Chapter-1

Introduction

1. Luffa Cylindrica (LC)

Luffa cylindrica (LC) is predominantly cultivated in sub-tropical regions across the Middle East, Asia, India, South America, Africa, Japan, and China. It is primarily cultivated in sub-tropical and tropical climates, as it prefers warm temperatures and ample sunlight. LC can be derived from agriculture and forests. Figure 1.1 shows the geographical distribution of LC in various regions and the availability of LC in various forms (Adeyanju et al. 2021; Akinwumi et al. 2021; Azeez, Bello*, and Adedeji 2013; J.-P. Chen et al. 2003; Kamran et al. 2021; Shendge and Belemkar 2018).

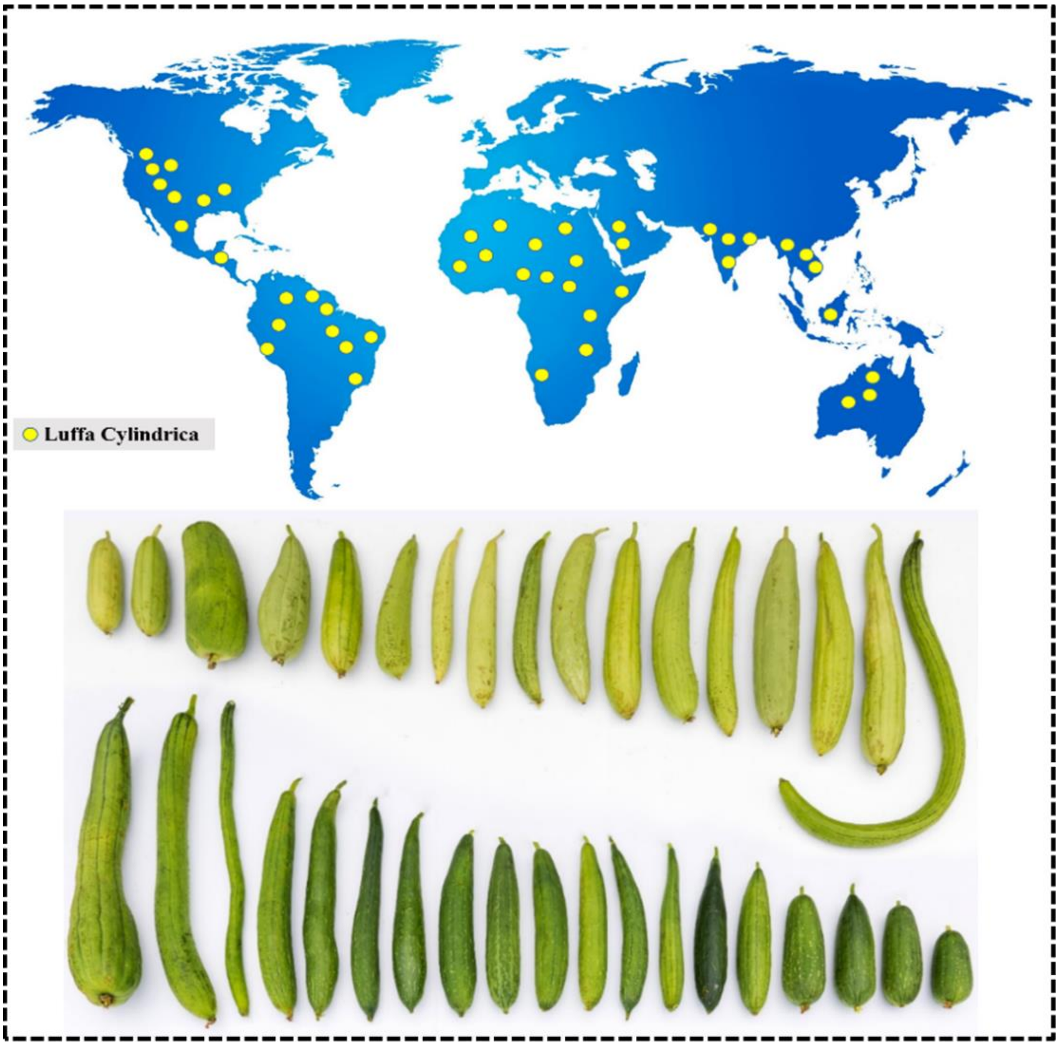


Figure 1.1: A representation of the geographical distribution of LC and various kinds of LC that are attainable (Dhillon et al. 2020).

The dried fruit of LC is a species of vine with tendrils widely recognized as sponge gourd. This versatile plant has a relatively short harvesting period and boasts an incredibly lightweight nature with a fibrous architectural structure. Luffa cylindrica belongs to the Cucurbitaceae family and falls under the Plantae kingdom, with three main variations: *Luffa aegyptiaca*, *Luffa acutangular and Luffa operculata* (Partap et al. 2012). The luffa plant, a climbing vine, displays vibrant yellow flowers and produces elongated cylindrical fruits measuring 20-25 cm in length. These fruits feature tapered ends and encase brownish-black seeds within a protective pod. Figure 1.2 illustrates the components of LC plant (Akinwumi et al. 2021; Mariod, Saeed Mirghani, and Hussein 2017). The dried fruit of LC is usually known by various names such as vegetable sponge, smooth luffa, dish gourd and bath sponge (Mariod, Saeed Mirghani, and Hussein 2017).

The fruit is edible when harvested at a primitive stage of development, allowing it to mature fully and form intricately intertwined fibers organized in a three-dimensional reticulation. These processed fibers find applications as organic bath sponges, shock absorber components, packing materials, soundproof linings, dishwasher scrub and body wash scrub (J.-P. Chen et al. 2003; JH Shen et al. 2012; Vishnu Vardhan S 2021; Partap et al. 2012). Unfortunately, scientific literature regarding this fruit’s structure, properties, and applications remains limited. However, it possesses notable medicinal properties, including laxative, depurative, emollient, anthelmintic, tonics, expectorant, carminative, and galactagogic that facilitate in curing fever, diabetes syphilis, tumors, bronchitis, splenopathy, and leprosy. Due to its purgative property, LC is employed in the traditional Indian Ayurveda medical practice for various ailments such as chronic bronchitis, dropsy, nephritis, chronic bronchitis and lung complaints (Jianhu Shen et al. 2012).

Figure 1.2: Luffa cylindrica: A) plant, B) LC flower, C) LC seeds, D) LC raw fruit, E) LC partially removed outer skin, and F) LC dried fruit.

1.1 Structure of luffa plant fiber

To develop composites from natural resources, a comprehensive understanding of plant fibers microstructure and chemical composition is essential. The plant fiber cell wall is divided into two cell walls: primary and secondary. The primary cell wall contains an irregular network of closely packed cellulose microfibrils. It forms long, linear chains that are bundled together to create microfibrils. These cellulose microfibrils provide structural strength and rigidity to the cell wall.

In contrast, the secondary cell wall consists of crystalline cellulose microfibrils arranged in a spiral pattern. These microfibrils range from 10 to 30 nm in diameter and have crystalline structures forming a strong and rigid framework. The secondary cell wall is composed of three distinct layers: outer layer (S1), middle layer (S2), and inner layer (S3). The S2 layer significantly influences the fibers mechanical properties. The tensile strength and modulus of the fiber depend on factors such as the degree of polymerization, crystalline cellulose content and microfibrillar angles. Fibers with higher degrees of polymerization, greater cellulose content and lower microfibrillar angles exhibit superior mechanical properties. The hollow cavity called the lumen facilitates a pathway for the movement of fluids; allowing the fiber to transport water, minerals and signaling compounds vital for various physiological processes within the plant. The middle lamella is predominantly composed of pectin. Pectin acts as a cementing agent, binding the fibers together, that helps in maintaining the structural integrity of tissues and allows them to function as a cohesive unit. The primary chemical composition of plant fibers is lignocellulose, which includes cellulose, hemicellulose, and lignin. Cellulose, a semi-crystalline polymer, contributes to the hydrophilic nature of plant fibers. Hemicellulose, an amorphous polysaccharide, acts as a cementing matrix between cellulose microfibrils, forming the cellulose/hemicellulose network.

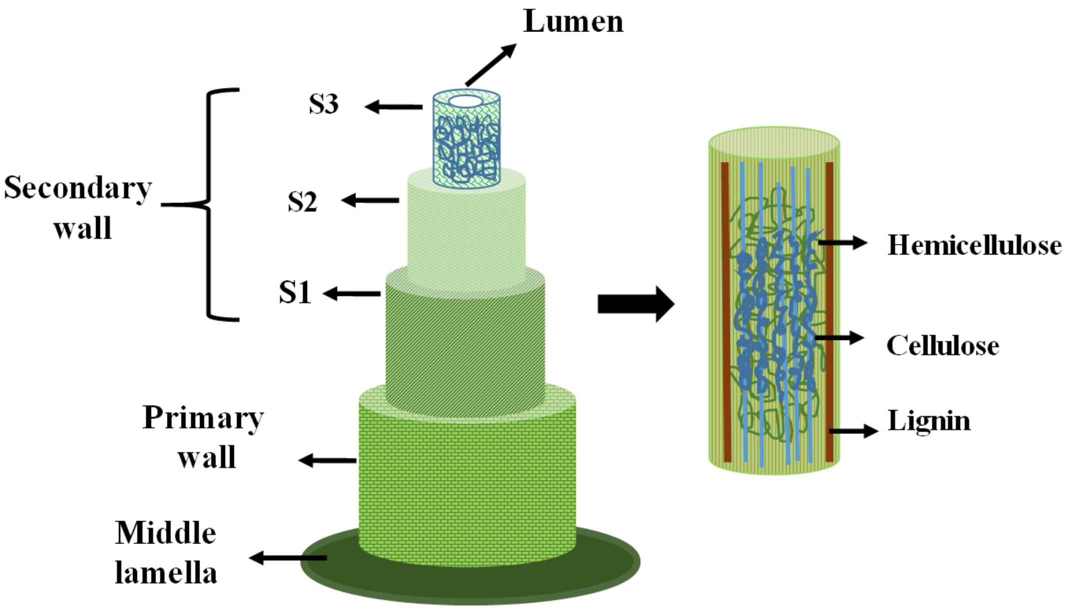


Figure 1.3: Structure of plant fiber illustrating the primary and secondary walls as well as the interior components of the fiber (Aminudin et al. 2017).

