability of a material to absorb energy when pulled apart or stretched, whereas impact toughness is the ability of a material to absorb sudden impact energy without breaking. The key to toughness is a good combination of ductility and strength. PLA possesses low crack initiation energy, which is determined by unnotched impact test, low crack propagation energy, which is measured by notched impact test, and it fails by crazing. Thus, PLA is an inherently brittle polymer, with less than 10% elongation at break and relatively poor impact strength. While it might be simple to improve ductility or elongation at break, it is very challenging to increase the impact toughness of PLA. Impact toughness is dependent on numerous intrinsic and extrinsic parameters. Intrinsic parameters include factors such as temperature, notch, mode of loading, specimen geometry, fracture behaviour and extrinsic parameters includes phase morphology, chain structure and entanglements. The strategies for toughening PLA commonly involve composite modification, blending, copolymerization and plasticization (Farah *et al*., 2016; Jin *et al*., 2019; Nagarajan *et al*., 2016; Peelman *et al*., 2015; Zhao *et al*., 2020).

Generally, the incorporation of organic and inorganic fillers into a polymer matrix can improve its mechanical properties, such as stiffness, toughness, and heat distortion temperatures amongst others. The ductility and toughness of PLA can be improved with several strategies, including plasticization, copolymerization, and melt blending with different tough polymers, rubbers, and thermoplastic elastomers (Chen *et al*., 2018; Farah *et al*., 2016; Zhao *et al*., 2020). As a result, research progress in toughening PLA based on these strategies can be found in several recent studies (Jin *et al*., 2019; Zhao *et al*., 2020).

Furthermore, similar to its monomer lactic acid, PLA is a chiral polymer and contains asymmetric carbon atoms with a helical orientation which permits ease of manipulation (Chen *et al*., 2018; Davachi & Kaffashi, 2015; Sikhosana *et al*., 2021). Due to the presence of a stereogenic centre in the main unit, PLA offers two different structures based on tacticity, that is, the isotactic and syndiotactic. Isotactic polymers have sequential stereogenic centres which possess the same configuration, whereas syndiotactic polymers possess sequential stereogenic centres of opposite configuration. Poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) are semi-crystalline, which makes them hard polymers, whereas poly(D,L-lactide) (PDLLA) is an amorphous polymer, which makes it a brittle material. It is only when the D- and L-unit sequences are completely alternating that PDLLA can be crystalline (Peelman *et al*., 2015). The thermal history and stereochemistry have a direct influence on PLA crystallinity and therefore on its general properties (Singhvi *et al*., 2019). Crystallinity influences many polymer properties, including hardness, modulus, tensile strength, stiffness, crease and melting points. As such, from the three enantiomeric states, the most widely used polylactides are PLLA and PDLA, respectively (Byun & Kim, 2014; Lopes *et al*., 2012; Peelman *et al*., 2015). PLA structural versatility presents many opportunities for viable modifications that may lead to the desired breakthrough (suitability of intended application).

To enhance the functionality of biopolymers such as PLA whilst maintaining an environmentally friendly material, composite material formation by integrating reinforcement matter within the micro/nano regime of the polymer matrix is a suitable approach. The combination of organic materials (i.e., cellulose) that are biodegradable (Hickey & Pelling, 2019; Prodanović & Milutinović, 2017) and reproducible bioplastics can be designed to meet the demands of specific applications, and their unique balance of properties may open a new market development opportunity for biocomposites, in the era of green materials. This growing development has provided room for biopolymers to pose as a potential alternative to the petroleum-derived polymers, which are synthetic and harmful to nature (Hickey & Pelling, 2019; Ibrahim *et al*., 2019; Prodanović & Milutinović, 2017). Ideal products for sustainable development are biodegradable composites where the matrix part is also biodegradable. Environmentally friendly composites from plant-derived fibres are novel materials of the 21st century. These materials are of great importance to the materials' world due to their potential to reduce the growing environmental threats. Furthermore, they present a possible solution to the uncertainty of the petroleum supply (Barkhad *et al*., 2020; Mohanty *et al*., 2005; Swain *et al*., 2018). Great emphasis remains on the development of composites based on fully bio-based thermoplastics matrices with biomaterials such as cellulose.

2.3. Cellulose

Cellulose is the most common natural polymer on the planet, which sometimes occurs in a nearly pure state in cotton (Lynd *et al*., 2002; Malafaya *et al*., 2007). Cellulose is composed of long chains of glucose units bound together by *β*-1,4-glycosidic bonds (Perez & Samain, 2010). The cellulose molecule comprises 2000 to 25000 glucose units (Segato *et al*., 2014). As a result, the number of monomers that make up each cellulose chain determines the degree of polymerization. There are two different ending groups at the edge of the cellulose chain. The one end of the chain has a non-reducing group with a closed ring structure, while the other end has a reducing group with both an aliphatic structure and a carboxyl group. Cellulose is therefore a polymerized molecule. New glucose residues assimilate at the non-reducing end by the cellulose synthase enzymes. The 1,4-linkages between the glucose units provide cellulose with unique structural features. It is, therefore, a water-insoluble polymer molecule with a stiff linear structure. Controlled cellulose biosynthesis allows for the formation of long linear chains that can be stacked one on top of the other, creating fibres with high mechanical strength. Cellulose has a tension resistance comparable to that of materials such as glass and steel (Hickey & Pelling, 2019; Malafaya *et al*., 2007; Lynd *et al*., 2002; Segato *et al*., 2014).

Although cellulose is produced by different organisms, including plants (trees, shrubs, herbs), animals (tunicates) and some microorganisms (Gluconoacetobacter) (Ochoa-Villarreal, 2012), cellulose is almost found exclusively in plant cell walls (Lynd *et al*., 2002; Tajeddin, 2014). Plants are the key players in cellulose production, with an annual turnover of 180 billion tons of cellulose (Festucci-Buselli *et al*., 2007). Cellulose is the major component of plant cell walls, and it is regarded as the major sink for atmospheric carbon in plants. Commercially important plant cell walls are made up of three layers and each layer has two phases (microfibrillar and matrix). Cellulose represents the microfibrillar phase, which is crystalline, while the matrix, which is a non-crystalline phase, is made up of polysaccharides (pectins and hemicelluloses), phenolic compounds (lignin and others), and protein. Despite significant variances in cell wall composition and anatomical structure among plant taxa, a high cellulose content, often in the range of 35 to 90%, is a unifying trait (Aridi, *et al*., 2021; Festucci-Buselli *et al*., 2007; Lynd *et al*., 2002). Cellulose is responsible for many distinctive traits in plants. For instance, cellulose microscopic fibres are layered in such a way that they form the backbone structure of a plant cell wall and through cell division, the different plant parts (roots, stems, leaves and branches) are derived (Festucci-Buselli *et al*., 2007; Hofte & Voxeur, 2017; Ioelovich, 2016; Sampath *et al*., 2016).

Due to its structural integrity, cellulose already acts as a reinforcing material in nature by providing structural support to plants. Its potential as a reinforcing agent has been demonstrated by its many attractive properties. Regardless of source, cellulose is generally a highly crystalline and high molecular weight polymeric material that usually forms highly crystalline fibres (Baghaei & Skrifvars 2020; Mwaikambo, 2006; Tajeddin, 2014). However, depending on the origin and method of extraction and regeneration, the geometry, polymerisation degree, fibrillary order, and crystallinity vary, hence the existence of the different polymorphs (cellulose I, II, III and IV). Plant cellulose is commonly known as native cellulose or cellulose I and the different polymorphs can be obtained from it through chemical or biological processes as outlined in Table 2.2. The elastic moduli of the crystalline regions of the different polymorphs of cellulose towards the chain axis differ, which is an indication that the polymer skeletons of these polymorphs are completely distinct from one another. This is because, during the crystallisation transition, the skeletal confirmations and intramolecular hydrogen bonds are altered. Although modifications are feasible, mechanical, and physical qualities can be chosen by selecting certain source materials. Different cellulose forms give rise to different properties. Cellulose extracted from various plant sources has an attractive combination of mechanical and thermal properties (Baghaei & Skrifvars, 2020; Festucci-Buselli *et al*., 2007; Lynd *et al*., 2002; Smole *et al.*, 2013; Tajeddin, 2014).

Table 2.2. Different polymorphs and their properties

Type	Structure	Treatment	Young's moduli (GPa)	References
I	Native (Micro-crystalline)	None	138	(Baghaei & Skrifvars, 2020; Hickey & Pelling, 2019; Ochoa-

				Villarreal, 2012; Smole *et al.*, 2013)
II	Crystal structure	Strong alkaline solution or precipitation from solution (Sodium hydroxide solution) mercerization	88	(Baghaei & Skrifvars, 2020; Hickey & Pelling, 2019; Ochoa-Villarreal, 2012; Smole *et al.*, 2013)
III_{I}	Crystal structure	Liquid ammonia	87	(Baghaei & Skrifvars, 2020; Hickey & Pelling, 2019; Ochoa-Villarreal, 2012; Smole *et al.*, 2013)
III_{II}	Crystal structure	Ammonia at -30 ºC	58	(Hickey & Pelling, 2019; Rojas, 2013)
IV	Lattice structure	Hot bath under stretch Heating (prepared from ammonia at -30 ºC) III with glycerol	75	(Baghaei & Skrifvars, 2020; Hickey & Pelling, 2019; Smole *et al.*, 2013)

Besides being primary building materials and providing structural support for plants, cellulose and its derivatives contribute to many other industrial applications (Kabir *et al*., 2020; Lavanya *et al*., 2011; Nawrath *et al*., 1995; Singla *et al*., 2017). Cellulosic materials have been used for water filtration, drug delivery, production of fibres, membranes, films and plastics, and sensors in a form of nanofibrous membrane (El-Saied *et al.*, 2020). Cellulose is commonly employed as a raw material in the development of commercial plastics (Nawrath *et al*., 1995) and/or as a nanofiller to address limitations suffered by other polymers (Egamberdieva *et al*., 2017; Mishra *et al*., 2018). Cellulose acetate is one of the most important derivatives of cellulose, commonly employed as films, membranes or fibres in various applications including packaging, photography, and optometry amongst others. Many other cellulose-based materials, including composites, have been widely explored in the fields of tissue engineering and packaging (Tajeddin, 2014; Zahan *et al*., 2020). Derivatives of cellulose are equally attractive because they retain most of the parent polymer properties such as abundance, environmental friendliness, relatively low cost, light weight, high specific strength, high thermal stability, biodegradability, and biocompatibility (Baghaei and Skrifvars, 2020; El-Hoseny *et al*., 2015; Sahana & Rekha, 2018). Additionally, cellulose exhibits an inherent structural feature that permits altering of the molecule to acquire any desired function. Cellulose can be improved by surface modification, which allows substitution of the hydroxyl functional groups with other functional groups at the surface of the molecule. Thus, all derivatives of cellulose are based on substituting the hydroxyl groups of cellulose with other functional groups (Baghaei & Skrifvars, 2020; Bao, 2015; El-Hoseny *et al*., 2015; Sahana & Rekha, 2018).

While cellulose is widely distributed in the plant kingdom, only a few plant species are exploited in excess for its production on a commercial scale. Commercial cellulose is primarily isolated from forest wood, agricultural residues, or by-products (corncob, risk husk and sugarcane bagasse), and a few herbaceous plants (hemp, flax, jute, ramie, kenaf and cotton). However, raw materials such as forest wood and agricultural waste used for manufacturing bio-based monomers and polymers, including cellulose, often compete with requirements for food-based, furniture, paper, and biofuels products among other things (La Rosa, 2016; Lynd *et al*., 2002; Rendón-Villalobos *et al*., 2016; Sonia & Dasan, 2013). Thus, there are concerns over their sustainability. Conversely, medicinal plants are almost exclusively used for medicinal purposes, with only a few species commercialised for biopolymer preparations. Relative to other industrial key players in cellulose production, medicinal plants produce highly crystalline cellulose with equivalent strength and stiffness in comparatively high quantities. Again, these cellulose-rich species that grow like weeds without special care provide an alternative source of fillers that cost less than traditional reinforcing materials such as glass and carbon (Chirayil *et al*., 2014; Coppola *et al*., 2021; Rendón-Villalobos *et al*., 2016; Rojas *et al*., 2015; Sonia & Dasan, 2013).

2.4. Medicinal plants

Medicinal plants are plants that are used in herbal medicine and are believed to contain extractable chemical compounds with therapeutic significance in their leaves, stems, flowers, and fruits. Extracts from medicinal plants are used in their natural state or as additives in various industries (Yalavarthi & Thiruvengadarajan, 2013). Nowadays, herbal products such as natural pigments, sweeteners, flavourings, drugs, cosmeceuticals, nutraceuticals, and many plant extracts symbolise safety in comparison to synthetic compounds that are considered unsafe to humans and the environment (Oladeji, 2016; Xie *et al*., 2016; Yalavarthi & Thiruvengadarajan, 2013). Medicinal plants cannot be differentiated from other plants by morphological features but by the chemical constituents through which they impart beneficial therapeutic effects on the animal body. Medicinal plants contain numerous interesting chemical compounds in their different parts that are responsible for their various traits. The 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 are essential for proper analysis and application (Alaribe *et al*., 2018; Bamola *et al*., 2018; Oladeji, 2016; Tiwari *et al*., 2011; Yalavarthi & Thiruvengadarajan, 2013).

Generally, plants have incredible biosynthetic potential, and they produce thousands of distinct chemical compounds through different metabolic pathways. Plant metabolism is sub-divided into primary metabolism (comprises reactions and pathways vital-essential for survival) and secondary metabolism (defence specialist). Primary metabolites are directly involved in growth, development, and reproduction, and often serve as precursors for synthesising tens of thousands of secondary metabolites. On the other hand, secondary metabolites are metabolic end-products that serve specialized roles such as attracting pollinators or protecting against diseases. The composition and distribution of bioactive compounds in medicinal plants vary significantly depending on the plant type. There is a variation of secondary metabolites in plants, but their accumulation in plant cells is in minute quantities compared to primary metabolites. Primary metabolites are the byproducts of vital metabolic processes, including the Krebs cycle and glycolysis, present in all living cells. Primary metabolites include energy-rich fuel molecules like sucrose, starch, and lipids, as well as structural components like cellulose, proteins, and lipoproteins, high molecular building blocks like amino acids and carboxylic acids, enzymes (proteins), informative molecules like DNA and RNA, and pigments (chlorophyll). Most of these primary metabolites form classes of biopolymers and some act as precursors for their biosynthesis. Molecular compounds such as carbohydrates, proteins, lipids, and nucleic acids, are considered major biomacromolecules (Koche *et al*., 2016; Pott *et al*., 2019; Sarraf *et al*., 2021).

Medicinal plants are the most abundant source of biomaterials, and polysaccharides have been discovered as major active components in these plants, which are responsible for a wide range of pharmacological activities. Biomacromolecules found in the kingdom Plantae not only serve as the building blocks of life but also have vital roles in the transmission of biological signals such as cell-to-cell communication, immunological recognition, and mitogenesis, among others. These compounds possess eminent pharmacological properties, including antioxidant activity, anticancer activity, wound healing effects, and immunostimulatory action. As such, plant polysaccharides can be used for industrial applications, including pharmaceuticals, biomaterials, foodstuff and nutrition, and biofuels among others. For instance, various indigestible plant polysaccharides such as cellulose, hemicelluloses, pectin, oligosaccharides, and gums were defined as dietary fibre by the FAO (Food and Agriculture Organization). Among these, cellulose and hemicellulose can directly induce bowel movement. Furthermore, cellulosic materials have been vastly used in the biomedical field. Lastly, lignocellulose biomass, which contains mainly polysaccharide sugars, has become one of the world’s leading biofuel resources with the greatest potential. These milestones in the development and commercialization of plant-based products have prompted a lot of research interest toward biomolecules from various plant species (Li *et al*., 2018; Ullah *et al*., 2019; Xie *et al*., 2016).

Biomolecules present great potential in many applications due to physico-chemical and biological properties such as high stability, biocompatibility, biodegradability, and non-toxicity. As such, several studies have reported on the extraction of polymeric materials whose biological activities are strongly influenced by their chemical structures and chain conformations from medicinal plants. The chemical structures have been correlated with biological and/or functional properties to address and advance the sustainable development and economic growth of mankind. In most instances, the traditional medicinal use of these plants has been used as a guiding thread to survey their potential (Li *et al*., 2018; Sarraf *et al*., 2021; Xie *et al*., 2015). Table 2.3 summarises a couple of bioactive polysaccharides extracted from different medicinal plants that have been developed into beneficial compounds for promoting good health and safe environmental conditions. These compounds, especially cellulose, can be further exploited for the development of other materials in various applications other than the ones mentioned in Table 2.3. Previous studies have demonstrated the extraction of cellulose fibres with different dimensions from various medicinal plants by mechanical treatment and acid hydrolysis or a combination of the two (Desmaisons *et al*., 2017; Khenblouche *et al*., 2019; Sonia & Dasan, 2013). The obtained cellulose was characterized using morphological investigations (optical, scanning electron and atomic force microscopy), as well as physicochemical techniques (infrared spectroscopy, X-ray diffraction and thermogravimetric analysis). The results illustrated the successful extraction of cellulose from the herbaceous plants with a percentage yield ranging between 52-90% (Khenblouche *et al*., 2019; Aridi, *et al*., 2021). The percentage yield depends on the raw material and the process of extraction. From Table 2.4, it can be seen that the cellulose obtained using the alkali and bleaching treatments revealed the highest cellulose yield and percentage crystallinity. The high cellulose content suggests the possibility to use the cellulose obtained from medicinal plants in the production of biodegradable composites with enhanced properties.

Table 2.3. Herb derived polysaccharides

Plant	**Medicinal use**	**Polysaccharide**	**Composition**	**Physiological activity**	**Application**	**Reference**
Various plants (e.g. flax, hemp, sisal)	Numerous (e.g. wound healing, constipation)	Cellulose	β-(1-4)-Linked-glucopyranose.	Biocompatibility, hydrophilicity, biodegradable, nontoxicity and antimicrobial properties.	Food, nutraceutical, biomedical, tissue engineering, materials.	(Li *et al*., 2018; Moťková *et al*, 2017; Neoh *et al*., 2012; Sindhu *et al*., 2014; Swapan *et al*., 2011; Xie *et al*., 2016)
Various plants (e.g. flax, hemp, sisal)	Numerous (e.g. wound healing, constipation)	Hemicellulose	Xylans, mannans, mixed linkage β-glucans, xyloglucans.	Immune regulation, bacteria inhibition, drug release, anti-caries, scaffold materials and anti-tumour.	Food, nutraceutical thickener, dietary fibre, medical and pharmaceutical field.	(Liu *et al*., 2019;
Hawthorns	Heart and blood vessel diseases	Pectin	α-(1-4)-d-Galacturonic acid and rhamnose.	Regulates intestinal inflammation	Food, nutraceutical	(Guo *et al*., 2018; Li *et al*., 2018)

				apart from its prebiotic effect.	thickener, dietary fibre, gelling agent.	
Eucommia ulmoides	Hypertension and diabetes.	*Eucommia ulmoides* polysaccharide	Mannose, galactose, glucose, arabinose, rhamnose, and galacturonic acid	Binding PDGF-BB growth factor and anti-inflammatory effect.	-	(Li *et al*., 2018; Yuqing, *et al*., 2019)
Lentinus edodes	Lowering blood cholesterol levels, treating prostate cancer.	Lentinan	β-(1→3)-D-glucan	Anti-tumour, anti-viral activities anti-aging.	Medical (cancer treatment), food and cosmetics.	(Xie *et al*., 2016)
Eucomis autumnalis	Cancer, wounds, and sexually transmitted infections.	No information available	No information available	Anti-microbial, anti-carcinogenic, anti-oxidative, anti-inflammatory, and anti-histaminic.	No information available	(Alaribe *et al*., 2018; Bamola *et al*., 2018; Yalavarthi &

						Thiruvengadarajan, 2013)
Opuntia polyacantha	Skin sores, infections, wounds, and back aches.	*Opuntia* polysaccharide	Galactose, galacturonic acid, xylose, arabinose, and rhamnose	Immunoregulatory and anti-inflammatory effect.	Medical and neutraceuticals.	Schepetkin *et al*., 2008
Juniperus scopulorum	Diaphoretic, disinfectant, febrifuge, haemostatic, laxative, sedative, and tonic	*Juniperus scopolorum* polysaccharide	β-Galactopyranose, and α-arabinofuranose	Immunomodulatory effect to the murine macrophages Exhibit anti-inflammatory, hypoglycemic, anti-bacterial, and anti-tumor activities	Medical	(Li *et al*., 2018; Schepetkin *et al*., 2008)

Table 2.4. Summary of the medicinal use, methods of extraction and percentage yield and crystallinity of the cellulose extracted from medicinal plants

Plant	**Traditional medicinal use**	**Phamarcological properties**	**Extraction method**	**Type of cellulose**	**% yield of cellulose**	**% crystallinity**	**References**
Helicteres Isora	Treating of diarrhoeal infections	Not specified	Alkaline treatment, bleaching, acidic steam treatment and homogenization	Nanofibrils	71	90	(Chirayil *et al*., 2014; Kumar & Singh, 2014; Rojas *et al*., 2015)
Retama raetam	Treating wounds, skin rash, kidney disorders, and microbiological infections	Not specified	Classical chemical treatments with adaptations in dewaxing, alkali and bleaching treatment processes	Microfibres	52	78	(Khenblouche *et al*., 2019)
Hibiscus sabdariffa	High blood pressure, liver	Antiscorbutic, emollient, diuretic,	Steam explosion and oxalic acid hydrolysis	Microfibres	82	79	(Bedi *et al*., 2020; Sonia & Dasan, 2013)

	diseases and fevers	refrigerant, and sedative					
Syngonanthus nitens	Not specified.	Antiulcerogenic, antioxidant, antibacterial, and antifungal activity	Alkali and bleaching treatments	Fibers	67	70-91	(Dos Santos Ramos *et al*., 2016; Siqueira *et al*., 2012)
Phormium tenax	Production of woven mats and ropes	Not specified	Acid hydrolysis	Nanocrystals	61	Not specified	(Fortunati *et al*., 2012)
Leacaena leucocephala	Used to cure skin ailments and eliminate body hair	Anti-inflammatory and anti-diabetic. Anti-microbial	Alkali and bleaching treatment	Nanocrystals	90	71	(Ab Rashid *et al*., 2019; Aderibigbe *et al*., 2011; Aridi, *et al*., 2021)
Eucomis autumnalis	Cancer, wounds, and sexually transmitted infections	Anti-microbial, anti-arcinogenic, anti-oxidative, anti-inflammatory, and anti-histaminic	Alkali and acid hydrolys (Current study)	Not specified.	21-38	Not specified.	(Alaribe *et al*., 2018; Bamola *et al*., 2018; Cheesman *et al*., 2010; Kulkarni *et al*., 2006)

2.5. Development of PLA/cellulose (extracted from medicinal plants) composites

The modification of PLA has become one of the most vital subjects in PLA research. Relative to PLA alone, PLA composites reinforced with suitable fillers exhibit mechanical and physiological properties that make them ideal for a variety of applications (Gumede *et al*., 2018, Sikhosana *et al*., 2021). Plant-derived fibres with increased availability, superior properties, and environmental benefits such as cellulose are among the many materials that have been used to modify PLA. The benefits of using such fillers in comparison to other potential reinforcing agents such as glass, talc, or carbon for improving the performance of polymers such as PLA include retaining the biodegradability of the composite while also exhibiting lower density, superior performance, and lower cost due to their widespread availability. Composites of PLA and cellulose extracted from agricultural plants or crops such as wheat straw, sugarcane bagasse and maize stalk among others have been extensively investigated. However, these composites have displayed higher tensile modulus and lower tensile strength compared with PLA alone. These shortcomings have been associated with factors that include weak interfacial interaction between the hydrophobic PLA matrix and the hydrophilic cellulose fibre and lack of fibre dispersion due to a high degree of fibre agglomeration (Liu *et al*., 2019; Xiao *et al*., 2012; Zhou *et al*., 2018).

Different methods of modifying the surface area of the cellulose have been explored to improve the interactions between the PLA and cellulose matrices. Improvements in cellulose properties and the development of better processing techniques are vital to produce PLA/cellulose composites with enhanced properties. Furthermore, factors including filler content, preparation method, and filler-matrix interaction have all been proven to have a significant impact on the characteristics of the final PLA/cellulose composite materials. On the other hand, searching for alternative sources of cellulose from different plant materials with the intent to produce cellulose with varying features that may suit specific fields of application is of particular interest, thus the current interest in cellulose extracted from medicinal plants in PLA matrices (Fortunati *et al.*, 2014; Sawpan, 2011; Zhou *et al*., 2018).

Cellulose has been extracted from various medicinal plants and incorporated in PLA matrices to produce composites with improved mechanical and physiological properties using different techniques including solvent casting, compounding and compression moulding among others. Table 2.5 provides a summary of medicinal plants used for cellulose extraction used in PLA matrices, improvement, non-improvements, and potential applications.

Table 2.5. PLA/cellulose extracted from medicinal plants

Herb	**Fabrication Technique**	**Improvement**	**Non-improvements**	**Target application**	**References**
Cannabis savita (hemp)	Compound moulding	• Increased PLA transcrystallinity • Improved chemical bonding	• It was found that covalent bonding between the treated fibre and PLA decreased compared to the untreated fibre and PLA sample.	Automative, building construction	(Sawpan, 2011)
Phormium tenax (New Zealand Flax)	Compounding using a Twin-screw microextruder	• Increased chain mobility resulted in a considerable increase in chain mobility and, as a result, a reduction in PLA's glass transition temperature.	• Not specified.	Active packaging	(Fortunati *et al.*, 2014)
Boemeria nivea (Ramie)	Heat pressing technology compression moulding, compounding	• Improved mechanical properties than pure PLA • Increased the interfacial	• Not specified	Aircraft's interior structures	(Choi & Lee, 2012)

		property between fiber and PLA matrix than that of PLA alone			
Hibiscus sabdariffa L. Linne Malvaceae (Kenaf)	Micro compounding and hot compression moulding	- Improvement in strength and modulus was achieved.	- Not specified	Various applications	(Neoh *et al*., 2012)
Linum usitatissimum (Flax)	Micro compounding and hot compression moulding	- Excellent tensile.	- The modified biocomposite did not pass V-0 rating in UL-94 test.	Flame retardant applications.	(Zhang *et al*., 2018)
Posidonia oceanica	Solvent casting	- PLA matrix's optical transparency was preserved. - PLA matrix has a porous structure.	- Not specified	Food packaging	(Fortunati *et al*., 2015)

Although to some extent there have been improvements when using medicinal plant-sourced cellulose in PLA matrices, their performance is still inferior in some applications. This further warrants continuation of the search for suitable cellulosic material from these poorly explored but promising ecosystems. Apart from herbs, other plant materials such as woody trees and agricultural crops have been used as sources of cellulose for reinforcing polymers since time immemorial. However, trees and agricultural crops are the most exploited in biocomposite preparations to date. Medicinal plants have been used selectively for this purpose and only a few species (kenaf, flax, hemp, jute, sisal, cotton, ramie) have been excessively exploited (Neoh *et al*., 2012; Swapan *et al*., 2011). As a result, the use of most medicinal plant-derived compounds is equitably documented in biomedical and pharmaceutical journals, but there is limited information on their use in biopolymer matrices such as PLA (Komal *et al*., 2019; Moreno *et al*., 2019). For example, the use of compounds derived from medicinal plants such as *E. autumnalis* has been solely limited to pharmaceutical products and therefore, it is worth exploring its potential as a possible source of cellulose to develop new green composites and broaden its practical applications.

2.6. References

Ab Rashid, N.A.N., Husin, M., Yury N., Othman, M., Shariff, Z.M., Kassim, H. & Li, A.R. **2019**. Cellulose isolation from *Leucaena leucocephala* seed: Effects on concentration sodium hydroxide. *Journal of Academia*. 7(2): 36–45.

Aderibigbe, S.A., Adetunji, O.A. & Odeniyi, M.A. **2011**. Antimicrobial and pharmaceutical properties of the seed oil of *Leucaena leucocephala* (Lam.) De Wit (Leguminosae). *African. Journal of Biomedical Research*. 14(1): 63–68.

Baghaei, B. & Skrifvars, M. **2020**. All-cellulose composites: A Review of recent studies on structure, properties and applications. *Molecules*. 25(12): 1–19. https://doi.org/10.3390/molecules25122836

Bao C. **2015**. Cellulose acetate/plasticizer systems: Structure, morphology and dynamics. (PhD Dissertation.) Claude Bernard University, Lyon, France.

Barkhad, M.S., Abu-Jdayil, B., Mourad A.H.I. & Iqbal M.Z. **2020**. Thermal insulation and mechanical properties of poly(lactic) acid (PLA) at different processing conditions. *Polymers*. 20(2091): 1–16. https://doi.org/10.3390/polym12092091

Bedi, P.S., Bekele, M. & Gure G. **2020**. Phyto-chemistry and pharmacological activities of *Hibiscus sabdariffa* Linn: A Review. *International Research Journal of Pure and Applied Chemistry*. 21(23): 41-54. https://doi.org/10.9734/irjpac/2020/v21i2330301

Byun, Y. & Kim, Y.T. **2014**. Bioplastics for food packaging: Chemistry and physics. In Han, J.H. (Eds). *Innovations in Food Packaging*. United States of America: Elsevier. pp 353–368. https://doi.org/10.1016/b978-0-12-394601-0.00014-x

Cheesman, L., Finnie, J.F. & Van Staden, J. **2010**. *Eucomis zambesiaca* baker: Factors affecting *in vitro* bulblet induction. *South African Journal of Botany.* 76(1): 543–549. https://doi.org/10.1016/j.sajb.2010.04.004

Chen, J., Zhang, T.-Y., Jin, F.-L. & Park, S.-J. **2018**. Fracture toughness improvement of poly(lactic acid) reinforced with poly(ε-caprolactone) and surface-modified silicon carbide. *Advances in Materials Science and Engineering*. 2018: 1–10. https://doi.org/10.1155/2018/6537621

Chirayil, C.J., Joy, J., Mathew, L., Mozetic, M., Koetz, J. & Thomas, S. **2014**. Isolation and characterization of cellulose nanofibrils from *Helicteres isora* plant. *Industrial Crops and Products*. 59(2014): 27–34. https://doi.org/10.1016/j.indcrop.2014.04.020

Choi, H. Y. & Lee, J. S. **2012**. Effects of surface treatment of ramie fibers in a ramie/poly(lactic acid) composite. *Fibers and Polymers*. 13(2): 217–223. https://doi.org/10.1007/s12221-012-0217-6

Choudhury, A.K. **2019**. Biopolymers in the textile industry. In Padinjakkara, A., Thankappan, A., Souza, F.G. & Thomas, S. (Eds.). *Biopolymers and Materials*. Canada: Apple Academic Press Inc. pp 1–18.