On the other hand, lignin, a hydrophobic component, retains water within the fibers and protects against biological degradation. It also imparts high stiffness to the stem, allowing it to resist gravity forces and wind. The general structure of plant fiber is depicted in figure 1.3 showing its non-homogeneous nature. The distribution of these components within the plant cell wall can be uneven, posing challenges in determining the composition and properties of the fibers (Ali et al., 2018).

1.2 Chemical Composition of luffa fibers

Chemical composition and percentage of luffa fiber can vary due to various factors, including plant origin, soil conditions, maturity of plant and fruit, climatic conditions, processing technique and cultivation methods (Partap et al. 2012). LC majorly composed of cellulose, hemicellulose and lignin. Cellulose content ranges from 55 to 90%, hemicellulose from 8 to 22%, lignin 10 to 23%, and other minor components, pectin, waxes, proteins, and minerals from 0.12% to 8% (Table 1.1). These variations in composition contribute to the unique properties and characteristics of luffa fibers; making them suitable for a wide range of applications (Adeyanju et al. 2021; Akgül et al. 2013). To enhance specific properties of the fibers for different applications, the chemical composition of luffa fibers can be modified through various processing methods such as retting, bleaching, and chemical treatments, which can alter the proportions of cellulose, hemicellulose and lignin (Figure 1.4).

Luffa fibers exhibit natural variations in their chemical composition influenced by various factors such as geographical location, plant origin environmental conditions like temperature, rainfalls, and soil quality that can influence the growth and development of luffa plants; consequently influencing the composition of their fibers (Akgül et al. 2013; Saw et al. 2013). Post-harvest processing techniques like retting, bleaching and chemical treatments can selectively modify cellulose, hemicellulose, lignin and minor components.

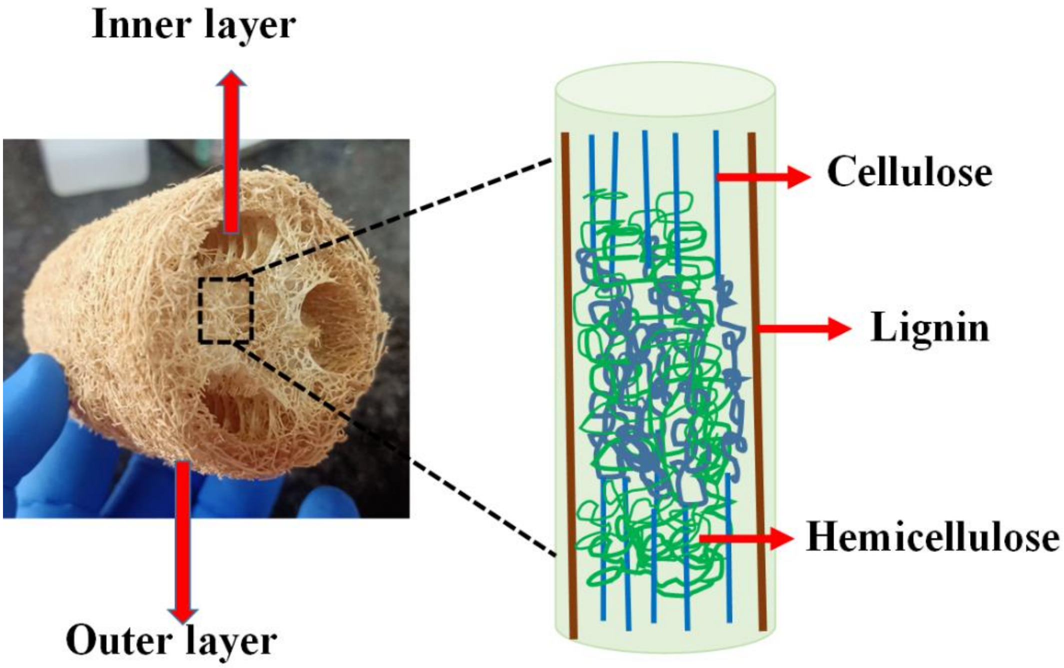


Figure 1.4: Illustrates the layers of dried luffa fruit and internal components inside the fiber (Image was captured in the Tissue Engineering and Biomicrofluidics Laboratory, School of Biomedical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi and generated in powerpoint).

Cellulose, comprising a significant portion of luffa fiber, provides strength and stiffness due to its crystalline structure. Higher cellulose content typically correlates with increased mechanical strength in luffa fiber-based composites. Hemicelluloses, contributing to fiber flexibility and toughness, also facilitate adhesion between fibers and matrix materials in composite structures (Cecen et al. 2016; Alhijazi et al. 2020; Moreno-Anguiano et al. 2021; Kamran et al. 2021). Variations in hemicellulose content can affect bonding and mechanical properties of the composite. Lignin, acting as a natural adhesive, provides rigidity and resistance to compression in luffa fiber. However, excessive lignin content can reduce fiber flexibility and increase brittleness (Wang et al. 2019; Alhijazi et al. 2020; Kamran et al. 2021).

The hygroscopic nature of luffa fibers makes them versatile and effective in absorbing water; rendering them suitable for use in household cleaning, water absorption, and even wastewater treatment. However, treatments like sodium hydroxide are commonly employed to enhance purity, strength and mechanical properties by improving tensile strength,elongation and firmness depending on specific application requirements (Saw et al. 2013; Mohanta and Acharya 2018; Zhang et al. 2019; Papanicolaou et al. 2022; Kharrati et al. 2022). The strength of LC-based scaffolds is influenced by factors such as fiber orientation, crosslinking density, processing techniques and porosity, crucial for efficient fluid transport and cell infiltration. Therefore, optimizing composite design and processing parameters is essential to achieve desired properties for tissue engineering applications; ensuring adequate mechanical support, flexibility, and resilience to physiological forces. Furthermore, the variations in LC-based scaffold chemical composition controlled through additives, surface modifications or inclusion of bioactive agents can influence cellular responses such as adhesion and proliferation in tissue engineering applications.

Table 1.1: Chemical composition of luffa fiber.

Chemical constituents	Percentage composition	References
Cellulose	65.5 ± 0.5%	(Akgül et al. 2013; AS, VJ, and GD 2015)
Hemicellulose	17.5 ± 0.5%	
Lignin	10.0 ± 1.0%	
Ash	0.7 ± 0.2%	
Extractives	3.1 ± 0.5%	

1.2.1 Cellulose:

Cellulose is a widely distributed polysaccharide in plants, animals and certain bacteria. It is the primary structural component of plant cell walls; making it the most abundant polysaccharide. The cellulose molecule consists of β-D-glucopyranose units linked by β (1-4)-glycosidic bonds, forming long, linear chains with intramolecular hydrogen bonding. Vander Waals forces and intermolecular and intramolecular hydrogen bonding contribute to the stability of cellulose

(Luan et al. 2022; Hu et al. 2020). These interactions give cellulose strength and rigidity. The chemical formula is $(C_6H_{10}O_5)_n$, where the degree of polymerization (DP) represents the number of glucose units, typically ranging from 700 to 1000, therefore it is considered insoluble dietary fiber (Frassoldati and Ranzi 2019). Cellulose predominantly exists in a crystalline form, where the glucose chains form numerous hydrogen bonds; forming stable microfibrils. These microfibrils exhibit alternating crystalline and amorphous regions. The crystalline regions have a highly ordered structure, while the amorphous regions are less ordered structured. This property is significant because the amorphous regions of cellulose are more reactive than the crystalline regions; making them more accessible to reagents (hu et al., 2020). Cellulose plays a crucial reinforcement component in polymer composites and has various applications. For instance, it serves as a binding agent in manufacturing acetaminophen tablets. However, higher concentrations of hemicelluloses and lignin in cellulose fibers can negatively affect the material's mechanical properties. Figure 1.5 represents the chemical structure of the cellulose.

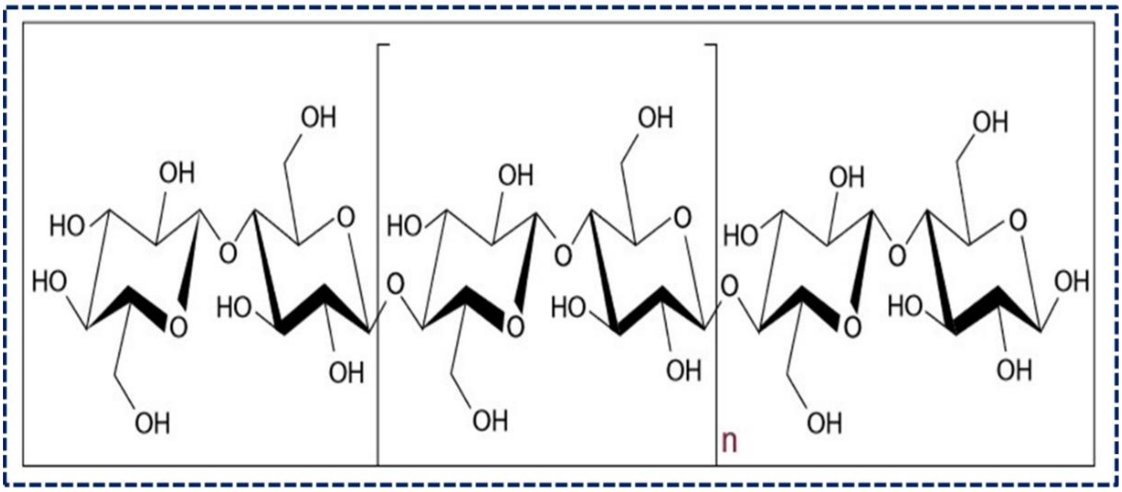


Figure 1.5: Represents cellulose chemical structure comprised of a series of β-D-glucopyranose units covalently connected by (1–4) glycosidic bonding (George and N 2015).

1.2.2 Hemicellulose:

Hemicellulose, a complex carbohydrate, is a significant component surrounding cellulose fibers in plant cells and other carbohydrates like pectins. It contains various sugar molecules, including galactan, glucuronoxylan, glucomannan and small amounts of other polysaccharides. These sugars such as glucans, xylans, galactan's, arabinans and mannans are linked together through a β-1,4 backbone. Xylan plays a crucial role, containing 1-4 linkages of xylopyranosyl units attached to α-4-O-methyl-D-glucuronopyranosyl branches of glucose, which connects with other xylose sugar monomers. Unlike cellulose, hemicellulose consists of 50-3000 sugar units, whereas cellulose contains 7000-15,000 glucose molecules per polymer (Shah et al. 2022).

Unlike the crystalline nature of cellulose, hemicellulose exhibits an amorphous structure; making it more susceptible to hydrothermal extraction and hydrolysis. It readily dissolves in water at temperatures above 180°C (Brunner 2014). Hemicellulose is closely associated with lignin through cinnamate acid ester linkages and with cellulose through extensive hydrogen bonding. Its hydrophilic nature contributes to both the rigidity and flexibility of the plant cell wall and water retention. In addition, the polymer chains of hemicellulose have short branches; making them partially soluble in water. Various chemical reactions can modify the properties of hemicellulose including esterification, acetylation, etherification, oleoylation, crosslink, fluorination and benzylation (hu et al., 2020). Figure 1.6 represents the chemical structure of hemicellulose.

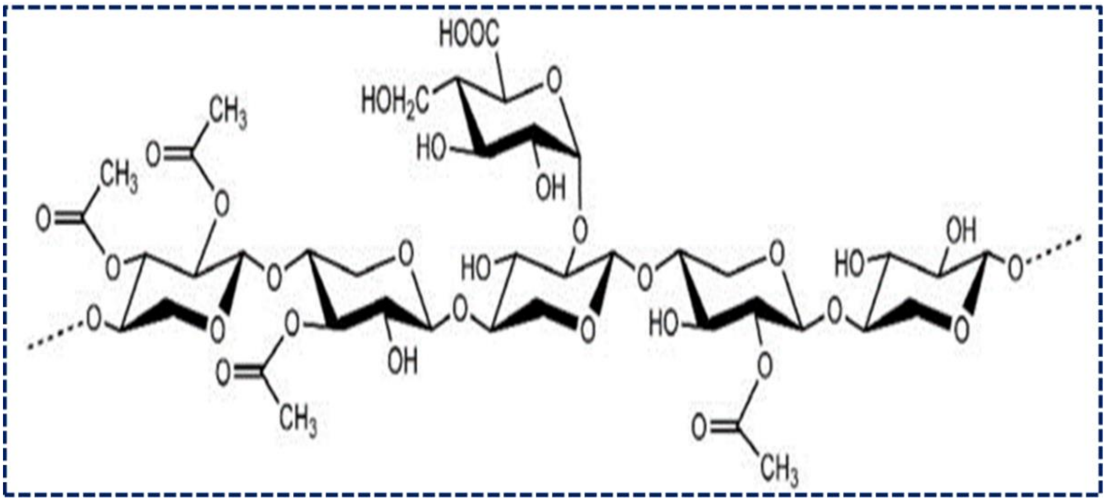


Figure 1.6: The chemical structure of hemicellulose (hu et al. 2020).

1.2.3 Lignin:

Lignin possesses a complex phenolic chemical structure that includes phenylpropane motifs. It acts as a filler between cellulose and hemicellulose, contributing to the cohesion of the lignocellulosic matrix; thereby enhancing the rigidity and impermeability of plant cells. The three main monomers in lignin are p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol (Figure 1.7), which make up 40% of the biomass. The lignin matrix comprises various types of bonds, including β-O-4 ether, β-5 phenylcoumaran, β-β′ pinoresinol, diphenyl ether 4-O5′, and β-1′ diphenyl methane. Linear or cyclic C-O-C bonds are considered the most significant bonding patterns in lignin. Its non-crystalline (amorphous) structure arises from its complex and branched configuration (Erfani Jazi et al. 2019; Hu et al. 2020).

Lignin deposition in the cell wall acts as the mechanical support of plant organs; it allows upright growth and large sizes, provides strength and rigidity to the cells, allows transport of water and solutes in the vascular system due to its hydrophobicity and mechanical resistance, and it is associated to protection against pathogens (Lourenço et al. 2016). The functions of lignin in the plant cell wall include structural support, water and nutrient transport, and protection against chemical or biological attacks (Lu et al. 2017).

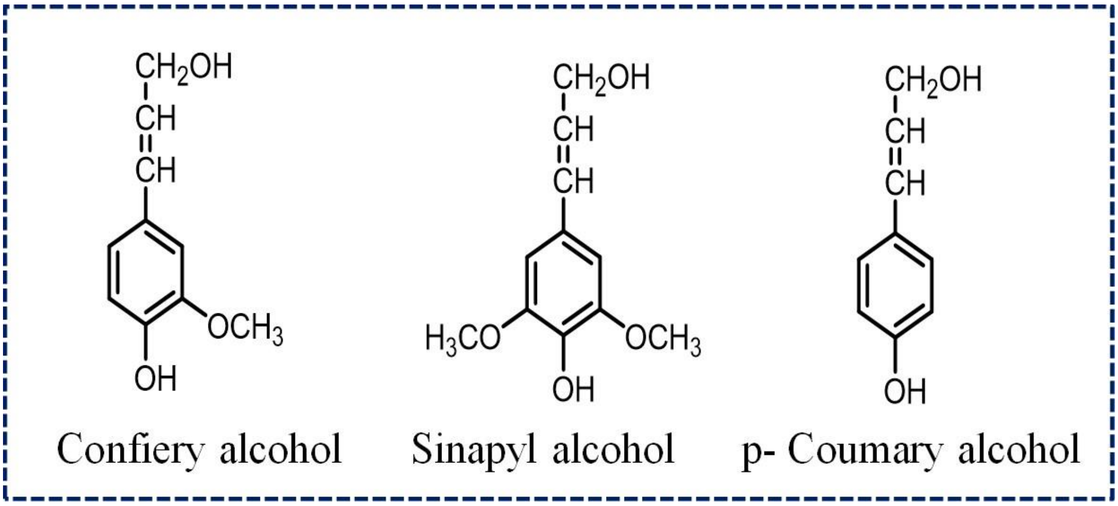


Figure 1.7: The three main components of the lignin structure.

1.3 Extraction and processing of luffa fibers

When natural fibers are used as composite reinforcing materials, their hydrophilic nature and high moisture absorption capacity pose challenges. The presence of moisture can lead to the development of microcracks in the composite, resulting in reduced mechanical strength and durability (Guo et al. 2019; Taimur -Al-Mobarak, Gafur, and Mina 2018). To mitigate these issues, chemical treatment of the fibers is necessary to minimize absorbency and enhance mechanical properties (Chakrabarti et al. 2020; Y. Chen et al. 2017; 2018). One commonly used method involves treating dried luffa fibers with a sodium hydroxide (NaOH) solution (Abdulrahman 2015). This treatment enhances the mechanical characteristics of the fibers by removing lignin and hemicellulose, which are responsible for water absorption. However, excessive alkali concentrations can lead to excessive delignification and weaken the fibers (L. Ghali et al. 2009). Another treatment approach is a mixed treatment, where luffa fibers are treated with a solution containing both NaOH and hydrogen peroxide (H_2O_2). This combination acts as a delignification and bleaching agent; removing lignin and hemicellulose and improving the adhesion between the fiber and the composite. However, this method can lower the mechanical qualities of the luffa fiber (L. Ghali et al. 2009). An alternative treatment method involves an initial treatment of luffa fibers with NaOH (alkali) followed by benzoyl chloride.

This treatment approach has shown promising results, as it improves the impact strength of the fibers and increases the material's flexural and tensile strength (N Mohanta and Acharya 2016). The controlled removal of lignin and hemicellulose from luffa fibers can enhance their mechanical stability and reduce water absorption (LAIB et al. 2021). However, excessive alkali concentrations during treatment can lead to high delignification, weakening and damaging fibers (Ubi and Salawu 2015).

It should be noted that this approach might lower the overall mechanical qualities of the luffa fiber (Alhijazi et al., 2020). Furthermore, other chemicals can be utilized to enhance the mechanical properties of luffa fibers. These include acetic acid, cyano-ethyl compounds, calcium hydroxide and silane (Mariod, Saeed Mirghani, and Hussein, 2017; Panneerdhass, Gnanavelbabu, and Rajkumar, 2014). The elimination of lignin and hemicellulose within tolerable limitations may have been useful at some locations where luffa is expected to provide high mechanical stability since they are primarily responsible for the fibers water-absorption capability (LAIB et al. 2021). However, larger alkali concentrations result in high delignification, weakening and damaged fibers (Ubi and Salawu 2015). However, this approach lowers the luffa fibers mechanical qualities (Alhijazi et al., 2020). An alternative treatment method involves the initial treatment of luffa fibers with NaOH (alkali) followed by benzoyl chloride. The fibers demonstrated great impact strength as well as an increase in the material's flexural and tensile strength (N Mohanta and Acharya 2016). Other chemicals that can be employed to improve the mechanical properties of luffa fibers include acetic acid, cyano-ethyl compounds, calcium hydroxide and silane (Mariod, Saeed Mirghani, and Hussein 2017; Panneerdhass, Gnanavelbabu, and Rajkumar 2014). Table 1.2 represents various treatments for modifying the surface and improving the overall mechanical characteristics of luffa fibers.

Table 1.2: Different chemical treatments utilized to modify luffa fibers.