Davachi, S.M. & Kaffashi, B. **2015**. Polylactic acid in medicine. *Polymer-Plastics Technology and Engineering*. 54(9): 944–967. https://doi.org/10.1080/03602559.2014.979507

Desmaisons, J., Boutonnet, E., Rueff, M., Dufresne, A. & Bras, J. **2017**. A new quality index for benchmarking of different cellulose nanofibrils. *Carbohydrate Polymers*. 174: 318–329. https://doi.org/10.1016/j.carbpol.2017.06.032

Dos Santos Ramos, M., de Toledo, L., Calixto, G., Bonifácio, B., de Freitas Araújo, M., dos Santos, L., de Almeida, M.T.G., Chorilli, M. & Bauab, T. **2016**. Syngonanthus nitens Bong. (Rhul.)-loaded nanostructured system for vulvovaginal candidiasis treatment. *International Journal of Molecular Sciences*. 17(8): 1–19. https://doi.org/10.3390/ijms17081368

Egamberdieva, D., Wirth, S., Behrendt, U., Ahmad, P. & Berg, G. **2017**. Medicinal plant harbours antagonistic rhizobacteria. Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. *Frontiers in Microbiology*. 8(199): 1–11. https://doi.org/10.3389/fmicb.2017.00199

El-Hoseny, S.M., Basmaji, P., De-Olyveira, G.M., Costa, L.M.M., Alwahedi, A.M., Da-Costa-Oliveira, J.D. & Francoza, G.B. **2015**. Natural EMC-bacterial cellulose wound healing – Dubai study. *Journal of Biotechnology and Nanobiotechnology*. 6: 237–246.

https://doi.org/10.4236/jbnb.2015.64022

El-Saied, H., Mostafa, A.M., Hasanin, M.S, Mwafy, E.M. & Mohammed, A.A. **2020.** Synthesis of antimicrobial cellulosic derivative and its catalytic activity. *Journal of King Saud University*. 32(1): 436–442. https://doi.org/10.1016/j.jksus.2018.06.007

Fairag, R., Rosenzweig, D. H., Ramirez-Garcialuna, J.L., Weber, M.H. & Haglund, L. **2019**.Three-dimensional printed polylactic acid scaffolds promote bone-like matrix deposition in vitro. *ACS Applied Materials and Interfaces*. 11(17): 15306–15315. https://doi.org/10.1021/acsami.9b02502

Festucci-Buselli, R.A., Otoni, W.C. & Joshi, C.P. **2007**. Structure, organization, and functions of cellulose synthase complexes in higher plants. *Brazilian Journal of Plant Physiology*. 19(1): 1–13. https://doi.org/10.1590/S1677-04202007000100001

Fortunati, E., Puglia, D., Monti, M., Peponi, L., Santulli, C., Kenny, J.M. & Torre, L. **2012**. Extraction of cellulose nanocrystals from *Phormium tenax* fibres. *Journal of Polymers and the Environment*. 21(2): 319–328. https://doi.org/10.1007/s10924-012-0543-1

Fortunati, E., Luzi, F., Puglia, D., Dominici, F., Santulli, C., Kenny, J.M. & Torre, L. **2014**. Investigation of thermo-mechanical, chemical and degradative properties of PLA-limonene films reinforced with cellulose nanocrystals extracted from *Phormium tenax* leaves*. European Polymer Journal*. 56(2014): 77–91. https://doi.org/10.1016/j.eurpolymj.2014.03.0

Fortunati, E., Luzi, F., Puglia, D., Petrucci, R., Kenny, J.M. & Torre, L. **2015**. Processing of PLA nanocomposites with cellulose nanocrystals extracted from *Posidonia oceanica* waste: Innovative reuse of coastal plant*. Industrial Crops and Products*. 67(2015): 439–447*.* https://doi.org/10.1016/j.indcrop.2015.01.075

Gumede, T.P., Luyt, A.S. & Muller, A.J. **2018**. Review on PCL, PBS, and PCL/PBS blends containing carbon nanotubes. *Express Polymer Letters*. 12(6): 505–529. https://doi.org/10.3144/expresspolymlett.2018.43

Guo, Q., Du, J., Jiang, Y., Goff, H. D., & Cui, S. W. **2018**. Pectic polysaccharides from hawthorn: Physicochemical and partial structural characterization. *Food Hydrocolloids*. https://doi.org/10.1016/j.foodhyd.2018.10.0

Haider, A., Haider, S., Kumar, M.R., Kamalt, T., Algyamah, A.A. & Khan, N. **2020**. Advances in the scaffolds fabrication techniques using biocompatible polymers and their biomedical applications: A technical and statistical review. *Journal of Saudi Chemical Society*. 24(1): 186–215. https://doi.org/10.1016/j.jscs.2020.01.002

Hickey, R.J. & Pelling A.E. **2019**. Cellulose biomaterials for tissue engineering. *Frontiers in Bioengineering and Biotechnology*. 7(45): 1–15. https://doi.org/10.3389/fbioe.2019.00045

Hofte, H. & Voxeur, A. **2017**. Primer: Plant cell walls. *Current Biology Magazine*. 27(17): 853–909. https://doi.org/10.1016/j.cub.2017.05.025

Ibrahim, S., Riahi, O., Said, S.M., Sabri, M.F.M. & Rozali, S. **2019**. Biopolymers from crop plants. *Reference Module in Materials Science and Materials Engineering*. https://doi.org/10.1016/b978-0-12-803581-8.11573-5

Ioelovich, M. Y. **2016**. Models of supramolecular structure and properties of cellulose. *Polymer Science Series A*. 58(6): 925–943. https://doi.org/10.1134/s0965545x16060109

Jamshidian, M., Tehrany, E.A., Imran, M., Jacquot, M. & Desobry S. **2010**. Poly-Lactic Acid: Production, applications, nanocomposites, and release Studies. *Comprehensive Reviews in Food Science and Food Safety*. 9(5): 552–571. https://doi.org/10.1111/j.1541-4337.2010.00126.x

Jem K.J. & Tan B. **2020.** The development and challenges of poly(lactic acid) and poly(glycolic acid). *Advanced Industrial and Engineering Polymer Research*. 3(2020): 60–70. https://doi.org/10.1016/j.aiepr.2020.01.002

Jin, F.-L., Hu, R.-R. & Park, S.-J. **2019**. Improvement of thermal behaviors of biodegradable poly(lactic acid) polymer: A review. *Composites Part B: Engineering*. 164(0): 287–296. https://doi.org/10.1016/j.compositesb.2018.10.078

Kabir E., Kaur R., Lee J., Kim K.-H. & Kwon E.E. **2020**. Prospects of biopolymer technology as an alternative option for non-degradable plastics and sustainable management of plastic wastes. *Journal of Cleaner Production*. 258 (2020): 1–15. https://doi.org/10.1016/j.jclepro.2020.120536

Kim I., Viswanathan K., Kasi G., Sadeghi K., Thanakkasaranee S. & Seo, J. **2019**. Poly(lactic acid)/ZnO bionanocomposite films with positively charged ZnO as potential antimicrobial food packaging materials. *Polymers*. 11(9): 1427/1–1427/17. https://doi.org/10.3390/polym11091427

Khenblouche, A., Bechki, D., Gouamid, M., Charradi, K., Segni, L., Hadjadj, M. & Boughali, S. **2019**. Extraction and characterization of cellulose microfibers from *Retama raetam* stems. *Polímeros*. 29(1): 1–8. https://doi.org/10.1590/0104-1428.05218

Koche, D., Shirsat, R. & Kawala, M. **2016**. An overview of major classes of phytochemicals: Their types and role in disease prevention. *Hislopia Journal*. 9(1/2): 1–11.

Kulkarni, M.G., Sparg, S.G. & Van Standen, J. **2006**. Dark conditioning, cold stratification and a smoke-derived compound enhance the germination of *Eucomis autumnalis* subsp. *autumnalis* seeds. *South African Journal of Botany*. 72(1): 157–162. https://doi.org/10.1016/j.sajb.2005.06.006

Kumar, N. & Singh, A. K. **2014**. Plant profile, phytochemistry and pharmacology of Avartani (*Helicteres isora* Linn.): A review. *Asian Pacific Journal of Tropical Biomedicine*. 4(1): S22–S26. https://doi.org/10.12980/apjtb.4.2014c872

La Rosa, A.D. **2016**. Life cycle assessment of biopolymers. In Pacheco-Torgal, F., Ivanov, V., Karak, N. & Jonkers, H. (Eds.) *Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials*. Cambridge: Woodmead Publishing. pp 57–78. https://doi.org/10.1016/b978-0-08-100214-8.00004-x

Lavanya, D., Kulkarni P.K., Dixit. M., Raavi, P.K. & Krishna, L.N.V. **2011**. A review: Sources of cellulose and their applications. *International Journal of Drug Formulation and Research*. 2(6): 19–37.

Li, Q., Niu, Y., Xing, P. & Wang, C. **2018**. Bioactive polysaccharides from natural resources including Chinese medicinal herbs on tissue repair. *Chinese Medicine*. 13(1): 1–11. https://doi.org/10.1186/s13020-018-0166-0

Liu, X., Lin, Q., Yan Y., Peng, F., Sun, R. & Ren, J. **2019**. Hemicellulose from plant biomass in medical and pharmaceutical application: A critical review. *Current Medicinal Chemistry*. 26(14): 2430–2455.

https://doi.org/10.2174/0929867324666170705113657

Lopes, M.S., Jardini, A.L. & Filho, R.M. **2012**. Poly(lactic acid) production for tissue engineering applications. *Procedia Engineering*. 42(2012): 1402–1413. https://doi.org/10.1016/j.proeng.2012.07.534

Lynd, L.R., Weimer, P.J., Van Zyl, W.H. & Pretorius, I.S. **2002**. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*. 66(3): 506–577. https://doi.org/10.1128/MMBR.66.3.506-577.2002

Malafaya P.B., Silva G.A. & Reis, R.L. **2007**. Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Advanced Drug Delivery Reviews*. 59(2007): 207–233. https://doi.org/10.1016/j.addr.2007.03.012

Marturano, V., Cerruti, P. & Ambrogi, V. **2017**. Polymer additives. *Physical Sciences Reviews*. 2(6): 1–22. https://doi.org/10.1515/psr-2016-0130

Mohanty, A.K., Misra, M., Drzal, L.T., Selke, S.E., Harte, B.R. & Hinrichsen, G. **2005**. In Mohanty, A.K., Misra, M. & Drzal, L.T. (Eds.). *Natural fibers, biopolymers, and biocomposites: An introduction*. United States of America: CRC Press Taylor and Francis Group.

Moťková, P., Brožková, I., Vytřasová, J. & Kukla, R. **2017**. Antimicrobial effect of OKCEL® H-D prepared from oxidized cellulose. *Folia Microbiologica,* 63(1): 57–62. https://doi.org/10.1007/s12223-017-0534-7

Mwaikambo, L.Y. **2006**. Review of the history, properties and application of plant fibres. *African Journal of Science and Technology (AJST): Science and Engineering.* 7(2): 120 – 133.

Nagarajan, V., Mohanty, A.K. & Misra, M. **2016**. Perspective on Poly(lactic) acid (PLA) based sustainable materials for durable applications: Focus on toughness and heat resistance. *ACS Sustainable Chemistry and Engineering*. 4(6): 2899–2916. https://doi.org/10.1021/acssuschemeng.6b00321

Naser, A.Z., Deiaba, I. & Darras, B.M. **2021**. Poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHAs), green alternatives to petroleum-based plastics: A review. *Royal Society of Chemistry* 28(11): 17151–17196. https://doi.org/10.1039/D1RA02390J

Nawrath, C., Poirier, Y. & Somervill, C. **1995**. Plant polymers for biodegradable plastic: Cellulose, starch polyhydroxylalkanoids. *Molecular Breeding*. 1(2): 105-122. https://doi.org/10.1007/BF01249696

Neoh, K. W., Tshai, K. Y., Khiew, P. S. & Chia, C. H. **2012**. Micro palm and kenaf fibers reinforced PLA composite: Effect of volume fraction on tensile strength. *Applied Mechanics and Materials*. 145(2012): 1–5. https://doi.org/10.4028/www.scientific.net/amm.145.1

Ochoa-Villarreal, M., Aispuro-Hernández, E., Vargas-Arispuro, I. & Martínez-Téllez M.Á. **2012**. Plant cell wall polymers: Function, structure and biological activity of their derivatives. *Polymerization*. Lodon: IntechOpen. pp 63–79. https://doi.org/10.5772/46094

Peelman, N., Ragaert, P., Ragaert, K., De Meulenaer, B., Devlieghere, F. & Cardon, L. **2015**. Heat resistance of new biobased polymeric materials, focusing on starch, cellulose, PLA, and PHA. *Journal of Applied Polymer Science*. 132(48): 2–15. https://doi.org/10.1002/app.42305 42305

Perez, S. & Samain, D. **2010**. Structure and Engineering of Celluloses. *Advances in Carbohydrate Chemistry and Biochemistry*. 64(1): 25–116. https://doi.org/10.1016/S0065-2318(10)64003-6

Petinakis, E., Yu, L., Simon, G. & De, K. **2013**. Natural fibre bio-composites incorporating poly(lactic acid). *Fiber Reinforced Polymers - The Technology Applied for Concrete Repair*. Melbourne: IntechOpen. pp 42–59. https://doi.org/10.5772/52253

Pott, D. M., Osorio, S. & Vallarino, J. G. **2019**. From central to specialized metabolism: An overview of some secondary compounds derived from the primary metabolism for their role in conferring nutritional and organoleptic characteristics to fruit. *Frontiers in Plant Science.* 10: 1–19. https://doi.org/10.3389/fpls.2019.00835

Prodanović, S. & Milutinović, M. **2017**. Some applications of biomaterials in automotive andustry. In: Pellicer, E., Nikolic, D., Sort, J., Baró, M., Zivic, F., Grujovic, N., Grujic, R. & Pelemis, S. (Eds.). *Advances in applications of industrial biomaterials*. Switzerland: Springer. pp 1–218. https://doi.org/10.1007/978-3-319-62767-0_12

Rendón-Villalobos, R., Ortíz-Sánchez, A., Tovar-Sánchez, E. & Flores-Huicochea, E (Eds). **2016**. *The Role of Biopolymers in Obtaining Environmentally Friendly Materials.* pp 151–152*.*
https://doi.org/10.5772/65265

Rojas, J (Ed). **2013***. Effect of Polymorphism on the Particle and Compaction Properties of Microcrystalline Cellulose*. IntechOpen: Melbourne. pp 27–46.
https://doi.org/10.5772/56591

Rojas, J., Bedoya, M. & Ciro, Y (Eds). **2015**. *Current Trends in the Production of Cellulose Nanoparticles and Nanocomposites for Biomedical Applications*. pp 193–228.
https://doi.org/10.5772/61334

Sampath, U., Ching, Y., Chuah, C., Sabariah, J. & Lin, P.-C. **2016**. Fabrication of porous materials from natural/synthetic biopolymers and their composites. *Materials*. 9(991): 1–32.
https://doi.org/10.3390/ma9120991

Sahana, T.G. & Rekha, P.D. **2018**. Biopolymers: Applications in wound healing and skin tissue engineering. *Molecular Biology Reports*. 45(1): 2857–2867.
https://doi.org/10.1007/s11033-018-4296-3