S.No	Treatment	Findings	References
1.	Sodium hydroxide (NaOH)	enhanced flexural and tensile strength as a result of better fiber-matrix interaction. Enhanced crystallinity index	(L. Ghali et al. 2009)
2.	Sodium hydroxide (NaOH) + hydrogen peroxide (H_2O_2)	modified luffa fiber arrangement from mat to fibril structure. Improved compression resilience	(L. Ghali et al. 2009; K. Zhang et al. 2019)
3.	Sodium hydroxide (NaOH) + benzoyl chloride (C_6H_5COCl)	enhanced tensile and flexural modulus improved impact strength	(Chakrabarti et al., 2020, Mohanta & Acharya, 2016)
4.	Acetic acid (CH_3COOH)	improved flexural strength, flexural modulus, and fracture strain. Improved fiber and matrix interaction	(Lassaad Ghali et al. 2011)
5.	Acrylonitrile solution (CH_2CHCN) + Sodium hydroxide (NaOH)	reduced water absorption and retention ratio. Improved fiber-matrix adhesion	(Alhijazi et al., 2020, Ghali et al, 2008)
6.	Calcium hydroxide $Ca(OH)_2$	increased fiber diameter as a result of the development of a surface coating. Improved ultimate tensile strength	(Kalusuraman et al. 2019)
7.	Silane (SiH_4)+ sodium hydroxide (NaOH)	improved ultimate tensile strength and decreased crystallinity index. Improved thermal stability	(Dharmalingam, Meenakshisundaram, and Kugarajah 2020; Kalusuraman et al. 2019)

1.4 Bioactive compounds of LC

Phytochemicals are considered secondary metabolites directly responsible for activity such as antioxidant, antimicrobial, antifungal, anti-cancer, and anti-inflammatory, among others. Therefore, screening chemical constituents in medicinal plants to assess their availability may provide new useful information to the scientific community and in claiming their therapeutic efficacies. These phytochemicals possess potent antioxidant properties and can combat bacteria by disrupting their cell wall and membrane, blocking protein synthesis, altering pH and other methods (Khameneh et al. 2021; Thakur, Singh, and Khedkar 2020). LC claims a diverse array of phytochemical components that provide luffa its therapeutic or antimicrobial properties. Among its notable constituents are carotenoids, chlorophylls, oleanolic acid, alkaloids, phenolic acid, saponins, tannins, flavonoids and triterpenoids (S and Vellapandian 2022; Al-Snafi 2019; Valerie et al. 2017). Saponins exhibit remarkable chemical complexity and are classified into three groups based on their aglycone structure: steroid, steroidal alkaloid or triterpenoid. Traditional medicinal practices utilize the immature fruits of LC for ailments such as fever, jaundice, bronchitis, and acne due to their potent antioxidant, anti-inflammatory and antimicrobial properties.

Consequently, exploring the therapeutic potential of these bioactive compounds requires further investigation and a comprehensive understanding of their mechanisms of action (S and Vellapandian, 2022; Al-Snafi, 2019). The immature fruits contain ascorbic acid, rhiboflavin, vitamin A, thiamine, niacin, protein, fats, carbohydrates, calcium, anthocyanins, flavonoids, triterpenoid and saponins. The key parts of luffa acutangula showed that luffa fruits were high in calcium, rough fiber and water. In addition, several other important elements were present, including magnesium, zinc and iron (Jaysingrao and Sunil 2014).

The LC leaves also contain flavonoids, saponins, cardiac glycosides and tannins. The flowers of the plant are rich in flavonoids and carotenoids. LC exhibits immune stimulant properties, acts as an oxytocic agent and is used in treating hypersensitive reactions. Additionally, the fruits possess anti-fungal and anti-bacterial properties. Traditional uses of the plant include the treatment of decayed teeth, bronchodilator effects, amenorrhea and parasitic infections using its leaves. The leaves and flowers also have antiemetic activity (Raut et al. 2021). In a study wherein luffa acutangula leaf extracts were tested against several bacterial isolates, it was observed that the highest suppression occurred against Streptococcus pyrogens and Candida albicans (Valerie et al. 2017). Potassium and calcium were found to be the two primary minerals present in the extract of the leaves of the luffa acutangula plant. Thus, it is generally claimed to play an important role in bone regeneration, development and growth, as well as limiting calcium loss from the bone and impeding osteoporosis, particularly in old age people (Kong et al. 2017; Seyi Valerie 2017; Vannucci et al. 2018). The kernel of the luffa acutangula seed is rich in minerals, phosphorus, magnesium and iron. Several studies concluded that luffa seeds are a great source of protein concentrate and oil. Table 1.3 and 1.4 describes the mineral and nutritional analysis of LC fruit, seed kernel and leaf extracts. In some nations, luffa fruit pulp was also utilized as a remedy for paralysis, fever, hemorrhoids, dyspnea, headaches, etc. Leaf extracts were used to cure snake bites, amenorrhea, granular conjunctivitis and other ailments (Akinwumi et al. 2021; Shendge and Belemkar 2018).

Table 1.3: Various studies on the mineral content of luffa leaf extract, fruit, and seed kernel. NA= not applicable.

Minerals	**Leaf Extract (mg/100 g)**		**Fruit (mg/100 g)**		**Seed kernel (mg/100 g)**		
Copper(Cu)	0.6	NA	0.9	0.1	2.18	3.33	3.33
Iron(Fe)	8.1	0.03	34.1	2.0	10.7	4.72	4.72
Magnesium (Mg)	12.4	0.44	27.38	224.0	330.0	28.98	28.98
Manganese (Mn)	0.9	0.01	2.34	1.0	NA	2.77	2.77
Calcium (Ca)	58.6	4.31	99.78	227.0	62.0	2.12	2.12
Zinc(Zn)	0.6	0.011	9.52	3.0	5.80	3.43	3.43
Sodium (Na)	14.4	0.31	NA	166.0	NA	8.18	8.18
Potassium (K)	143.6	2.16	NA	1280.0	NA	13.86	13.86
Phosphorous (P)	NA	0.12	NA	414.0	1050.0	30.63	30.63
References	(Alagbe John 2019; Seyi Valerie 2017)		(Jaysingrao et al. 2014; Ravella et al. 2015)		(Kamel 1982; Ogunyemi et al. 2020)		

Table 1.4: Various findings on the nutritional value of luffa leaf extract, fruit, and seed kernel.

Specimen	Moisture	Ash	Carbohydrates	Crude protein	Crude fiber	Fat	References
Leaf Extract (%)	10.6	6.3	71.4	2.6	4.0	5.1	(Osuagwu, A. N, and H. O 2014; Seyi Valerie 2017)
	0.19	0.23	97.46	0.30	12.0	0.006	
Fruit (%)	94.6	0.26	3.86	0.46	42.94	0.1	(Hussain et al., 2009; Jaysingrao et al., 2014)
	92.45	8.0	66.05	13.43	10.25	2.33	
Seed kernel (%)	5.56	4.13	3.91	74.6	2.82	44.3	(Kamel 1982; Nnaji, Okolo, and Onukwuli 2020)
	9.30	2.16	60.46	21.88	8.70	6.80	

1.5 Properties of LC for scaffold fabrication

Luffa cylindrica (LC), a plant material, has been widely utilized in scaffold fabrication due to its beneficial properties. It possesses a high porosity level, reaching up to 90%, allowing for effective diffusion of nutrients and oxygen to support cell growth on the scaffold. Moreover, its porosity provides ample space for the development of new tissue.

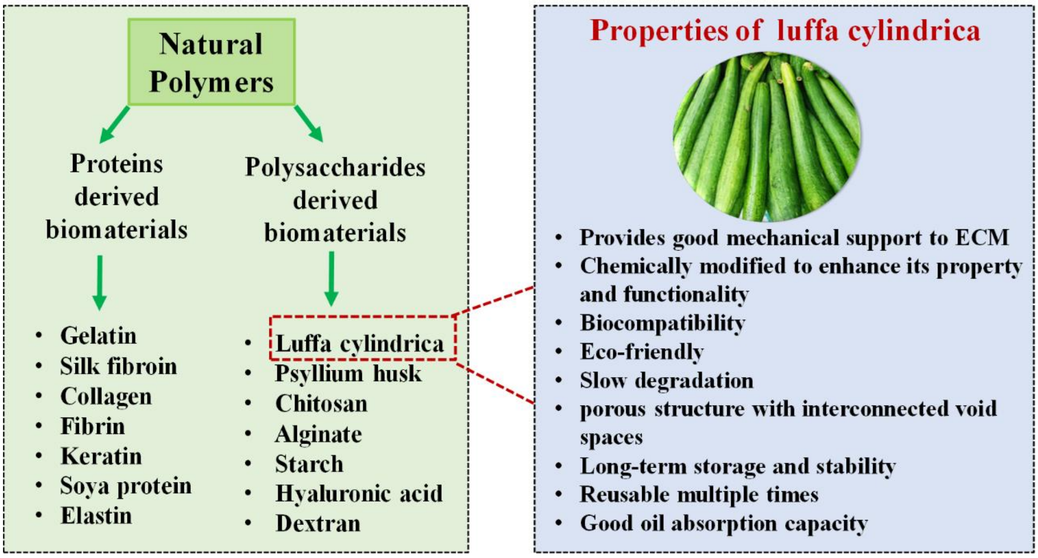


Figure 1.8: Represents the types of natural polymers and the properties of luffa cylindrica (Paul, Das, and Sharma 2021; Oun et al. 2021; Cecen et al. 2016).

LC exhibits favorable mechanical properties, enabling it to withstand the forces encountered during implantation. This resilience prevents the collapse or deformation of the scaffold, which could potentially harm the growing cells. Importantly, LC is biocompatible, ensuring it does not pose toxicity risks to cells. This compatibility safeguards the well-being of cells attached to the scaffold. Additionally, LC stands out as an abundant and inexpensive material; making it a cost-effective choice for scaffold fabrication (Figure 1.8).

1.5.1 Physical, chemical and mechanical properties of LC

A cross-sectional examination of the luffa fibers revealed that the luffa architecture is comprised of a large central canal or core with a honeycomb-like structure surrounded by smaller shells and external shells in which the fibers are dispersed at an angle of roughly 90 degrees to one another (Bal, Bal, and Lallam 2004; BOYNARD and D'ALMEIDA 2000). The luffa fibers are also found to be significantly shorter in length (Bal, Bal, and Lallam 2004). Morphological examinations are essential for comprehending the material properties and architecture, and assessing the effect of chemical treatment on fiber morphology (Table 1.5), crystallinity index, etc. (Alhijazi et al. 2020; Norul Izani et al. 2013).

Table 1.5: Physical properties of treated and untreated luffa fibers.

Luffa Material	Diameter (μm)	Density (kg^{-3})	Crystallinity index (%)	References
Untreated luffa fibers	≈ 290	1247	50.0	(L. Ghali et al. 2009a; Ighalo et al. 2021; Kalusuraman et al. 2019)
Alkali-treated luffa fibers (NaOH)	≈ 170	1337	66.32	

For instance, Chen et al. reported that the bulk of luffa fibers was markedly reduced after performing an alkali treatment. Additionally, there was a slight decrease in volume. During compression tests, cracks and ridges were seen on the surface of untreated luffa fibers.