Sarraf, A., Veron, E., Addoun, N., Boual, Z., Ould El Hadj, M. D., El Alaoui-Talibi, Z., Modafar, E.L., Abdelkaf, S., Fendri, I., Delattre, C., Dubessay, P., Michaud, P. & Pierre, G. **2021**. Polysaccharides and derivatives from Africa to address and advance sustainable development and economic growth in the next decade. *Applied Sciences*. 11(11): 1–29.
https://doi.org/10.3390/app11115243

Sawpan, M. A., Pickering, K. L. & Fernyhough, A. **2011**. Effect of fibre treatments on interfacial shear strength of hemp fibre reinforced polylactide and unsaturated polyester composites. *Composites Part A: Applied Science and Manufacturing*. 42(9): 1189–1196.
https://doi.org/10.1016/j.compositesa.2011.05.003

Schepetkin, I.A., Xie, G., Kirpotina, L.N., Klein, R.A., Jutila, M.A. & Quinn, M.T. **2008**. Macrophage immunomodulatory activity of polysaccharides isolated from Opuntia polyacantha. *International Immunopharmacology.* 8(10): 1455–1466.
https://doi.org/10.1016/j.intimp.2008.06.003

Segato, F., Damásio, A.R.L., de Lucas, R.C., Squina, F.M. & Prade, R.A. **2014**. Genomics review of holocellulose deconstruction by *Aspergilli. Microbiology and Molecular Biology Reviews*. 78(4): 588–613. https://doi.org/10.1128/mmbr.00019-14

Shamsuddin, I.M., Jafar, J.A., Shawai, A.S.A., Yusuf, S., Lateefah, M. & Aminu, I. **2017**. Bioplastics as better alternative to petroplastics and their role in national sustainability: A Review. *Advances in Bioscience and Bioengineering*. 5(4): 63–70. https://doi.org/10.11648/j.abb.20170504.13

Shankar, S. & Rhim, J. 2**018**. Bionanocomposite films for food packaging applications. In Knoerzer, K. & Muthukumarappan, K. (Eds*.). Innovative Food Processing Technologies*. Australia: Elsevier. pp 234–243. https://doi.org/10.1016/B978-0-12-815781-7.21875-1

Sikhosana, S.T., Gumede, T.P., Malebo, N.J. & Ogundeji, A.O. **2021**. Poly(lactic acid) and its composites as functional materials for 3-D scaffolds in biomedical applications: *A mini-review of recent trends*. *Express Polymer Letters*. 15(6): 568–580. https://doi.org/10.3144/expresspolymlett.2021.48

Sindhu, K.A., Prasanth, R. & Thakur, V.K. **2014**. Medical Applications of Cellulose and its Derivatives: Present and Future. *Nanocellulose Polymer Nanocomposites*. 437–477. https://doi.org/10.1002/9781118872246.ch16

Singhvi, M.S., Zinjarde, S.S. & Gokhale, D.V. **2019**. Polylactic acid: synthesis and biomedical applications. *Journal of Applied Microbiology*. 127: 1612–1626. https://doi.org/10.1111/jam.14290

Singla, R., Soni, S., Patial, V., Kulurkar, P.J., Kumari, A., Mahesh, S., Padwad, Y.S. & Yadav, S.K. **2017**. Cytocompatible anti-microbial dressing of *Syzygium cumin* cellulose nanocrystals decorated with silver nanoparticles. Accelerated acute and diabetic wound healing. *Scientific reports*. 7(1): 1–13. https://doi.org/10.1038/s41598-017-08897-9

Siqueira, G., Abdillahi, H., Bras, J. & Dufresne, A. **2009**. High reinforcing capability cellulose nanocrystals extracted from *Syngonanthus nitens (Capim Dourado). Cellulose*. 17(2): 289–298. https://doi.org/10.1007/s10570-009-9384-z

Smole, S.M., Hribernik, S., Stana, K. & Kree, T. **2013**. Plant fibres for textile and technical applications. In Grundas, S. & Stepniewski, A. (Eds.). *Advances in Agrophysical Research*. London: IntechOpen. pp 370–398. https://doi.org/10.5772/52372

Sonia, A. & Dasan, P.K. **2013**. Chemical, morphology and thermal evaluation of cellulose microfibers obtained from *Hibiscus sabdariffa*. *Carbohydrate Polymers*. 92(1): 668–674. https://doi.org/10.1016/j.carbpol.2012.09.015

Swain, S.K., Pattanayak, A.J. & Sahoo, A.P. **2018**. Functional biopolymer composites. In: Thakur, V.K. & Thakur, M.K. (Eds.). *Functional Biopolymers*. Switzerland: Springer International Publishing GA. pp 159–182. https://doi.org/10.1007/978-3-319-66417-0_6

Tajeddin, B. **2014**. Cellulose-based polymers for packaging applications. In Thakur, V.K. (Ed.). *Lignocellulosic Polymer Composites*. Massachusetts: Scrivener Publishing LLC. pp 477–498. https://doi.org/10.1002/9781118773949.ch21

Tiwari, P., Kumar, B., Kaur, G. & Kaur, H. **2011**. Phytochemical screening and extraction: A review. *International Pharmaceutica Sciencia*. 1(1): 98–106.

Ullah, S., Khalil, A. A., Shaukat, F. & Song, Y. **2019**. Sources, extraction and biomedical properties of polysaccharides. *Foods*. 8(8): 1- 23. https://doi.org/10.3390/foods8080304

Wankhade, V. **2020**. Animal-derived biopolymers in food and biomedical technology. In Kunal, P. Banerjee, I., Sarkar, P., Kim, D., Deng, W.-P., Dubey, N.K. & Majumder, K. (Eds.). *Biopolymer-Based Formulations*. Amsterdam: Elsevier. pp 139–152. https://doi.org/10.1016/b978-0-12-816897-4.00006-0

Xiao, L., Mai, Y., He, F., Yu, L., Zhang, L., Tang, H. & Yang, G. **2012**. Bio-based green composites with high performance from poly(lactic acid) and surface-modified microcrystalline cellulose. *Journal of Materials Chemistry*. 22(31): 15732–15739. https://doi.org/10.1039/c2jm32373g

Yalavarthi, C. & Thiruvengadarajan, V.S. **2013**. A review on identification strategy of phyto-constituents present in herbal plants. *International Journal of Research in Pharmaceutical Sciences*. 4(2): 123-140.

Yuqing, D., Fenbo, M., Ruiz-Ortega, L.I., Ying, P., Ye, T., Wenqi, H. & Bin, T. **2019**. Fabrication of strontium Eucommia ulmoides polysaccharides and in vitro evaluation of their osteoimmunomodulatory property. *International Journal of Biological Macromolecules.* 140(2019): 727–725. https://doi.org/10.1016/j.ijbiomac.2019.08.14

Zahan, K.A., Azizul, N.M., Mustapha, M., Tong, W.Y., Abdul Rahman, M.S. & Sahuri, I.S. **2020**. Application of bacterial cellulose film as a biodegradable and antimicrobial packaging material. *Materials Today: Proceedings*. 31(2020): 83–88. https://doi.org/10.1016/j.matpr.2020.01.201

Zhao, X., Hu, H., Wang X., Yu, X., Zhou, W. & Peng, S. **2020**. Super tough poly(lactic acid) blends: a comprehensive review. *Royal Society of Chemistry*. 10: 13316–13368. https://doi.org/10.1039/d0ra01801e

CHAPTER 3

Methodology

3.1. Materials

3.1.1. *Eucomis autumnalis*

The source of cellulose used in this study was *Eucomis autumnalis*, a medicinal plant collected from the District of Maseru in Lesotho (Figure 3.1.a-b). To prepare plant material for all experiments, the leaves, stem, and bulbs of *E. autumnalis* were separately stripped, dried, and ground into fine powder (Figure 3.2).

Figure 3.1. (a) Flowering plant of *E. autumnalis*, and (b) Bulb of *E. autumnalis*

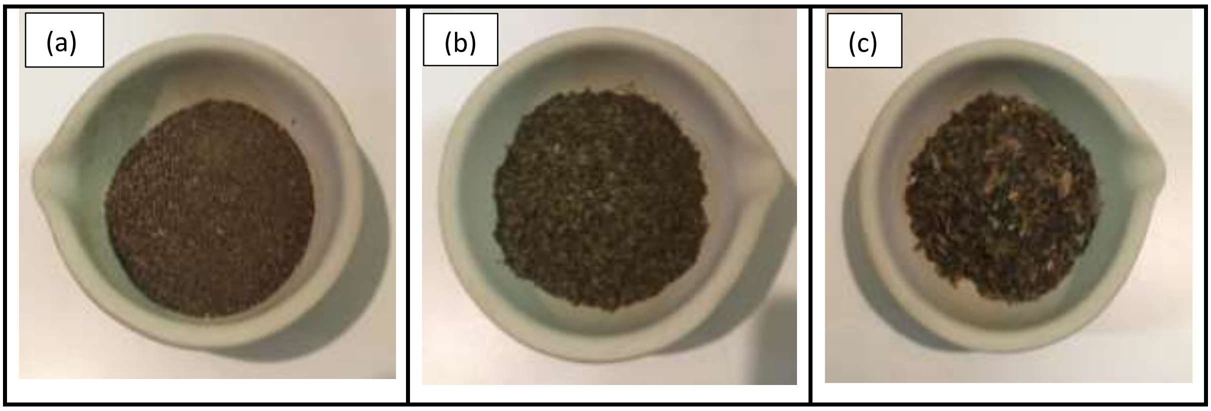


Figure 3.2. Dried and finely ground tissue of *E. autumnalis* (a) bulbs, (b) leaves and (c) stems

3.1.1.1. Delignification of cellulose

The dried and finely ground tissue of *E. autumnalis* leaves, stems, and bulbs were bleached distinctly in 0.7% (w/v) sodium chloride solution (NaCl, Lasec, Bloemfontein, South Africa), fibre to liquor ratio of 1:50. The pH of the mixtures was adjusted to 4 with a solution of 5% acetic acid (CH_3COOH, Lasec, Bloemfontein, South Africa). To remove lignin, the mixtures were heated at 110 °C in a water bath (Biobase, 20L) for 5 hours and then washed with distilled water. The neutral residuals were further heated in a 250 ml solution of 5% (w/v) sodium sulphite (Na_2SO_3, Lasec, Bloemfontein, South Africa) at 110 °C in a water bath for 5 hours, followed by thorough washing with distilled water to completely remove lignin and then hemicellulose.

3.1.1.2. Isolation of cellulose

Residues of the delignification step were treated with 250 ml of 18% (w/v) sodium hydroxide (NaOH, Lasec, Bloemfontein, South Africa) solution in a water bath set at 110 °C for 5 hours to remove hemicellulose thoroughly. The resultant product was recovered by filtration, washed with distilled water until neutral, and air-dried. The recovered cellulose from the leaves, stems and bulbs was treated with dimethylsulfoxide (DMSO, Merck, Johannesburg, South Africa) (50 ml) to remove any other impurities. It was then washed with distilled water and air-dried (Figure 3.3). Before final washing and drying, a clear gel-like substance that completely dissolves in DMSO to form a suspension was obtained from both the bulbs and stems. This is a typical characteristic feature of nanocellulose. However, this was not the case with the leaves. They required more processing to obtain this characteristic, and this was not possible due to low product yield. Although comparable features were observed in celluloses from the bulbs and stems, the following: (i) the slight difference in the chemical structure, and (ii) the lower yield in bulb cellulose (see Table 3.1) led to the utilization of cellulose from the stems for nanocomposite preparation in this study.

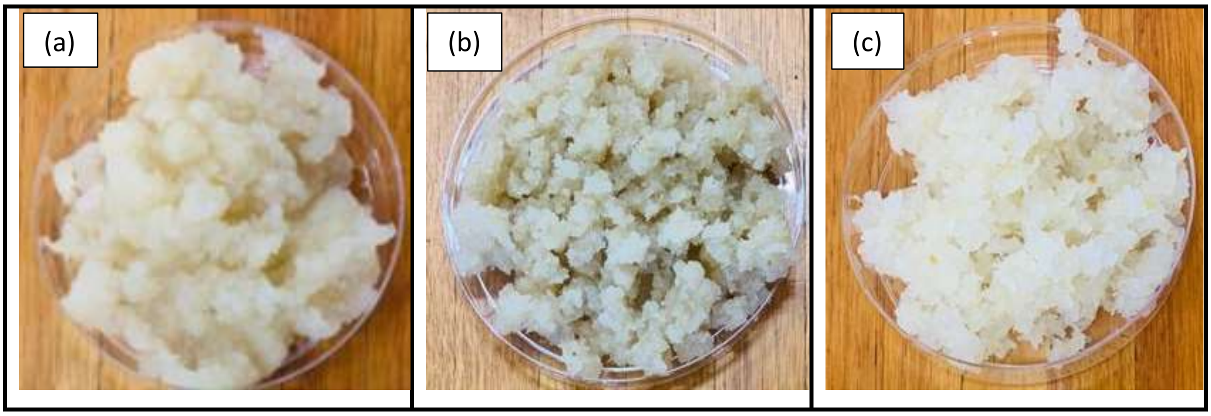


Figure 3.3. Visibly different textures of the extracted cellulose from the bulbs (a), leaves (b) and stems (c) before drying

Table 3.1. Chemical composition of cellulose in *E.automnalis*

Sample	Mass (g) (ground powder)	Mass (g) (cellulose mass)	Cellulose % yield
Cellulose from the bulbs	60.0251	12.8568	21
Cellulose from the leaves	60.0211	23.0226	38
Cellulose from the stem	60.0514	19.6572	33

3.1.2. Polylactic acid (PLA)

The polylactic acid (PLA, Merck, Johannesburg, South Africa) used in this study was a commercial-grade (Corbion LX175). It possessed an L-isomer content of 96%, a density of 1.24 g cm^{-3}, a glass transition temperature of 60 °C, and a melting temperature of 155 °C.

3.2. Sample preparation

3.2.1. Preparation of PLA/nanocellulose composites

3.2.1.1. Torque Rheomixer

The nanocomposites were prepared by utilizing a Haake PolyLab OS Rheomix with a counter-rotating roller at the following operating conditions: 165 °C, 100 rpm, for 5 min.

The calculated weight percentages of the different components in each of the investigated samples are shown in Table 3.2.

Table 3.2. Weight percentages of the components in the nanocomposites

PLA (wt.%)	Cellulose from the stems (wt.%)
100.0	0.0
99.5	0.5
97.0	3.0
0.0	100.0

3.2.1.2. Compression moulding

Prior to compression moulding, the nanocomposites were crushed into a powder to a particle size of less than 2 mm. Afterwards, the ground samples were dried overnight using a desiccator drier at 60 °C. The nanocomposites were then compression-moulded into tensile specimen using a Carver, model 973214A. Table 3.3, summarises the operational conditions.

Table 3.3. Operational Conditions

Temperature (°C)	Holding time before applying pressure (minutes)	Applied pressure (MPa)	Holding time under applied pressure (Minutes)
180	6	1	3

3.3. Sample characterisation

Various characterisation techniques such as FTIR, SEM, XRD, TGA and Tensile tester were employed in this study to analyse the chemical structures, surface morphology, and structural properties, as well as thermal and mechanical properties of the samples.

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

FTIR is a powerful analytical tool used for evaluating various materials, especially identifying unknown materials. Among the different methods used for characterisation, the FTIR is usually the initial step of analysis for polymeric materials and provides crucial information on the molecular structure of organic and inorganic components (Dyamenahalli *et al*., 2015; Kohli and Mittal, 2019).