However, treated fibers showed substantial fractures compared to non-treated fibers (Y. Chen et al. 2019).

The alkali treatment of luffa fibers facilitated the conversion of hydrophilic fibers to hydrophobic fibers. This may be probably because alkali treatment roughened the surface of the fiber and helped in the removal of the fiber's non-polar covalent components, such as xyloses, wax and other similar substances (Alhijazi et al. 2020; Verma and Goh 2021). With increasing alkali concentration (NaOH), the hydroxyl groups became less accessible (Abdulrahman 2015). This may be yet another explanation for the change in the hydrophilic to the hydrophobic properties of luffa fibers. The solubility of luffa in all solutions, except alcohol-benzene, was equivalent to that of hardwood. It displayed the highest possible solubility of around 16.38% in 1% NaOH. They exhibited a greater degree of solubility in cold water as compared to hot water (Alhijazi et al., 2020). Table 1.4 displays the percentage solubilities of luffa fiber.

The thermogravimetric approach enables a technique to analyze the response of luffa fibers against temperature change. It provides a comprehensive analysis of the change in mass of the substance over time concerning the temperature (Camuffo 2019). Thermogravimetric analysis (TGA) curve is plotted using the collected data from thermogravimetric measurements. The thermogravimetric curve had three distinct degenerative phases (TG curve). The first degradation curve is associated with moisture evaporation from the fiber and occurs in the temperature range of 32˚C to 100˚C (Premalatha et al. 2021). Approximately 7.25% of weight reduction occurs during this period (Alhijazi et al. 2020). The second degradation corresponds to thermal depolymerization of hemicellulose, lignin, and cellulose, and the weight losses varied for raw and treated luffa fibers, and this occurs at temperatures ranging from 200 to 250˚C (Alhijazi et al. 2020; Premalatha et al. 2021), raw luffa fibers lost 15.68% of their original weight, while alkali-treated luffa fibers lost only 12.23%. Aromatic ring cleavage took place at much greater temperatures. This phase resulted in a weight loss of approximately 57% (Premalatha et al. 2021). At even higher temperatures, aromatic ring cleavage occurred. The weight loss in this phase was about 57% (Premalatha et al., 2021). Table 1.6 shows the mass loss percentages for treated and untreated luffa fibers. Alkali-treated luffa, or chemically modified luffa in general, had superior thermal stability to untreated luffa and, consequently, a lower mass loss percentage (Alhijazi et al. 2020; V. O. A. Tanobe et al. 2014). This could be attributed to eliminating amorphous content, cellulose and lignin fibers, resulting in a higher crystallinity index and degradation temperature (Rajulu, Devi, and Venunadhan 2006; Tanobe et al. 2014).

Tensile testing is critical for identifying a material's strength and elongation characteristics and producing substitute composites with improved tensile attributes (Niharika Mohanta, 2013; Rahman and Zhafer Firdaus Syed Putra, 2019). Several investigations revealed that alkali-treated luffa fibers outperformed untreated luffa fibers regarding tensile qualities. The maximum stress of treated fiber was found to be higher than that of untreated fiber, and as tensile strength refers to the greatest stress a material can withstand before breakage, chemically modified fibers had a higher tensile strength (Chakrabarti et al. 2020; Pal et al. 2022). Additionally, it was found that the chemically treated fiber had a steeper stress-strain curve than the untreated fiber. Therefore, compared to untreated fiber, the chemically treated fiber had a higher tensile modulus (Chakrabarti et al., 2020). Chemically treated fibers could improve their chemical bonding; allowing them to endure greater tensile loads than untreated fibers (Saw 2014). It is also expected that the removal of hemicellulose and lignin improved the crystallinity index and consequently enhanced the tensile properties and the fact that NaOH, being a base, can donate an electron pair and thus improving the fiber and matrix interaction, and thereby increasing the fiber's strength (Shahzad 2012; Thangaraju and Aravindakumar 2016).

Table 1.6: Mass loss percentage for untreated and chemically altered luffa and mechanical properties of luffa fibers.

Luffa Material	Chemical modification			Mechanical properties			References
	30 - 100˚C	200 - 250˚C	>300˚C	Tensile Strength (MPa)	Tensile Modulus (MPa)	Compressive Strength (MPa)	
Untreated luffa	7.25%	15.68%	54.11%	178.29	4263.84	0.74	(Premalatha et al. 2021; Alhijazi et al. 2020; Saw 2014)
Alkali-treated luffa	≈ 7%	12.23%	57.14%	192.70	5184.62	107	

1.6 Review of the existing literature

Although natural fibers possess several advantages, such as being cheap, abundant, and renewable, and have good specific properties such as tensile strength and stiffness; making attractive reinforcements for composite materials. The untreated natural fiber composites have performed low potential capabilities and have therefore not been used extensively in the polymer industry due to poor interfacial bonding between the cellulose fiber and polymer matrix, limited thermal stability, high moisture absorption and biodegradability of the fibers. According to existing literature in Ayurveda, LC is considered a medicinal plant (Dhamargava) owing to its purgative properties; its roots, leaves, fruits and seeds possess phytochemical properties that aid in treating many ailments. Using the whole plant in a medical application has great advantages for restoring lost functionality, lowering inflammation responses and several other therapeutic utilities. Luffa exhibits the potential to be utilized in treating various conditions, including jaundice, liver enlargement, skin issues, wound healing, nephritis, chronic bronchitis, diabetes and arthritis. Extracting and isolating compounds from LC has shown many benefits, including synergistic pharmacological effects (e.g., immunomodulatory, antioxidant, anti-cancer and anti-inflammatory) (Azeez, Bello*, and Adedeji 2013; Seyi Valerie 2017; Shendge and Belemkar 2018).

Natural fibers are favored due to their renewable nature, environmentally benign, cost-effective and abundant availability (Niharika Mohanta 2016). Luffa fibers, in particular, possess several desirable qualities, such as non-toxicity, long-term stability, high fibrous content and high porosity (Adie, Igboro, and Daouda 2013; K et al. 2019; Kamran et al. 2021). These appealing features have attracted researchers to explore various applications (Figure 1.9) of luffa fibers, including drug administration, medicine, biotechnology, therapeutics, plant cell immobilization, cosmetology, biotic cleanser, pharmaceuticals, tissue engineering and template for diverse purposes (Alshaaer 2017; Ha et al. 2015; K et al. 2019).

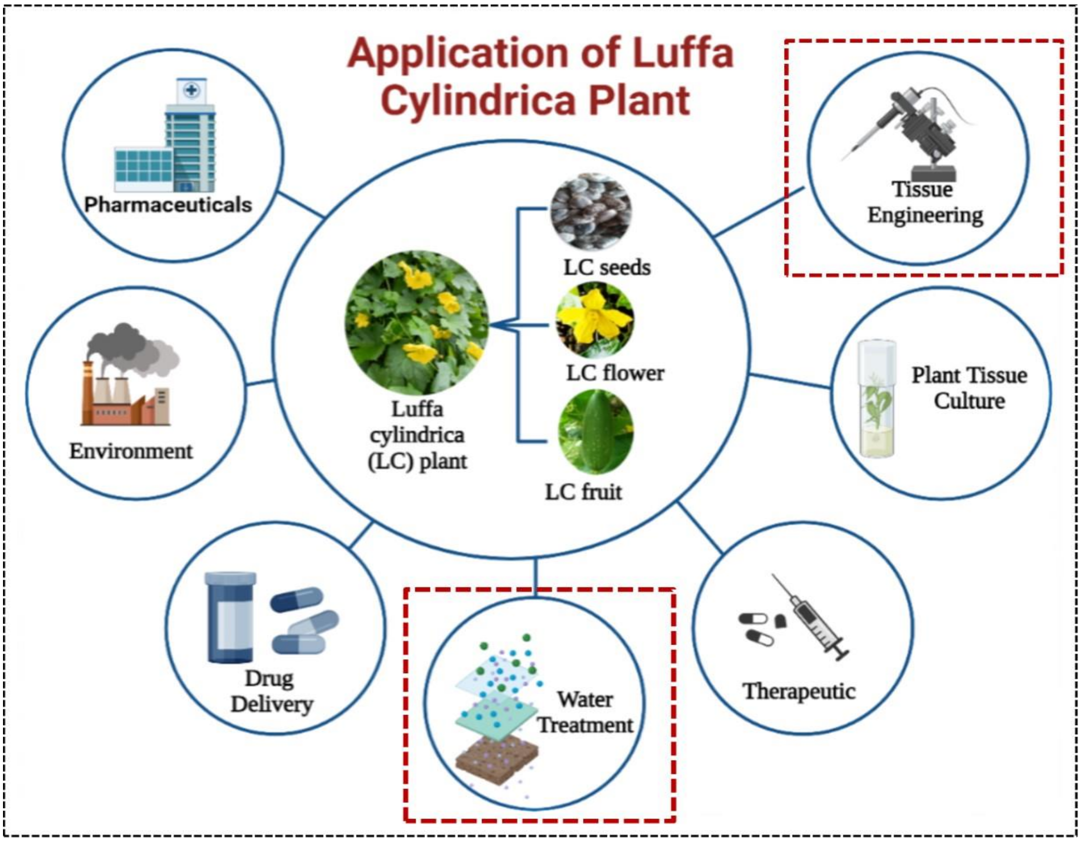


Figure 1.9: A graphical illustration of the various applications using luffa-based materials.

1.7 The significance of LC in biological and environmental applications

1.7.1 Biological

Using a luffa sponge as a template enables the fabrication of scaffolds with a wide range of porosities. The outer core of the luffa sponge is employed to create a three-dimensional interconnected porous network; allowing regulation of properties such as geometry, porosity and pore size. This approach facilitates significant scaffold porosity while maintaining desirable mechanical characteristics (Alshaaer 2017). In a study by Chen et al., a luffa-based scaffold was developed for liver tissue engineering. The Luffa sponge served as a three-dimensional scaffold for the growth of hepatoblastoma (C3A/HepG2) cells. The luffa cubes provided a higher secretion of α-fetoprotein and albumin by C3A/HepG2 cells compared to polyurethane foam, indicating the competency of luffa sponge as a scaffold for high-density growth of the human hepatocyte cell line and the potential expression of liver-specific activities (J.-P. Chen et al. 2003). Another research study utilized Luffachitin from the sponge-like dried fruit of Luffa aegyptiaca to develop a skin equivalent. The white residue of Luffa aegyptiaca's dried fruit, woven into a thin, porous membrane through filtering and lyophilization, was used as a skin substitute for investigating wound healing in rats. The Luffachitin membrane demonstrated high yield, excellent manufacturing qualities and an appealing appearance (Jiang et al. 2014).