In this study, FTIR was used to confirm the purity of nanocellulose extracted from the *Eucomis autumnalis* as well as to detect the presence of specific impurities. The powdered sample (i.e., leaves, stem, and bulbs) was placed on the microscope stage and clamped. The light emerging from the sample was channelled into a detector and then analysed on the screen using an attenuated total reflectance 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)

SEM is the most useful tool for acquiring a surface image of a material using an electron beam. Unlike the modern light microscope that uses light, SEM produces images of the sample using a high-energy beam of electrons to scan over the sample. An incident beam of electrons is rapidly scanned across the sample surface, and as electrons hit the sample, the signal is varied to form an image of the surface (Kohli and Mittal, 2019; Rajeswari, 2020; Yan, 2010).

To qualitatively analyse the surface texture of neat PLA, nanocellulose, and PLA/nanocellulose composites, the samples were mounted on aluminium stubs (Cambridge pin type, 10 mm) using an epoxy glue. The mounted samples were sputter coated with Iridium (± 5 nm) Leica EM ACE600 coater for conductivity before SEM analysis. The surface morphology of the specimens was imaged with a JSM-7800F Extreme-resolution Analytical Field Emission Scanning Electron Microscopy at an accelerating voltage of 5.0 kV.

3.3.3. Structural properties: X-Ray Diffraction (XRD)

XRD is an effective non-destructive analytical technique used for determining the structural properties of crystalline materials. A diffraction pattern occurs when a material is irradiated with a collimated beam of x-rays. However, amorphous materials such as glass lack a periodic array with long-range order and therefore do not form diffraction patterns (Kaliva, 2020). The constructive interference of a monochromatic X-ray beam scattered at specified angles from each set of lattice planes in the specimen produces XRD peaks. The intensities of the peaks are determined by the atomic locations within the lattice planes. Thus, the XRD pattern is specific to the periodic atomic arrangement of a given material (Kohli and Mittal, 2019: Rajeswari *et al*., 2020).

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

3.3.4. Thermal stabilities: Thermogravimetric analysis (TGA)

TGA is an analytical technique ideal for the characterisation of properties and behaviour such as composition, purity, decomposition reactions, decomposition temperatures, and absorbed moisture content of materials such as plastics, elastomers and thermosets, mineral compounds, and ceramics as well as chemical and pharmaceutical products.

In this study, TGA analyses were carried out using a PerkinElmer Pyris 1 TGA Q500 instrument to determine the purity and decomposition temperature of the investigated samples. The samples with a mass 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. An alternative and/or complementary presentation of TGA curves is the first derivative of the TGA curves for temperature or time. It displays the rate at which the mass changes and is known as the differential thermogravimetric or DTG curve (Ng *et al*., 2018; Vyazovkin, 2012).

3.3.5. Mechanical properties: Tensile testing analysis

Tensile testing is one of the fundamental techniques in polymer science that involves applying controlled tension to a sample until it fails. The test involves mounting a specimen that fairly represents the body of the material in question in an electromechanical or hydraulic testing machine and subjecting it to tension. When a polymer material is stretched to its limit (high-strain deformation), it undergoes plastic deformation (permanent distortion) and eventually fails. The reasons for performing tensile tests may vary, especially during material developmental stages where the suitability of materials and processes need to be determined and validated for the intended use. Most important is the strength of the material, which can be defined as the amount of stress required to cause visible deformation or the maximum stress that the material can withstand.

Another point of interest is the ductility of the material, which is the measure of how much it can be deformed before it fractures. Features that can be assessed during a tensile test include maximum elongation, ultimate tensile strength, and area reduction. With these properties, values for yield strength, Poisson's ratio, and Young's modulus may all be calculated. As a quality assurance measure, tensile properties are often included in material specifications (Davis, 2004: Milisavljević *et al*., 2012).

The tensile testing analysis of the compression moulded samples was carried out using an Instron 5966 tester (Instron Engineering Corporation, USA, ASTM 638D), with a load cell of 10 kN, and a universal testing machine at a crosshead speed of 5 mm min^{-1} to determine the strength and ductility of the materials. The dumbbell shaped samples had a Gauge length of 25 mm, a thickness of 3 mm, and a width of 3 mm. Approximately nine samples of each composition were tested, and average values with standard deviations are presented.

3.4. References

Davis, J.R. **2004**. Introduction to Tensile Testing. In Davis, J.R. (Ed.). *Tensile Testing*. United States: ASM International. pp 1–12.

De Nardo, L. & Farè, S. **2017**. Dynamico-mechanical characterization of polymer biomaterials. In Tanzi, M.C. & Farè, S. (Eds.). *Characterization of Polymeric Biomaterials*. pp 203–232. https://doi.org/10.1016/B978-0-08-100737-2.00009-1

Drzeżdżon, J., Jacewicz, D., Sielicka, A. & Chmurzyński, L. **2019**. Characterization of polymers based on differential scanning calorimetry based techniques. *TrAC Trends in Analytical Chemistry*. 110(1): 51-56. https://doi.org/10.1016/j.trac.2018.10.037

Dyamenahalli, K., Famili, A. & Shandas, R. **2015**. Characterization of shape-memory polymers for biomedical applications. In L'Hocine, Y. (Ed.). *Shape Memory Polymers for Biomedical Applications*. England: Woodhead Publishing. pp 35–63. https://doi.org/10.1016/b978-0-85709-698-2.00003-9

Kaliva, M. & Vamvakaki, M. **2020**. Nanomaterials characterization. In Narain, R. (Ed.). *Polymer Science and Nanotechnology*. Amsterdam: Elsevier. pp 401–433. https://doi.org/10.1016/b978-0-12-816806-6.00017-0

Kohli, R. & Mittal, K.L. **2019**. Methods for assessing surface cleanliness. *Developments in Surface Contamination and Cleaning*. 12(1): 23–105. https://doi.org/10.1016/b978-0-12-816081-7.00003-6

Kumar, S. **2014**. Selective laser sintering/melting. *Comprehensive Materials Processing*. 10(1): 93–134. https://doi.org/10.1016/b978-0-08-096532-1.01003-7

Menard, K.P. & Menard, N.R. **2015**. Dynamic mechanical analysis in the analysis of polymers and rubbers. In Mark, H.F. (Ed.). *Encyclopedia of Polymer Science and Technology*. United States: John Wiley & Sons. pp 1–33. https://doi.org/10.1002/0471440264.pst102.pub2

Milisavljević, J., Petrović, E., Ćirić, I., Mančić, M., Marković, D. & Đorđević, M. **2012**. (Paper presented at the DAS-29, 29th Danubia-Adria Symposium: "Tensile testing for different types of polymers" held at the University of Belgrade in Serbia) University of Belgrade, Serbia.

Ng, H.M., Saidi, N.M., Omar, F.S., Ramesh, K., Ramesh, S. & Bashir, S. **2018**. Thermogravimetric analysis of polymers. In Mark, H.F. (Ed.). *Encyclopedia of Polymer Science and Technology*. United State: John Wiley and Sons. pp 1–29. https://doi.org/10.1002/0471440264.pst667

Rajeswari, A., Jackcina Stobel Christy, E., Gopi, S., Jayaraj, K. & Pius, A. **2020**. Characterization studies of polymer-based composites related to functionalized filler-matrix interface. In Goh, K.G., Aswathi, M.K., De Silva, R.T. & Thomas, S. (Eds.). *Interfaces in Particle and Fibre Reinforced Composites*. England: Woodhead Publishing. pp 219–250. https://doi.org/10.1016/b978-0-08-102665-6.00009-1

Schick, C. **2009**. Differential scanning calorimetry (DSC) of semicrystalline polymers. *Analytical and Bioanalytical Chemistry*. 395(6): 1589–1611. https://doi.org/10.1007/s00216-009-3169-y

Vyazovkin, S. **2012**. Thermogravimetric analysis. In Kaufmann, E.N. (Ed.). *Characterization of Materials*. United State: John Wiley and Sons. pp 1–12. https://doi.org/10.1002/0471266965.com029.pub2

Yan, Y. **2010**. Tribology and tribo-corrosion testing and analysis of metallic biomaterials. In Niinomi, M. (Ed.). *Metals for Biomedical Devices*. England: Woodhead Publishing Limited. pp 178–201. https://doi.org/10.1533/9781845699246.2.178

CHAPTER 4

Results and Discussion

4.1. Cellulose extracted from *Eucomis autumnalis*

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

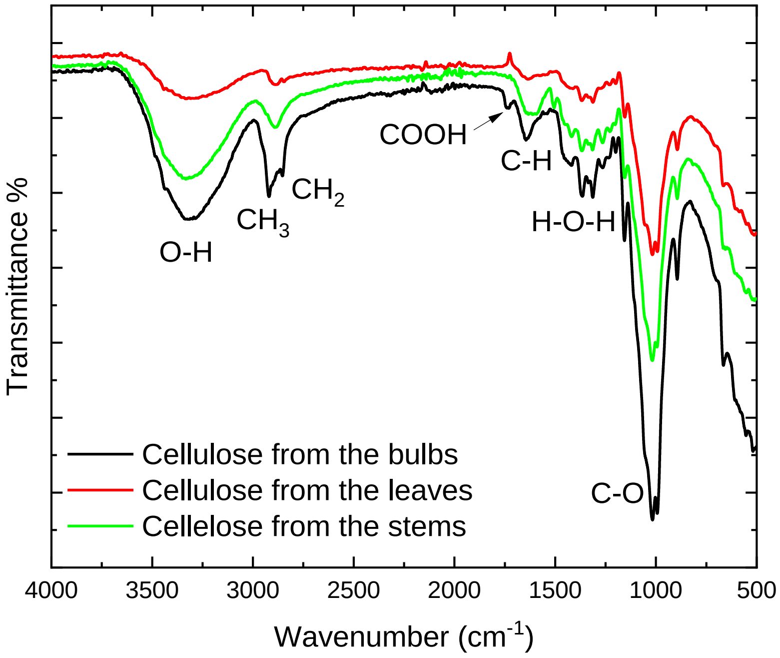


Figure 4.1. FTIR spectra of celluloses extracted from the bulbs, leaves, and stem

The chemical composition of the cellulose extracted was studied by FTIR spectroscopy and the results are presented in Figure 4.1. The cellulose spectra present a distinctive broad absorption band at 3316 cm^{-1} that corresponds to O-H groups stretching cellulose vibrations. Other cellulose feature groups were detected from peaks at 2826 cm^{-1}, 1644 cm^{-1}, 1423–1368 cm^{-1} and 1161–1023 cm^{-1} which represent C–H stretch, absorbed water H_2O bending, C–O ether stretch and alcohol respectively (Hivechi *et al*, 2020; Inácio *et al*., 2020; Morán *et al*, 2008). However, the spectra corresponding to cellulose obtained from the bulbs and leaves display two absorption peaks at 2922 cm^{-1} and 2853 cm^{-1}, indicating the presence of long alkyl chains. These peaks are attributed to –CH3 and –CH2 asymmetrical stretching vibrations and symmetrical stretching vibrations, respectively, which are commonly associated with methylcellulose (Gao *et al*., 2015; Hu *et al*., 2017; Nadour *et al*., 2017). Also noted on the curve of cellulose obtained from the bulbs is a characteristic peak primarily due to CH_3COO and COOH functionality of oleanolic acid associated with saponins, at 1741 cm^{-1} (Almutairi and Ali, 2014; El Aziz *et al*., 2019;). The appearance of these stretching vibrations, including the peak at 2922 $cm^{-1,}$ could be due to excessive amounts of saponins verified by the presence of increased foaming in all samples during extraction (Li *et al*., 2013). However, these two peaks cannot be seen on the leaf and stem spectrums, which confirms thorough removal of the saponin from both samples during chemical treatment and washing with distilled water. Saponins represent a class of plant chemicals responsible for the biological activity of some medicinal plants including *E. autumnalis* (Alaribe *et al*., 2018). Glycosylation of their hydrophobic aglycone leads to the formation of copious foam when agitated in water (Cherian *et al.*, 2012).

Saponins may likely be found in excess in the bulbs than the other plant parts. They are produced in large quantities for protection against fungi, especially in bulbs that are buried in the soil where scores of microbial populations can be found (Gentsch *et al*., 2020; Papadopoulou *et al*., 1999; Trdá *et al*., 2015). Also, the life of flowering plants like *E. autumnalis* stems from the bulbs, which are regarded as storage organs and facilitators of nutrient transfer throughout the plant (Flores, H.E., & Flores, 1997). Other studies have associated this observation with hemicellulose, but this can only be true for woody plants that are highly lignified and sometimes resist the alkali treatment (Hu *et al*., 2017; Morán *et al*., 2008). Herbaceous plants are very low in cementing materials such as lignin and hemicellulose, thus not very rigid and easily hydrolysable. They are known to possess higher cellulose content and produce an increased yield compared to woody plants (Chen *et al*., 2017; Novaes *et al*., 2010; Sorieul *et al*., 2016). Lastly, the Cs spectrum is typical for cellulose and this observation corresponds to results obtained by other researchers (Hivechi *et al*, 2020; Inácio *et al*., 2020; Morán *et al*, 2008). Since FTIR only detects the purity and functional groups present in a material, Scanning Electron Microscopy (SEM) was used to confirm the morphology of the extracts.

4.1.2. Surface morphology analysis: Scanning electron microscopy (SEM)

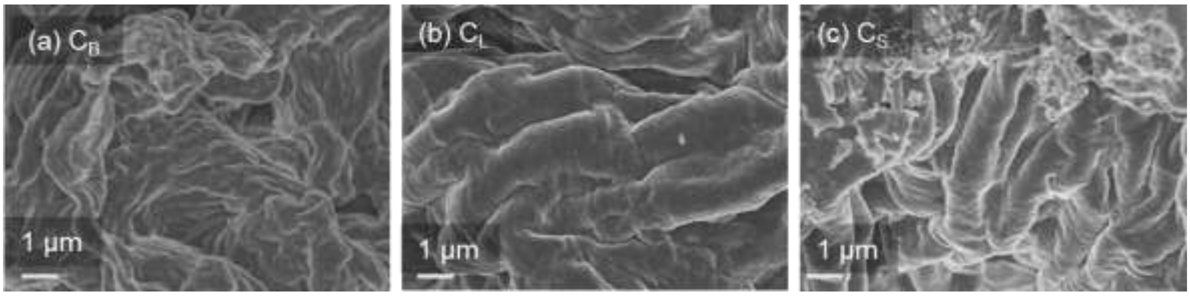


Figure 4.2. SEM micrographs for the celluloses extracted from the (a) bulbs C_B, (b) leaves C_L, and (c) stem C_S.

The SEM micrographs of cellulose obtained from three different parts of *E. autumnalis*, that is, the bulbs, leaves and stems are presented in Figure 4.2a, b and c, respectively. In Figure 4.2a the surface of the short and thin cellulose fibres appears smooth probably due to the existence of highly surface active saponins as confirmed by the FTIR analysis. The image shows multiple terraces, steps, and bends that resulted from the hydrolysis (Zhao *et al*., 2007). Figures 4.2b and c exhibit well organised and uniform cellulose surfaces that indicate the partial separation of fibre bundles, confirming that all hemicellulose and other non-cellulosic components have been removed (Asrofi *et al*., 2018; Zhao *et al*., 2007). A careful zoom into Figure 4.2c Cs also reveals different coarse aggregates of smaller dimensions. In addition to fibrous cells, the primary cell wall also consists of loose brick-like cells known as parenchyma cells. These cells are mainly composed of cellulose microstructures and are very common in the leaves and stems of herbaceous plants. When hydrolyzed, they produce microfibrillar crystals of cellulose. This observation is often associated with native cellulose (Abe & Yano, 2010; Gao *et al*., 2015; He *et al*., 2013). While SEM was useful for revealing the surface morphology of the samples, X-ray diffraction analysis (XRD) was required to determine the crystallinity, size, and orientation of the cellulose extracts.