Furthermore, Stella et al. employed chemical modification to obtain natural cellulose from a luffa sponge and subsequently electrospun it into nanofibrous scaffolds to achieve consistent, bead-free fibers with the desired diameter. Different ratios of hydroxyapatite (HAP): polylactic acid (PLA) were evaluated and compared with HAP-treated cellulose (TC)-PLA. All nanofibrous scaffolds exhibited excellent cytocompatibility (Mary Stella and Vijayalakshmi 2019). In bone tissue engineering, bone scaffolds must possess biocompatibility, biodegradability, load-bearing capacity, and appropriate porosity for nutrient distribution, waste removal, and angiogenesis (Ghassemi et al., 2018). Osteoblasts, bone-forming cells ranging in size from 10 to 50 µm , prefer pore sizes between 100 and 400 µm to promote bone repair and enhance vascularization (Abbasi et al., 2020; Alshaaer, 2017). However, excessively high scaffold porosity may negatively influence the material's mechanical properties. Thus, it is crucial to determine the appropriate pore size to balance effective angiogenesis and satisfactory mechanical characteristics (Alshaaer 2017).

LC-based scaffolds can be used to support the growth of bone cells, such as MG-63 osteoblast-like cells which can then help to repair or regenerate damaged bone tissue. In vitro studies have shown that luffa-based scaffolds can promote the adhesion, proliferation and differentiation of MG-63 osteoblast-like cells. These scaffolds have also been shown to support the growth of new bone tissue in animal models. In a research conducted by Alshaaer et al., a novel method was developed to synthesize porous bioceramics containing hydroxyapatite using natural luffa cylindrical fibers as templates. The bioceramics exhibited bimodal porosity, with smaller pores of 10-30 µm and cylindrical macropores of 100-400 µm. They were also bioactive and had mechanical properties comparable to natural spongy bones. In vitro characterization showed that cells attached to the apatite crystals proliferated in the scaffolds. These results suggest that the novel method has promising applications in bone tissue engineering (Alshaaer 2017).

Drug delivery refers to effectively distributing medications or drugs to specific target tissues for therapeutic purposes (Tiwari et al. 2012). In drug delivery systems, carriers are utilized to transport drugs to desired organs or cells, enabling precise drug distribution, controlled release at the target site, enhanced bioactivity and prolonged drug administration (Lavik, Kuppermann, and Humayun 2013; Tiwari et al. 2012).

Porous carrier systems have gained significant attention in drug delivery due to their large surface area, variable pore size and other beneficial properties (Ahuja and Pathak, 2009). These porous materials allow drug absorption and controlled release; providing a more predictable and regulated profile. Researchers have been exploring the use of luffa fibers in drug delivery systems due to their highly porous structure (Bal, Bal, and Lallam 2004). The typical pore diameter of luffa fibers has been measured to be approximately 736 µm (Tuncel et al. 2014). The microporous and macroporous nature of luffa fibers facilitates drug adsorption and serves as micro-reservoirs for drug administration. This property has been utilized in the targeted release of silver nanoparticles in wound dressing, where the microporous nature of luffa fibers enhances the incorporation and transport of metal nanoparticles to the desired location (K et al. 2019; Kupnik et al. 2020). Another application of luffa fibers in drug delivery is their use as a natural binder in the production of acetaminophen tablets (Macuja, Ruedas, and España 2015). Besides providing mechanical strength to the tablets, binding agents have been found to improve drug release properties, which play a crucial role in influencing the bioavailability of the integrated drug (Macuja, Ruedas, and España 2015; Patil et al. 2014). The use of cellulose from luffa fibers as a natural binder has been demonstrated to enhancement in tablet hardness and disintegration rate; thereby improving the bioavailability of the medication (Bhagavan and Wolkoff 1993; Macuja, Ruedas, and España 2015).

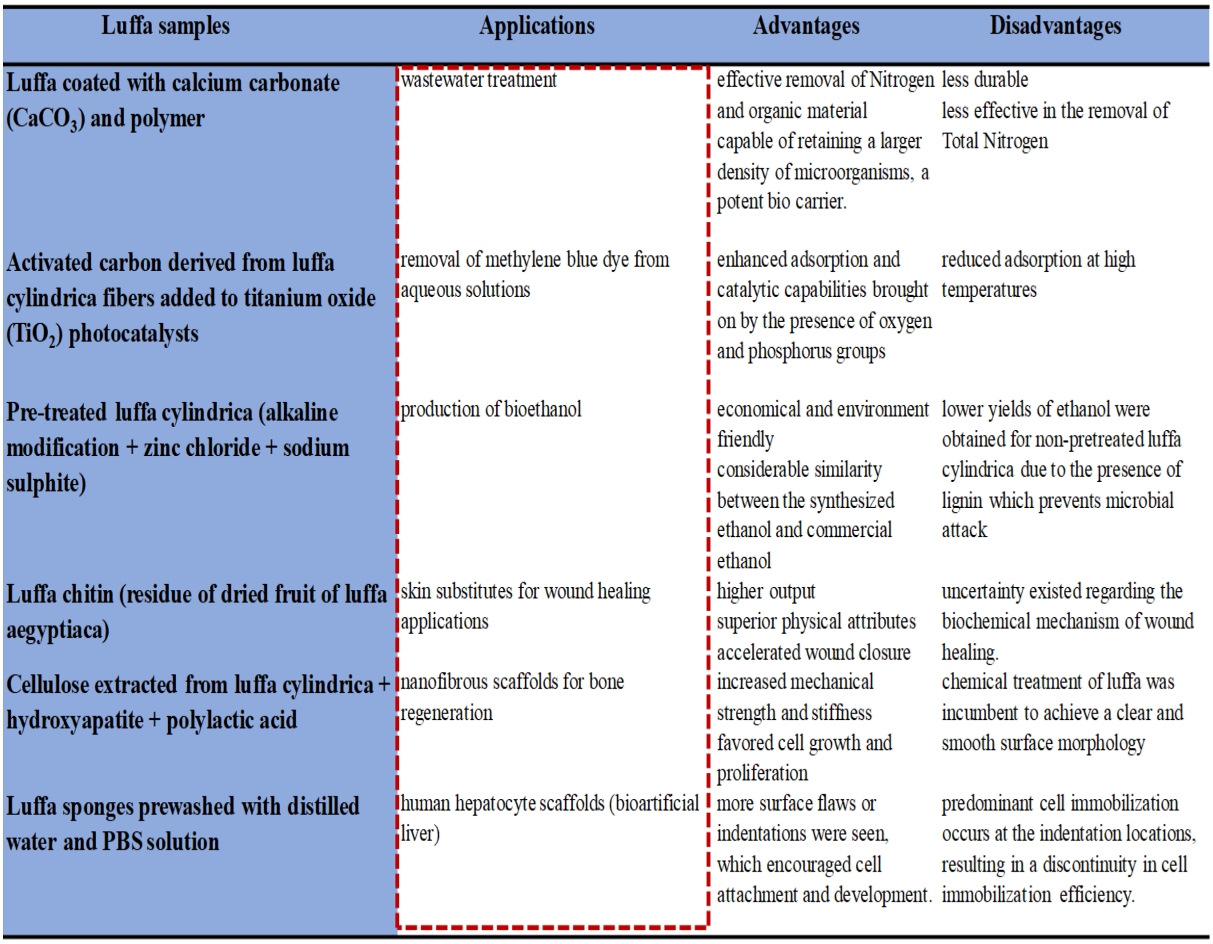
Plant cell immobilization is a fundamental technique employed in plant cell culture, which entails anchoring cells to a solid substrate or a membrane to provide stability and protection against environmental fluctuations and shear forces while allowing for efficient production (Nartop 2016). Hierarchically porous immobilization carriers are commonly used as a robust support system, as they possess high porosity, large surface area, high permeability and rapid mass transfer; facilitating effective cellular immobilization (Xin et al. 2018; Zhu 2007). Luffa sponge, characterized by its high porosity, biodegradability and non-toxic nature, has garnered attention and potential applications as a support structure for plant or microbial cells in immobilization processes (Liu et al. 1998). The core region of the luffa sponge exhibits greater porosity and larger pore size compared to the peripheral regions, which have relatively lower porosity and smaller pores. This porosity heterogeneity between the luffa structure's core and periphery renders it a promising material with significant potential for cell immobilization (Y. Chen et al. 2017; Y.-K. Liu et al. 1998).

1.7.2 Environmental

As a natural material, Luffa is commonly used as a filtration medium due to its hydrophilic properties, high porosity, and substantial surface area (J. Zhang et al. 2019). It has effectively removed contaminants, including total suspended solids (TSS), ammonia nitrogen, bacteria, turbidity, and color. Chemical stability tests have shown that luffa sponges used for filtration maintain their structural integrity well, indicating excellent resistance to chemical degradation (J. Zhang et al. 2019). Assessing the efficiency of luffa sponge in wastewater treatment can involve tests measuring biological oxygen demand (BOD) and chemical oxygen demand (COD), which provide insights into the degree of pollution (Tasnim Alam, 2015). While luffa sponge has shown efficacy in removing microbes and heavy metals, its high porosity poses limitations in effectively removing TSS, allowing relatively smaller particles to pass through. Furthermore, its effectiveness in reducing BOD is relatively limited, suggesting that luffa may not be optimal for addressing certain aspects of potable water, such as turbidity and hardness. Therefore, luffa can be utilized as an initial filtration medium in water treatment processes but may not be suitable for producing potable water.

Luffa sponge effectively removes microbes and heavy metals; however, it has limitations in removing TSS due to its high porosity, which allows relatively smaller particles to pass through. Its effectiveness in reducing BOD is relatively limited, suggesting that luffa may not be optimal for addressing key aspects of potable water, such as turbidity and hardness. Consequently, luffa can serve as an initial filtration medium in water treatment processes but may not be suitable for producing potable water (Adie, Igboro, and Daouda, 2013). The various applications of luffa-based scaffold are shown in Table 1.7.