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

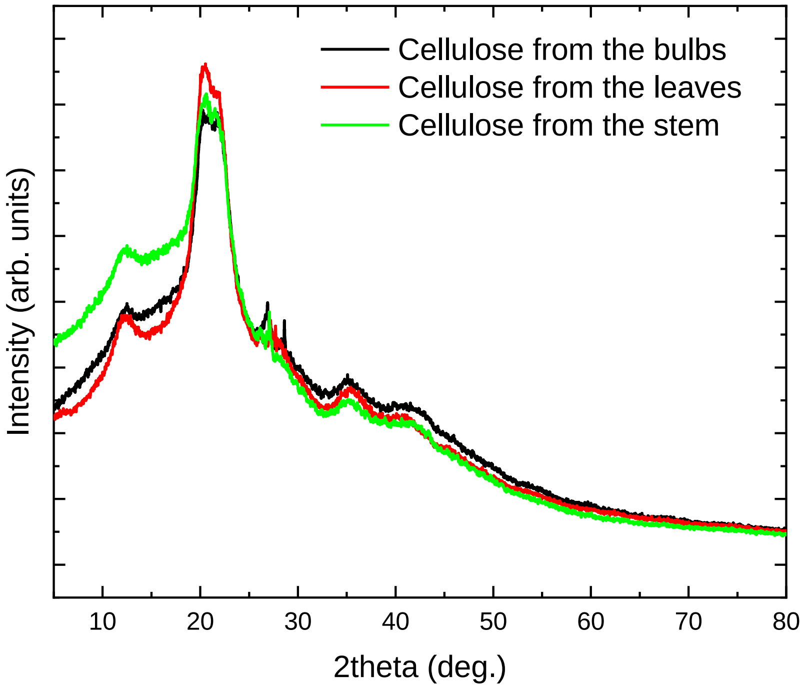
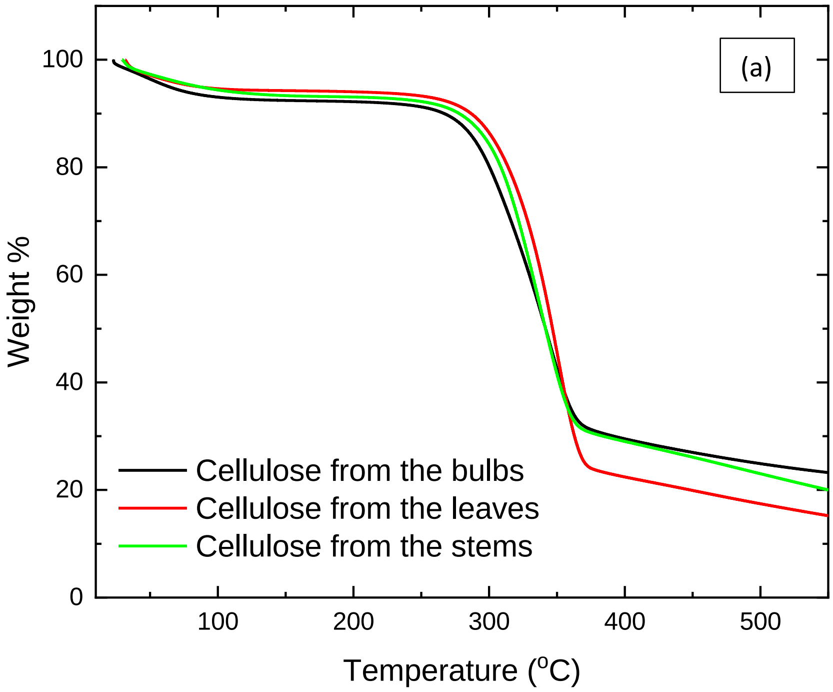


Figure 4.3. XRD diffractograms for the celluloses extracted from the bulbs, leaves, and stem

The X-ray diffraction patterns of celluloses from the bulbs, leaves and stems are shown in Figure 4.3. All three diffractograms display 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) (Naduparambath *et al*., 2017; Nie *et al*., 2019). Cellulose from the leaves shows higher crystallinity compared to celluloses from the bulbs and stems. This result may be due to the leaves having better lignification than the other parts of the plants, and the chemical treatment seems to have not influenced the microcrystalline structure of leaf cellulose as it did the bulb and stem celluloses (Chen *et al*., 2017; Novaes *et al*., 2010; Sorieul *et al*., 2016). Although the crystalline structure of cellulose fibres will not be altered by an increase or decrease in lignin content, in the presence of increased lignin during plant processing, removing this protective structure to access cellulose is generally challenging (Zhang *et al*., 2019). According to Poovaiah *et al*., (2014) lignin occludes cellulose and hemicellulose by physically shielding them from any form of degradation.

The supramolecular network of complex carbohydrates and aromatic polymers gives the cell adequate mechanical strength and rigidity, but it also makes the lignocellulosic materials naturally resistant to chemical and enzymatic treatments during processing (Himmel *et al*., 2007; Vanholme *et al*., 2008). The low crystallinity of the bulb and stem celluloses can be attributed to three factors: (i) the occurrence degradation to obtain isolated nanofibers (Liu *et al*., 2010), (ii) mechanical grinding, which is an efficient method of separating the microfibrils, may cause damage to the high crystalline ordered structure because of the reduced selectivity of high shearing force, leading to reduced crystallinity (Liu *et al*., 2016), (iii) the soft green stems of medicinal plants that lack lignification and whose cell wall contents are easily accessible did not only make it easy for hydrolysis to attack randomly ordered structures in cellulose but also altered the crystalline phase (Pattathil *et al*., 2015; Chen *et al*., 2017). The degree of crystallinity is one of the most important factors influencing cellulose's thermal degradation behaviour. Cellulosic materials with a high degree of crystallinity possessed usually have higher thermal stability (Johnsy & Sabapath, 2015). The thermal stability of the investigated samples was analysed using Thermogravimetric analysis (TGA).

4.1.4. Thermal stability: Thermogravimetric analysis (TGA)



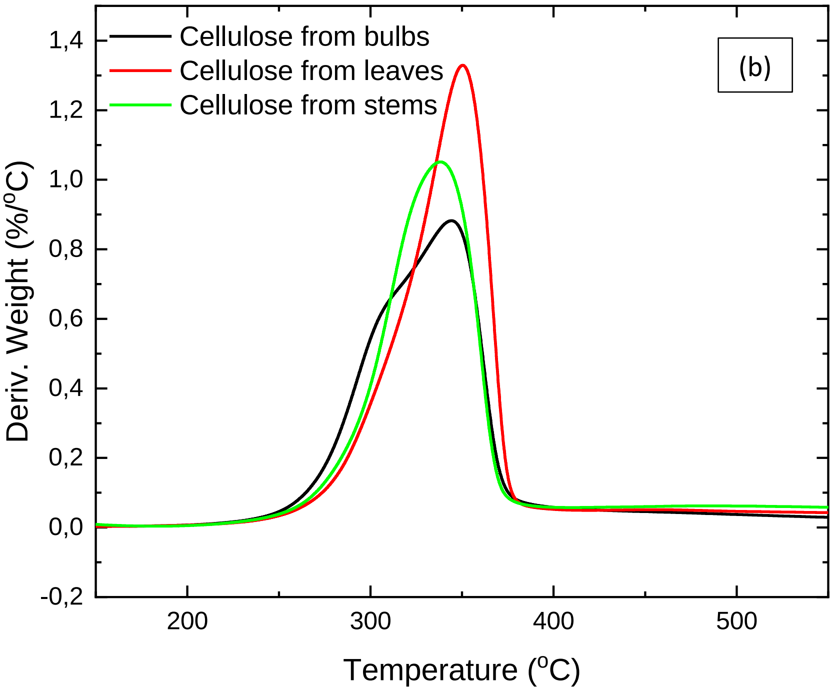


Figure 4.4. (a) TGA curves for the celluloses extracted from the bulbs, leaves, and stem. (b) TGA derivative curves for the celluloses extracted from the bulbs, leaves, and stem.

Thermal decomposition of the samples was investigated using TGA from 25 °C to 600 °C at 10 $°C.min^{-1}$ and, the weight loss curves (TG) are shown in Figure 4.4a. The thermograms show that all samples follow similar degradation patterns. Generally, the stages shown in the TG curves involve dehydration, depolymerisation and pyrolytic decomposition. At increased temperatures, this decomposition leads to the formation of H_2O, CO_2 and CH_4 (Naduparambath *et al*., 2017). The first minor loss occurred from 25 to 110 °C for all samples. This weight loss is basically due to the loss of absorbed and adsorbed water molecules from the polysaccharide structures through evaporation (Cabrales & Abiddi, 2019; Oliveira *et al*., 2015). The second and drastic weight loss is observed at the range 250–345 °C and is attributed to the breakdown of non-cellulosic and cellulosic materials. Among these samples, the most notable differences are at W_{460} (%), residual char weight at 460 °C. The char yield represents non-volatile carbonaceous material generated during pyrolysis presented by the residual weight after the decomposition step at 460 °C, is higher for bulb cellulose at 24%, followed by the stem (20%) and leaf cellulose (15%), respectively (Azubuike & Okhamafe, 2012). Higher weight residue in bulb cellulose may be due to saponins altering their structure by adding the CH_2 and COOH molecules as observed in the FTIR spectra. Interactions between high molecular weight saponins and cellulose lead to the hydrophobicity of the cellulose molecule, thereby increasing interfacial adhesion that leads to improved physical and mechanical properties (Cherian *et al*., 2012). Furthermore, medicinal plant isolated amorphous solid saponins, which contain 27 to 30 carbon atoms in the non-saccharide portion, will form part of the non-volatile carbonaceous represented by the 24% char yield (El Aziz et al., 2019; Azubuike & Okhamafe, 2012).

The thermal decomposition peaks for maximum weight loss of the different celluloses (bulbs, leaves and stems) are presented on the DTG curve in Figure 4.4b. The three well determined peak degradation temperatures of celluloses confirm their various degradation pathways (Naduparambath *et al*., 2017). The different midpoint temperature data are carefully outlined in Table 4.1. Another differentiable factor between the cellulose samples is observed in the onset and inflection point temperatures. The leaf cellulose has the highest onset point temperature, followed by the stem, and then the bulb celluloses. Onset temperature defines the thermal stability of a specific material. Thus, higher onset temperatures are associated with higher thermal stability (Azubuike & Okhamafe, 2012; Shameer & Nishath, 2019). The higher onset temperatures of leaf cellulose could be attributed to the presence of highly thermal stable lignin residues that were not fully hydrolysed by the chemical treatment. 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 (Nurazzi *et al*., 2021; Watkins *et al*., 2015). This is because lignin contains oxygenated functional groups with varied thermal stability values depending on the chain scission (Mariana *et al*., 2021). The presence of minute residues of lignin was confirmed on FTIR by the diminishing peak valley structures forming between 1750 and 1550 cm^{-1}, assigned to the C=O of lignin while a budding weak C-H band of cellulose forms (Inácio *et al*., 2020; Tian *et al*., 2017; Hu *et al*., 2017).

Table 4.1. Different midpoint temperature data

Sample	Temperature (ºC)
Cellulose from the bulbs (C_B)	344.2
Cellulose from the leaves (C_L)	350.4
Cellulose from the stem (C_S)	338.3

4.2 PLA/nanocellulose (stems) composites

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

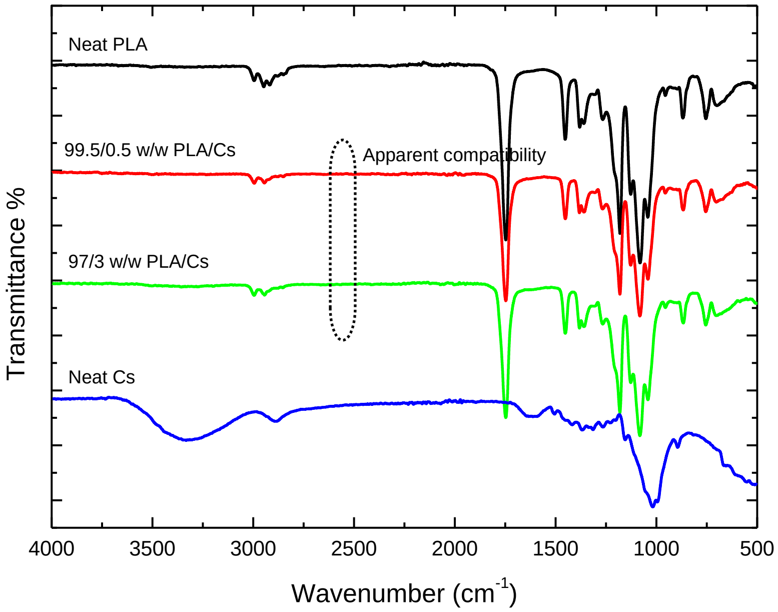


Figure 4.5. FTIR spectra of neat PLA, neat cellulose (stems) and the PLA/cellulose (stems, Cs) composites

Figure 4.5 shows the FTIR spectra of neat PLA, neat cellulose (stem) (Cs), as well as PLA/cellulose composites from the stems. Neat PLA is characterised by absorption bands located at 3000 cm^{-1} due to the C–H asymmetric stretching vibration, needle-like C=O bonds of the carbonyl group in 1750 and 1440 cm^{-1}, C–H stretching in the CH_3 at 1440 cm^{-1} and 1375 cm^{-1} and C-O stretching vibrations at 1175 and 1075 cm^{-1} (Salmieri *et al*., 2014; Mohamad *et al*., 2021). On the other hand, neat cellulose (stem) (Cs) shows characteristic peaks at 3316 cm^{-1} (O−H groups stretching vibrations), 2826 cm^{-1} (asymmetric and symmetric stretching methyl and methylene C−H groups), 1644 cm^{-1} (O−H group bending vibration or absorbed water), 1423–1368 (C−H bending) and 1161–1023 cm^{-1} (C-O stretching vibrations) (Hivechi *et al*, 2020; Inácio *et al*., 2020; Morán *et al*, 2008). The infusion of PLA and Cs features characteristic peaks or bands of both polymers, confirming the successful fabrication of the composites. Due to the uneven proportions of these components in the mixture, PLA vibrations are more dominant than Cs vibrations. Also, the position of PLA bands remains the same in the composites. However, there is a shift in the C-O groups of Cs to higher frequencies to form a single peak with C-O of PLA as well as the development of as well as the emergence of two new peaks around 3000 cm^{-1} and overlapping of the C-O groups can be observed. This suggests the occurrence of chemical interactions between PLA and Cs. The disappearance of Cs vibration at 3316 cm^{-1}, 2826 cm^{-1} and 1644 cm^{-1}, along with PLA peaks at 3000 cm^{-1} in the composites is probably due to the apparent compatibility between PLA and Cs (Trang Mai *et al*., 2012). Changes in the FTIR spectra of a composite material may confirm chemical interactions between the two materials. While the presence of new functional groups confirms covalent bonding, the shift of spectral bands characteristic of the material may indicate non-covalent interactions (Kowalczuk and Monika Pitucha, 2019). In terms of assessing the effect of Cs content in the PLA matrix, it can be seen that there are no visible differences in the FTIR spectra with the increase in Cs content. This is because FTIR focuses on revealing the various functional groups that exist in a molecule rather than its external morphology (Chen *et al*., 2018; Dyamenahalli *et al*., 2015; Kohli and Mittal, 2019). As a result, SEM was used to confirm any morphological changes that had occurred in the composites.