Table 1.7: Represents luffa-based samples utilized in various applications with advantages and disadvantages.



Luffa samples	Applications	Advantages	Disadvantages
Luffa coated with calcium carbonate ($CaCO_3$) and polymer	wastewater treatment	effective removal of Nitrogen and organic material capable of retaining a larger density of microorganisms, a potent bio carrier.	less durable less effective in the removal of Total Nitrogen
Activated carbon derived from luffa cylindrica fibers added to titanium oxide (TiO_2) photocatalysts	removal of methylene blue dye from aqueous solutions	enhanced adsorption and catalytic capabilities brought on by the presence of oxygen and phosphorus groups	reduced adsorption at high temperatures
Pre-treated luffa cylindrica (alkaline modification + zinc chloride + sodium sulphite)	production of bioethanol	economical and environment friendly considerable similarity between the synthesized ethanol and commercial ethanol	lower yields of ethanol were obtained for non-pretreated luffa cylindrica due to the presence of lignin which prevents microbial attack
Luffa chitin (residue of dried fruit of luffa aegyptiaca)	skin substitutes for wound healing applications	higher output superior physical attributes accelerated wound closure	uncertainty existed regarding the biochemical mechanism of wound healing.
Cellulose extracted from luffa cylindrica + hydroxyapatite + polylactic acid	nanofibrous scaffolds for bone regeneration	increased mechanical strength and stiffness favored cell growth and proliferation	chemical treatment of luffa was incumbent to achieve a clear and smooth surface morphology
Luffa sponges prewashed with distilled water and PBS solution	human hepatocyte scaffolds (bioartificial liver)	more surface flaws or indentations were seen, which encouraged cell attachment and development.	predominant cell immobilization occurs at the indentation locations, resulting in a discontinuity in cell immobilization efficiency.

1.8 Natural biomaterials and their potential in scaffold fabrication

Natural biomaterial resources are experiencing rapid growth as a sustainable, cost-effective, and environmentally friendly option for fabricating composite materials. The increasing concerns regarding environmental sustainability have drawn the interest of researchers, manufacturers, and end-users toward the fabrication of composite materials using natural biomaterials (Mia et al., 2020). The abundant biomass, forestry resources, and agricultural residues found in nature are all widely utilized as valuable commodities for use in different forms of renewable energy production. Various plants, crops, and agricultural byproducts are recognized as major sources of natural materials for polymer composites. These bio-based materials' research, development, and advancement can directly contribute to the ecosystem. The use of natural fibers as reinforcing materials in composites has substantially improved due to the increased emphasis on ecological sustainability and the use of renewable materials in constructing a greener society. Researchers, material scientists, and industry have recently begun to focus on natural fibers due to the inherent benefits natural fibers possess over traditional synthetic fibers. These benefits include reduced tool wear, cost-effectiveness, ease of disposal without causing harm to the environment, lower density, increased toughness, better energy recovery, substantial biodegradability and renewablity (Saba, Tahir, and Jawaid 2014; Mia et al. 2020).

1.8.1 Optimal Properties of Scaffolds

Scaffolds must be precisely designed to fulfill their intended functions while adhering to specific design criteria and possessing appropriate mechanical attributes (Echeverria Molina, Malollari, and Komvopoulos 2021). Several important considerations come into play during scaffold design to ensure their effectiveness in supporting tissue regeneration.

Firstly, the scaffold must exhibit biocompatibility to avoid triggering an immune response upon implantation. This necessitates the careful selection and thorough verification of a biomaterial compatible with the biological system. Biocompatible materials should be non-toxic, non-inflammatory, and able to integrate with the surrounding tissues. The scaffold's architecture directly influences the distribution of nutrients, oxygen and waste products within the tissue-engineered construct

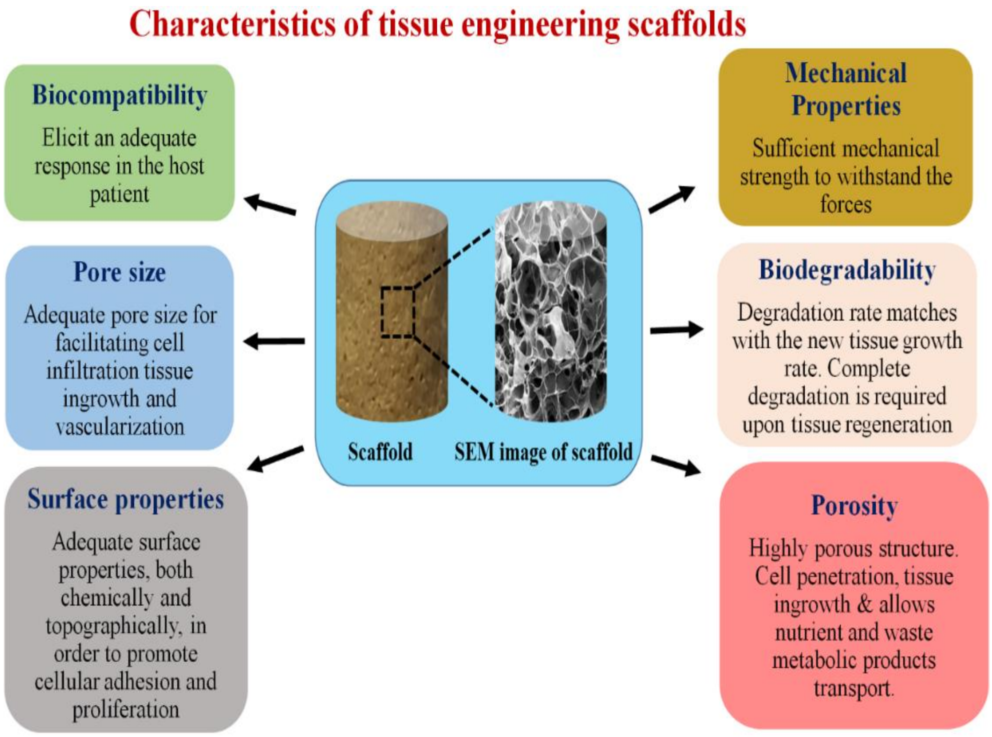


Figure 1.10: Characteristics of ideal scaffolds for tissue regeneration (Arjunan et al. 2021).

It also affects the mechanical properties of the scaffold, such as its stiffness and elasticity, which are important for mimicking the native tissue's mechanical environment. Therefore, carefully considering factors such as pore size, porosity, interconnectivity and overall geometry of the scaffold are needed to optimize its architectural properties (Echeverria Molina, Malollari, and Komvopoulos 2021; O'Brien 2011). Figure 1.10 represents the desired properties of scaffolds.

1.8.2 Scaffold fabrication techniques

Incorporating biomimicry is imperative in scaffold design, as the scaffolds must emulate the composition, architecture, and bioactive cues of the indigenous extracellular matrix (ECM) to facilitate cellular responses and tissue regeneration. Multiple methodologies are utilized in the production of three-dimensional scaffolds (L. Chen et al. 2019). These include 3D printing, which allows precise layer-by-layer deposition of materials based on computer-aided design (CAD) models, offering versatility in scaffold design and incorporation of bioactive molecules. Electrospinning generates nanofibers from polymer solutions, providing high surface area-to-volume ratios and interconnected porosity similar to the ECM (Xie et al. 2020; Luo, Wei, and Huang 2019; Kang et al. 2016). Self-assembly techniques use molecular interactions to organize materials into defined structures, while decellularization involves removing cellular components from native tissues, preserving the ECM architecture. Furthermore, porogen-based methodologies employ sacrificial substances to generate interconnected pores within scaffolds (Xie et al. 2020; O'Brien 2011). These requirements and fabrication techniques (Figure 1.11) are critical in developing functional and biomimetic scaffolds for tissue engineering applications.

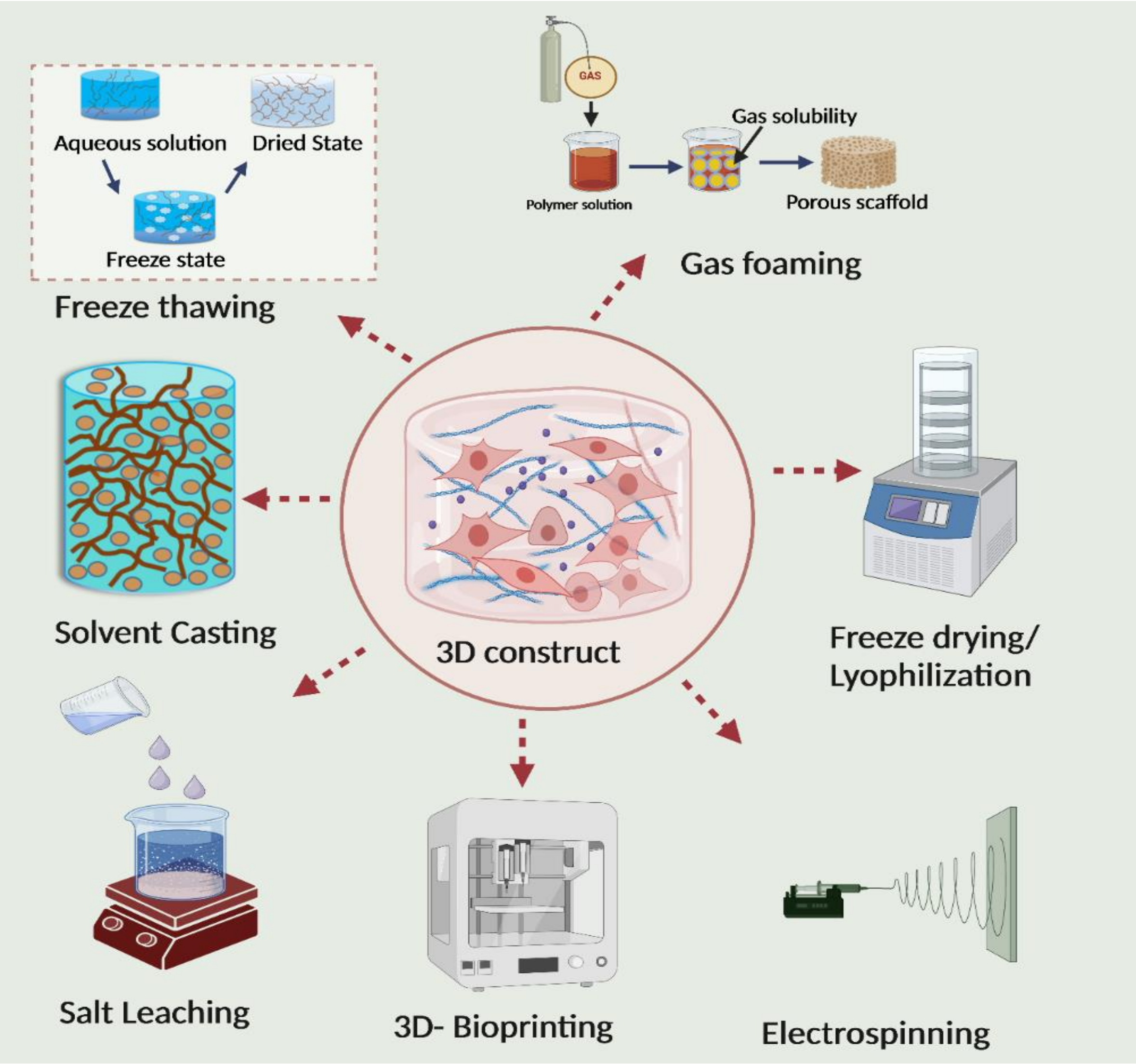


Figure 1.11: Various approaches for fabricating a wide range of 3D scaffolds (Kumar Sahi et al. 2023).