4.2.2. Surface morphology: Scanning electron microscopy analysis (SEM)

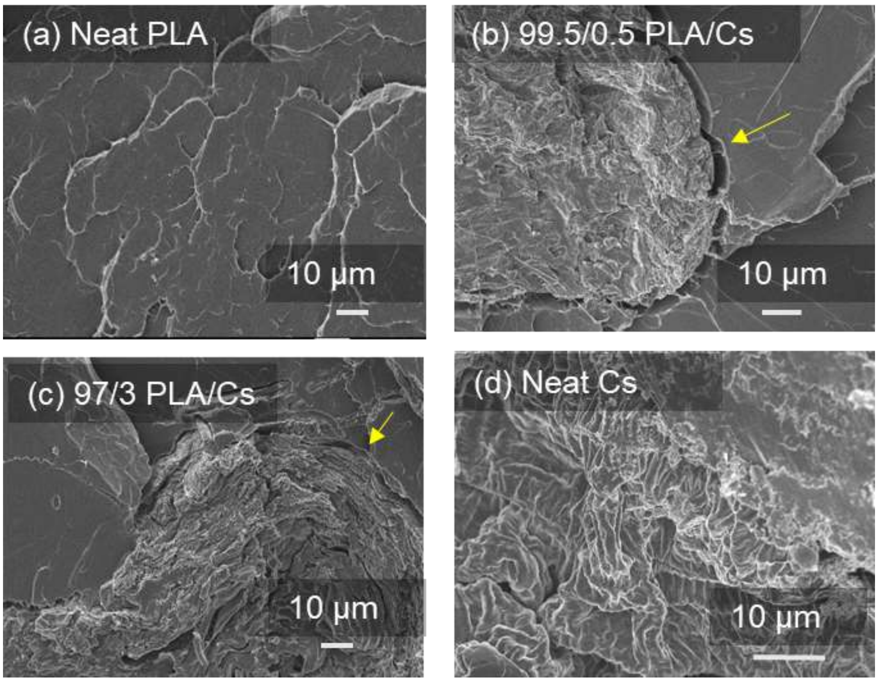


Figure 4.6. SEM micrographs for (a) neat PLA, (b) 99.5/0.5 PLA/Cs, (c) 97/3 PLA/Cs and (d) neat cellulose (stems) (Cs)

The morphology of PLA nanocellulose composites was investigated using SEM as shown in Figure 4.6. This technique was used to understand the effect of nanocellulose on fracture behaviour, including its distribution in a PLA matrix. Neat PLA (Figure 4.6(a)) exhibits a smooth brittle fracture (Eyholzer *et al*., 2012; Lu *et al*., 2016), while neat cellulose displays well organised and uniform surfaces with partial separation of fibre bundles and different coarse aggregates of smaller dimensions (Abe & Yano, 2010; Gao *et al*., 2015; He *et al*., 2013; Zhao *et al*., 2007). For the PLA/cellulose composites, the fractured surfaces of both composites seem rough and uneven after adding Cs in the PLA matrix, suggesting a significant matrix deformation. Voids and cracks feature between the fibres and matrix, which signifies that interface bonding is not uniform across interfaces (Frone *et al*., 2011; Singh *et al*., 2020). Also, fibre pull-outs are present, particularly at 0.5% Cs loading due to voids and cracks around the fibres (Kimura *et al*., 2019; Tanpichai & Wootthikanokkhan, 2018). As the filler content increases in the composites (i.e., 3.0 wt%), there is a reduction in the size of voids or cracks as shown by the arrow. There is also better distribution of fibres in the PLA matrix compared to the composites at 0.5% Cs content. These findings suggest that increasing the concentration of Cs in the PLA/Cs composites may have improved interfacial adhesion.

4.2.3. Thermal stabilities: Thermogravimetric analysis (TGA)

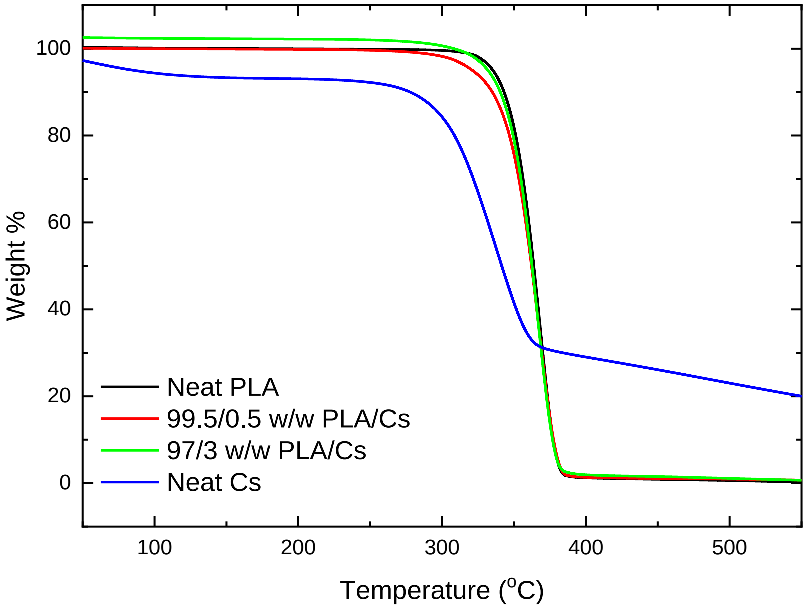


Figure 4.7. TGA curves of neat PLA, neat cellulose (stems) and the PLA/cellulose (stems) samples

Thermal decomposition of neat PLA, neat cellulose (stem)(Cs) and PLA/cellulose composites were studied using TGA from 25 °C to 600 °C at 10 °C.min-1. The weight loss curves (TG) are shown in Figure 4.7. Unlike PLA, which undergoes a single-step degradation process for complete disintegration, two degradation steps that lead to a fairly high residual mass can be observed for Cs following loss of moisture. The breakdown of cellulose in the PLA/Cs composites was not observed as an independent step but as a single degradation step mimicking that of neat PLA. Seemingly, PLA whose degradation is at elevated temperatures protected Cs from lower temperature degradation, and as a result, the composites started degrading at higher temperatures (Mofokeng *et al*., 2011). This was to be expected given that PLA is more thermally stable than cellulose. In a similar case, Kucharczyk *et al*. suggested that the one-step degradation observed is an indication that all components degraded simultaneously.

4.2.4. Mechanical properties: Tensile testing

Table 4.2. Summary of tensile testing results for all the investigated samples

Sample	σ_y/**MPa**	σ_b/**MPa**	ε_b/**%**	**E/MPa**
Neat PLA	107.9 ± 9.5	107.4 ± 9.3	4.9 ± 0.8	3447.5 ± 207.7
PLA/cellulose stems w/w 99.5/0.5	102.5 ± 4.6	101.7 ± 5.0	4.3 ± 0.4	3402.1 ± 278.8
PLA/cellulose stems w/w 97/3	98.0 ± 2.7	98.9 ± 2.9	3.8 ± 0.4	3466.2 ± 352.7

σ_y—stress at yield; σ_b—stress at break; ε_b—strain at break; E—Young's modulus.

Mechanical properties of neat PLA and PLA/cellulose (stem) (Cs) composites at two different filler matrix concentrations were evaluated by tensile and the results are reported in Table 4.2. The results show decreasing patterns for stress at yield, stress at break and strain at break as the content of cellulose increases in the PLA matrix. A slight decrease in Young's modulus can be observed with low filler content (i.e., 0.5 wt.%). However, this decrease is followed by a significant increase with higher Cs content. This value increased from 3447.5 MPa for neat PLA to 3466.2 MPa for the composite with 3.0 wt.% Cs. Greater values of Young's modulus translate to increased material stiffness, which is determined by the presence of reinforcement in the matrix. The interfacial bonding between Cs (filler) and matrix PLA (matrix) was confirmed by SEM micrographs of PLA/Cs at 3.0 wt.%. It is apparent from the readings that incorporating 3.0 wt.% Cs into the PLA matrix restricted flexibility and deformation by introducing a mechanical restraint. In the presence of some filler materials (such as Cs), the restriction of polymeric molecular diffusion, including that of PLA, occurs due to effective attraction potential between chain segments and the repulsive potential that the polymer is exposed to when it is close to the filler particles. The extent of particle restriction depends on the properties of individual components in the mixture. Thus, the interactions formed at interphase contribute to the improvement in composite modulus (Fu *et al*., 2008; Gumede *et al*., 2019; Gumede *et al*., 2019). In general, cellulose is a stiff material with inherently high chain rigidity and its adhesion to PLA leads to improved mechanical properties and is thus evident in the fabricated composites (Yang *et al*., 2019).

4.3. References

Abe, K. & Yano, H. **2009**. Comparison of the characteristics of cellulose microfibril aggregates of wood, rice straw and potato tuber. *Cellulose*. 16(6): 1017–1023. https://doi.org/10.1007/s10570-009-9334-9

Almutairi, M.S. & Ali, M. **2014**. Direct detection of saponins in crude extracts of soapnuts by FTIR. *Natural Product Research: Formerly Natural Product Letters*. https://doi.org/10.1080/14786419.2014.992345

Asrofi, M., Abral, H., Kasim, A., Pratoto, A., Mahardika, M., Park, J.-W. & Kim, H.-J. **2018**. Isolation of nanocellulose from water hyacinth fiber (WHF) produced via digester-sonication and its characterization. *Fibers and Polymers*. 19(8): 1618–1625. https://doi.org/10.1007/s12221-018-7953-1

Azubuike, C.P. & Okhamafe, A.O. **2012**. Physicochemical, spectroscopic and thermal properties of microcrystalline cellulose derived from corn cobs. *International Journal of Recycling of Organic Waste in Agriculture*. 1(9): 1–7. https://doi.org/10.1186/2251-7715-1-9

Cabrales, L. & Abidi, N. **2019**. Kinetics of cellulose deposition in developing cotton fibers studied by thermogravimetric analysis. *Fibers*. 7(78): 1–16. https://doi.org/10.3390/fib709007

Chen, Y., Tan, T., Lee, H. & Abd Hamid, S. **2017**. Easy fabrication of highly thermal-stable cellulose nanocrystals using Cr(NO3)3 catalytic hydrolysis system: A feasibility study from macro- to nano-dimensions. *Materials*. 10(1): 1–24. https://doi.org/:10.3390/ma10010042

Chen, J., Zhang T.-Y., Jin, F.-L. & Park, S.-J. **2018**. Fracture toughness improvement of poly(lactic acid) reinforced with poly(ε-caprolactone) and surface-modified silicon carbide. *Advances in Materials Science and Engineering*. 2018(0): 1–10. https://doi.org/10.1155/2018/6537621

Cherian, B.M., Leão, A.L., da Silva-Caldeira, M., Chiarelli, D., de Souza, S.F., Narine, S. & de Morais-Chaves, M.R. **2012**. Use of Saponins as an effective surface modifier in cellulose nanocomposites. *Molecular Crystals and Liquid Crystals.* 556(1): 233–245. https://doi.org/10.1080/15421406.2012.635969

El Aziz, M.M.A., Ashour, A.S., Melad, A.S.G. **2019**. A review on saponins from medicinal plants: chemistry, isolation, and determination. *Journal of Nanomedicine Research*. 7(4): 282–288. https://doi.org/10.15406/jnmr.2019.07.00199

Eyholzer, C., Tingaut, P., Zimmermann, T. & Oksman, K. **2012**. Dispersion and reinforcing potential of carboxymethylated nanofibrillated cellulose powders modified with 1-hexanol in extruded poly(Lactic Acid) (PLA) composites. *Journal of Polymers and the Environment*. 20(4): 1052–1062. https://doi.org/10.1007/s10924-012-0508-4

Flores, H.E. & Flores, T. **1997**. Biology and biochemistry of underground plant storage organs. In Johns, T. & Romeo, J.T. (Eds.) *Functionality of Food Phytochemicals*. New York. pp113–132. https://doi.org/10.1007/978-1-4615-5919-1_5

Fu, S.-Y., Feng, X.-Q., Lauke, B & Mai, Y.-W. **2008**. Effects of particle size, particle/matrix interface adhesion and particle loading on mechanical properties of particulate–polymer composites. *Composites: Part B*. 39(6): 933–961. https://doi.org/10.1016/j.compositesb.2008.01.002

Frone, A.N., Berlioz, S., Chailan, J.-F., Panaitescu, D.M. & Donescu, D. **2011**. Cellulose fiber-reinforced polylactic acid. *Polymer Composites*. 32(6): 976–985. https://doi.org/10.1002/pc.21116

Gao, X., Chen, K., Zhang, H. & Peng, L. **2015**. The preparation of soluble cellouronic acid sodium salt by 4-acetamide-TEMPO mediated oxidation of ultrasound-pretreated parenchyma cellulose from bagasse pith. *Archives of Acoustics*. 39(2): 267–275. https://doi.org/10.2478/aoa-2014-0031

Gao, Z., Ma, M., Zhai, X., Zhang, M., Zang, D. & Wang, C. **2015**. Improvement of chemical stability and durability of superhydrophobic wood surface via a film of TiO2 coated CaCO3 micro-/nano-composite particles. *RSC Advances*. 5(79): 63978–63984.
https://doi.org/10.1039/c5ra04000k

Gentsch, N., Boy, J., Batalla, J.D.K., Heuermann, D., von Wirén, N., Schweneker, D., Feuerstein, U., Groß, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Céspedes, F.C. & Guggenberger, G. **2020**. Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. *Biology and Fertility of Soils*. 56(7): 943–957. https://doi.org/10.1007/s00374-020-01475-8

Gumede, T.P., Luyt, A.S., Hassan M.K., Pérez-Camargo, R.A., Tercjak, A. & Müller, A.J. **2019**. Morphology, nucleation, and isothermal crystallization kinetics of poly(ε-caprolactone) mixed with a polycarbonate/MWCNTs masterbatch. *Polymers*. 11(0): 1–18.
https://doi.org/10.3390/polym11040682

Gumede, T.P., Luyt, A.S., Tercjak, A. & Müller, A.J. **2019**. Isothermal crystallization kinetics and morphology of double crystalline PCL/PBS blends mixed with a polycarbonate/MWCNTs Masterbatch. *Polymers*. 11(4): 1–18. https://doi.org/10.3390/polym11040682

He, W., Jiang, S., Zhang, Q. & Pan, M. **2013**. Isolation and characterization of cellulose nanofibers from *Bambusa rigida*. *BioResources*. 8(4): 5678–5689.
https://doi.org/10.15376/biores.8.4.5678-5689

Himmel, M.E., Ding, S.-Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W. & Foust, T.D. **2007**. Biomass Recalcitrance: Engineering plants and enzymes for biofuels production. *Science*. 315(5813): 804–807.
https://doi.org/10.1126/science.1137016

Hivechi, A, Bahrami, S.H., Siegel, R.A., Milan, P.B. & Amoupour, M. **2020**. *In vitro* and *in vivo* studies of biaxially electrospun poly(caprolactone)/gelatin nanofibers, reinforced with cellulose nanocrystals, for wound healing applications. *Cellulose*. 27 (0): 5179–5196. https://doi.org/10.1007/s10570-020-03106-9

Hu, Y., Hamed, O., Salghi, R., Abidi, N., Jodeh, S. & Hattb, R. **2017**. Extraction and characterization of cellulose from agricultural waste argan press cake. Agricultural wastes. *Cellulose Chemistry and Technology*. 51 (3-4): 263–272.

Inácio, E.M., Souza, D.H.S. & Dias, M.L. **2020**. Thermal and crystallization behavior of PLA/PLLA-grafting cellulose nanocrystal. *Material Sciences and Applications*. 11(0): 44–57. https://doi.org/10.4236/msa.2020.111004

Johnsy, G., & Sabapath, S.N. **2015**. Cellulose nanocrystals: synthesis, functional properties, and applications. Nanotechnology, Science and Applications. 8(0): 45–54. https://doi.org/10.2147/NSA.S64386

Kimura, M., Watanabe, T., Takeichi, Y. & Niwa, Y. **2019**. Nanoscopic origin of cracks in carbon fibre-reinforced plastic composites. *Scientific Reports*. 9(1): 1–9. https://doi.org/10.1038/s41598-019-55904-2

Kowalczuk, D. & Pitucha, M. **2019**. Application of FTIR method for the assessment of immobilization of active substances in the matrix of biomedical materials. *Materials*. 12(18): 1–13. https://doi.org/10.3390/ma12182972

Kucharczyk, P., Pavelková, A., Stloukal, P. & Sedlarík, V. **2016**. Degradation behaviour of PLA-based polyesterurethanes under abiotic and biotic environments. *Polymer Degradation and Stability.* 0(0): 1–27. *https:*//doi.org/10.1016/j.polymdegradstab.2016.04.019

Li, R., Wua, Z.L., Wang, Y. & Li, L.L. **2013**. Separation of total saponins from the pericarp of *Sapindus mukorossi* Gaerten. by foam fractionation*. Industrial Crops and Products*. 51(2013): 163–170. https://doi.org/10.1016/j.indcrop.2013.08.079

Liu, C., Li, B., Du, H., Lv, D., Zhang, Y., Yu, G., Mu, X. & Peng, H. **2016**. Properties of nanocellulose isolated from corncob residue using sulfuric acid, formic acid, oxidative and mechanical methods. *Carbohydrate Polymers*. 151(2016): 716–724. https://doi.org/10.1016/j.carbpol.2016.06.025

Liu, D.Y., Yuan, X.Y, Bhattacharyya, D. & Easteal, A.J. **2010**. Characterisation of solution cast cellulose nanofibre – reinforced poly(lactic acid). *Express Polymer Letters*. 4(1): 26–31. https://doi.org/10.3144/expresspolymlett.2010.5

Lu, F., Yu, H., Yan, C. & Yao, J. **2016**. Polylactic acid nanocomposite films with spherical nanocelluloses as efficient nucleation agents: effects on crystallization, mechanical and thermal properties. *RSC Advances*. 6(51): 46008–46018. https://doi.org/10.1039/c6ra02768g

Mariana, M., Tata A., Abdul-Khalil H.P.S., Yahya, E.B., Olaiya N.G., Nuryawan, A., Mistar, E.M., Abdullah, C.K., Abdulmadjid, S.N. & Ismail, H. **2021**. A current advancement on the role of lignin as sustainable reinforcement material in biopolymeric blends. *Journal of Material Research and Technology*. 15(0): 2287–2316. https://doi.org/10.1016/j.jmrt.2021.08.139

Mofokeng, J.P., Luyt, A.S., Tabi, T., Kovacs, J. **2011**. Comparison of injection moulded, natural fibre-reinforced composites with PP and PLA as matrices. *Journal of Thermoplastic Composite Materials*. 25(8): 927–948. https://doi.org/10.1177/0892705711423291

Mohamad, S.N.K., Ramli, I., Abdullah, L.C., Mohamed, N.H., Islam, M.S., Ibrahim, N.A. & Ishak N.S. **2021**. Evaluation on structural properties and performances of graphene oxide incorporated into Chitosan/Poly-Lactic acid composites: CS/PLA versus CS/PLA-GO*. Polymers*. 13(11): 1–17. https://doi.org/10.3390/polym13111839

Morán, J.I., Alvarez, V.A., Cyras, V.P. & Vázquez, A. **2008**. Extraction of cellulose and preparation of nanocellulose from sisal fibers. *Cellulose*.15(1): 149–159. https://doi.org/10.1007/s10570-007-9145-9

Nadour, M., Boukraa, F., Ouradi, A. & Benaboura, A. **2017**. Effects of methylcellulose on the properties and morphology of polysulfone membranes prepared by phase inversion. *Materials Research*. 20(2): 339–348. https://doi.org/10.1590/1980-5373-mr-2016-0544

Naduparambath, S., Jinitha T.V., Shaniba V., Sreejith M.P., Balan, A.K. & Purushothaman, E. **2017**. Isolation and characterisation of cellulose nanocrystals from sago seed shells. *Carbohydrate Polymers*. 180(0): 13–20. https://doi.org/10.1016/j.carbpol.2017.09.088

Nie, K., Song, Y., Liu, S., Han, G., Ben, H., Ragauskas, A.J. & Jiang, W. **2019**. Preparation and characterization of microcellulose and nanocellulose fibers from *Artemisia Vulgaris* bast. *Polymers*. 11(5): 1–10. https://doi.org/10.3390/polym11050907

Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H. & Sederoff, R. **2010**. Lignin and biomass: A Negative correlation for wood formation and lignin content in trees. *Plant Physiology*. 154(2): 555–561. https://doi.org/10.1104/pp.110.161281

Nurazzi, N.M., Asyraf, M.R.M., Rayung, M., Nurrrahim, M.N.F., Shazleen, S.S., Rani, M.S.A, Shafi, A.R., Aisyah, H.A., Radzi, M.H.M., Sabaruddin, F.A., Ilyas, R.A., Zainudin, E.S. & Abdan, K. **2021**. Thermogravimetric analysis properties of cellulosic natural fiber polymer composites: A review on influence of chemical treatments. *Polymers*. 13(16): 1–33. https://doi.org/10.3390/polym13162710

Oliveira, R.L., Vieira, J.G., Barud, H.S., Assunção, R.M.N., Rodrigues Filho, G., Ribeiro, S.J.L. & Messadeqq, Y. **2015**. Synthesis and characterization of methylcellulose produced from bacterial cellulose under heterogeneous condition. *Journal of the Brazilian Chemical Society*. https://doi.org/10.5935/0103-5053.20150163

Papadopoulou, K., Melton, R.E., Leggettt, M., Daniels, M.J. & Osbourn, A.E. **1990**. Compromised disease resistance in saponin-deficient plant. *Proceedings of the National Academy of Sciences of the United States of America*. 96(22): 12923–12928. https://doi.org/10.1073/pnas.96.22.12923

Pattathil, S., Hahn, M.G., Dale, B.E. & Chundawat, S.P. S. **2015**. Insights into plant cell wall structure, architecture, and integrity using glycome profiling of native and AFEXTM-pretreated biomass. *Journal of Experimental Botany*. 66(14): 4279–4294. https://doi.org/10.1093/jxb/erv107

Poovaiah, C.R., Nageswara-Rao, M., Soneji, J.R., Baxter, H.L. & Stewart, C.N. **2014**. Altered lignin biosynthesis using biotechnology to improve lignocellulosic biofuel feedstocks. *Plant Biotechnology Journal*. 12(9): 1163–1173. https://doi.org/10.1111/pbi.12225

Salmieri, S., Islam, F., Khan, R.A., Hossain, F.M., Ibrahim, H.M.M., Miao, C., Hamad, W.Y. & Lacroix, M. **2014**. Antimicrobial nanocomposite films made of poly(lactic acid)-cellulose nanocrystals (PLA-CNC) in food applications: part A—effect of nisin release on the inactivation of *Listeria monocytogenes* in ham. *Cellulose*. 21(3): 1837–1850. https://doi.org/10.1007/s10570-014-0230-6

Shameer, M.P. & Nishath, M.P. **2019**. Exploration and enhancement on fuel stability of biodiesel. In Azad, A.K. & Rasul, M. (Eds.). *Advanced Biofuels*. English: Woodhead Publishing. pp181–213. https://doi.org/10.1016/B978-0-08-102791-2.00008-8

Singh, A.A., Genovese, M.E., Mancini, G., Marini, L. & Athanassiou, A. **2020**. Green processing route for polylactic acid–cellulose fiber biocomposites. *ACS Sustainable Chemistry & Engineering*. 8 (10): 4128–4136. https://doi.org/10.1021/acssuschemeng.9b06760

Sorieul, M., Dickson, A., Hill, S. & Pearson, H. **2016**. Plant fibre: Molecular structure and biomechanical properties, of a complex living material, influencing its deconstruction towards a biobased composite. *Materials*. 9(8): 1–36. https://doi.org/10.3390/ma9080618

Subair, N., Jinitha, T.V., Shaniba V., Sreejith M.P., Balan, A.K., Purushothaman, E. **2017**. Isolation and characterization of cellulose nanocrystals from sago seed shells. *Carbohydrate Polymers*. 0(0): 1–26. https://doi.org/10.1016/j.carbpol.2017.09.088

Tanpichai, S. & Wootthikanokkhan, J. **2018**. Reinforcing abilities of microfibers and nanofibrillated cellulose in poly(lactic acid) composites. *Science and Engineering of Composite Materials*. 25(2): 395–401. https://doi.org/10.1515/secm-2016-0113

Tian, W., Li, H., Zhou, J. & Guo, Y. **2017**. Preparation, characterization and the adsorption characteristics of lignin/silica nanocomposites from cellulosic ethanol residue. *RSC Advances*. 7(65): 41176–41181. https://doi.org/10.1039/c7ra06322a

Trang Mai, T.T., Thuy N., Thi T., Duong Le Q., Ngoan Nguyen T., Cham Ba T., Binh Ngu H., Hoa Phan T.B., Lam Tran D., Nguyen X. P. & Seo Park J. **2012**. A novel nanofiber Cur-loaded polylactic acid constructed by electrospinning. *Advances in Natural Sciences: Nanoscience and Nanotechnology*. 3(2): 1–4 (2012). https://doi.org/10.1088/2043-6262/3/2/025014

Trdá, L., Janda, M., Macková, D., Pospíchalová, R., Dobrev, Petre I., Burketová, L., Matušinsky, P. **2019**. Dual mode of the saponin aescin in plant protection: Antifungal agent and plant defense elicitor. *Frontiers in Plant Science*. 10(0): 1–14. https://doi.org/10.3389/fpls.2019.01448

Trang-Mai, T.-T., Thuy-Nguyen, T.-T., Duong-Le, Q., Ngoan-Nguyen, T., Cham-Ba, T., Binh-Nguyen, H., Hoa-Phan, T.-B., Lam-Tran, D., Nguyen, X.P. & Seo-Park, J. **2012**. A novel nanofiber Cur-loaded polylactic acid constructed by electrospinning. *Advances in Natural Sciences: Nanoscience and Nanotechnology*. 3(2): 1–4. https://doi.org/10.1088/2043-6262/3/2/025014

Vanholme, R., Morreel, K., Ralf, J. & Boerjan, W. **2008**. Lignin engineering. *Current Opinion in Plant Biology*. 11(3): 278–285. https://doi.org/10.1016/j.pbi.2008.03.005

Watkins, D., Nuruddin, M., Hosur, M., Tcherbi-Narteh, A. & Jeelani, S. **2015**. Extraction and characterization of lignin from different biomass resources. *Journal of Materials Research and Technology*. 4(1): 26–32. https://doi.org/10.1016/j.jmrt.2014.10.009

Yang, Z., Li, X., Si, J., Cui, Z. & Peng, K. **2019**. Morphological, mechanical and thermal properties of poly(lactic acid) (PLA)/cellulose nanofibrils (CNF) composites nanofiber for tissue engineering. *Journal of Wuhan University of Technology-Mater*. 34(1), 207–215. https://doi.org/10.1007/s11595-019-2037-7

Zhao, H., Kwak, J., Conradzhang, Z., Browm, H., Arey, B. & Holladay, J. **2007**. Studying cellulose fiber structure by SEM, XRD, NMR and acid hydrolysis. *Carbohydrate Polymers*. 68(2): 235–241. https://doi.org/10.1016/j.carbpol.2006.12.013

Zhang, N., Tao, P., Lu, Y. & Nie, S. **2019**. Effect of lignin on the thermal stability of cellulose nanofibrils produced from bagasse pulp. *Cellulose*. 26(0): 7823–7835. https://doi.org/10.1007/s10570-019-02657-w

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CHAPTER 5

Conclusion

The primary goal of this chapter is to give a concise description of the data and results obtained in this study. The first objective was to collect and process *E. autumnalis* (drying and milling), which included acid-alkali treatment of the plant fibres to obtain isolated fibres and/or cellulose crystals. The second objective was to select the best suitable filler for the desired matrix using different characterization techniques such as FTIR, SEM, XRD and TGA. The third objective was to blend the selected cellulose with PLA to yield PLA/cellulose nanocomposites, and to analyse the composites using standard analytical tools such as FTIR, SEM, XRD, TGA and Tensile tests.

5.1. Achievement of objectives

The current study used a multifaceted approach to isolate and characterise cellulose from different parts of *E. autumnalis*, an indigenous South African medicinal plant known for its efficacy against a variety of ailments. These parts included stems, leaves, and bulbs, each of which had unique structural characteristics. As a result of the acid-alkali treatment, three distinct cellulose extracts were obtained. However, the yields were lower than expected due to a lack of specialised extraction equipment. Structural and morphological analysis by FTIR, SEM, and XRD confirmed the varying chemical structures and morphological features of the three celluloses obtained from the leaves, bulbs, and stems. The visual observations (as explained in section 3.1.1.2) and analytical observations revealed associations with cellulose I, cellulose II, and methylcellulose. This section of the study sought to demonstrate that cellulose is the most common biopolymer found in all plant species on the planet. It also confirms that cellulose exists in various forms, with each molecule being unique to the source. The thermal behaviour of cellulose varies greatly depending on its treatment and raw material source. As a result, it is among some of the most promising bio-renewable resources for lowering and replacing petroleum-based plastics.

Green composites containing PLA are fabricated using different PLA/Cs concentrations (99.5/0.5 and 97.0/3.0), assuming that cellulose (stem) (Cs) bears most, if not all, of the properties of nanocellulose. The aim was to investigate the interactions between the hydrophilic Cs and the hydrophobic PLA at the interface. FTIR revealed the formation of new bands that do not appear on the original bands of the individual materials, indicating apparent compatibility between the two materials. At higher filler content (3.0 wt.%), SEM micrographs revealed better distribution of Cs in the PLA matrix, thus improved interfacial bonding in the composites. The apparent compatibility between Cs and PLA interfaces can be regarded as an excellent factor for improving mechanical properties such as elastic modulus as observed at 3.0 wt.%. However, there was no apparent improvement in thermal properties. Therefore, the fabricated composites are limited to current applications (i.e., biomedical applications and short-term packaging). A status attained as a result of no weight loss at 260 C. More research into PLA/cellulose composites is therefore required.

5.2. Shortcomings

- Low cellulose yield resulting from lack of specialised extraction equipment.
- Limited plant samples (resulting from recollection constraints due to the COVID-19 pandemic and restrictions imposed on travel) made it impossible to repeat procedures and/or explore other methods for sample purity and optimum product recovery.
- Limited access to facilities with relevant equipment for characterisation techniques, thus no flexibility for repetition of techniques in a case of dissatisfaction with the outcomes.

5.3. Future recommendations

- Conduct the same study in a different setting using a soxhlet extractor for the extraction of cellulose to optimize product recovery.
- Carry out the entire procedure for cellulose extraction including the nanocellulose conversion step, which involves further treatment with sodium hydroxide, sodium hypochlorite and potassium hydroxide.
- Study the effect of cellulose (extracted from different plant parts) in a PLA matrix or other biopolymer and determine a suitable field of application.

- Fabricate wound healing dressing from bulb cellulose and evaluate its effect on wounds.

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