1.8.2.1 Freeze thawing

The freeze-thawing technique is frequently employed in scaffold production for tissue engineering. As mentioned earlier, the methodology pertains to the creation of supportive structures utilizing the solidification and subsequent melting of polymer solutions or suspensions. The polymer solution is subjected to rapid cooling to initiate the generation of ice crystals (Brougham et al. 2017; Shivalkar and Singh 2017). Freezing water molecules results in the segregation of polymer molecules, ultimately leading to a porous structure. Subsequently, the configuration mentioned above is conserved through a meticulous process of thawing the congealed substance, which permits the dissolution of ice crystals and the formation of a mesh-like system of interlinked cavities. Utilizing the freeze-thawing technique provides numerous benefits, including its straightforwardness, economical nature, and the capacity to regulate the scaffold's porosity and pore size by manipulating freezing and thawing parameters (Brougham et al. 2017; Kim et al. 2019). Applying this technique involves utilizing diverse biocompatible polymers, including collagen, gelatin, and chitosan to fabricate scaffolds that exhibit favorable characteristics for tissue regeneration. In addition, this methodology maintains the bioactivity of the biomolecules that have been integrated and fosters cellular attachment, proliferation, and differentiation within the scaffold. Utilizing the freeze-thawing method is a significant approach in scaffold manufacturing, which facilitates the creation of porous scaffolds that imitate the inherent extracellular matrix. These scaffolds provide support for the growth and regeneration of tissues (Katrilaka et al. 2023; Merivaara et al. 2021).

1.8.2.2 Gas foaming

The gas foaming technique has been observed to be a proficient approach to producing three-dimensional scaffolds. The methodology entails the integration of gaseous bubbles into a polymer solution or molten state, leading to the creation of a porous framework. At the outset, a gaseous substance is solvated within the polymer solution by applying pressure. Subsequently, the solution enriched with gas is exposed to an abrupt decrease in pressure, leading to the gas expansion and bubbles within the solution. The process of bubble expansion induces the formation of voids and interconnected pores within the scaffold material. The scaffold pore size and porosity adjustment to meet specific tissue engineering requirements can be achieved by regulating gas concentration, processing parameters, and polymer properties (W. Chen et al. 2012; Montjovent et al. 2007). The utilization of gas foaming presents various benefits in the process of scaffold production. The technique is a straightforward and economical approach that enables the fabrication of scaffolds with customized porosity, pore size and mechanical characteristics (Rao et al. 2019; W. Chen et al. 2012). The porous scaffolds from this process facilitate tissue regeneration by promoting cell attachment, proliferation and nutrient diffusion.

1.8.2.3 Freeze drying

The process of freeze-drying entails the elimination of moisture from a frozen sample through sublimation, which leads to the development of a porous architecture. The water content of the polymer solution or suspension is solidified through initial freezing. Following this, a vacuum is utilized to facilitate the sublimation process of the frozen sample, whereby the ice undergoes a direct transition from a solid to a gaseous state without undergoing a liquid phase. Consequently, the ice crystals change to a gaseous state, forming a porous structure with an interconnected three-dimensional network within the scaffold material. The process of freeze-drying presents various benefits in the context of scaffold fabrication (Katrilaka et al. 2023; Merivaara et al. 2021). The preservation of scaffold structure, porosity and mechanical integrity is facilitated by preventing material collapse during water removal. The methodology additionally preserves the bioactivity of integrated molecules, such as growth factors or pharmaceuticals; thereby augmenting cellular interactions within the scaffold. In addition, the process of freeze drying facilitates the utilization of diverse biocompatible polymers and biomaterials, encompassing natural polymers such as gelatin, collagen, and chitosan and synthetic polymers such as polylactic acid (PLA) or poly(lactic-co-glycolic acid) (PLGA) and polyvinyl alcohol (PVA). The scaffolds exhibit a porous architecture with a substantial surface area, which promotes cellular adhesion, proliferation and nutrient diffusion. The freeze-drying method is a significant asset in the production of scaffolds, enabling the development of biomimetic scaffolds that facilitate tissue regeneration in a regulated and consistent manner (Figure 1.12).

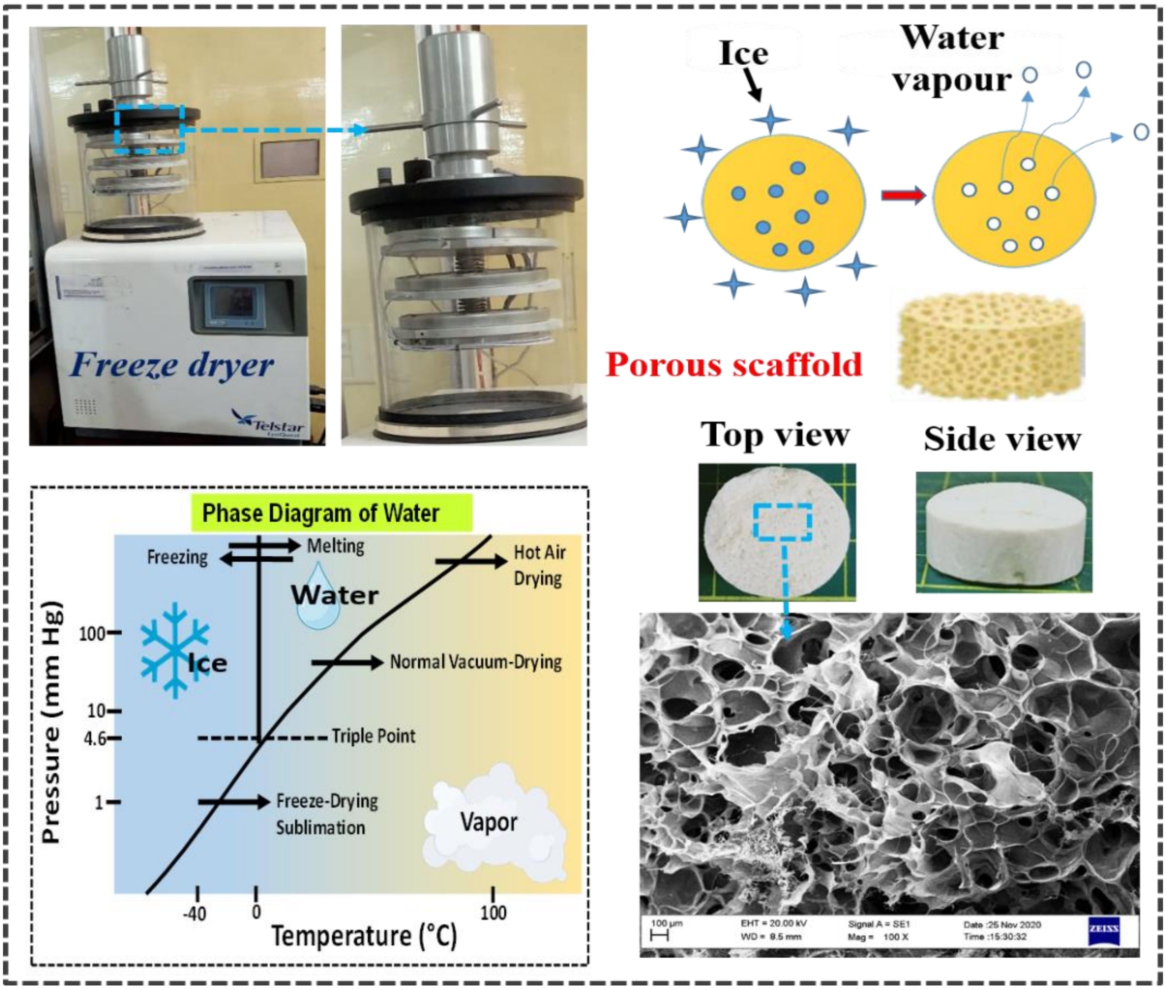


Figure 1.12: Porous scaffold and scanning electron microscopic (SEM) image of the fabricated scaffolds using freeze-drying process (Lim et al. 2016).

1.8.2.4 Electrospinning

It is a fiber spinning method in which an electrically charged polymer stream in a molten state is drawn into ultrafine fiber through electrostatic forces. Electrospinning provides precise control over fiber diameter and high surface area-to-volume ratio; mimicking the extracellular matrix (ECM) structure in native tissues (Aytac et al. 2021; Frohbergh et al. 2012). The fine fiber morphology enhances cell adhesion, proliferation, and differentiation; promoting tissue regeneration (Campiglio et al. 2019; Kumar Sahi et al. 2023; Poddar et al. 2021). However, Electrospinning also has limitations that need to be addressed. Achieving uniform and homogeneous fiber deposition is challenging, resulting in random or misaligned fiber orientations (Kumar Sahi et al. 2023). The scalability of the electrospinning process for large-scale scaffold production remains a concern (Ravichandran et al., 2011). Generally, nanofibers like gelatin and collagen with high porosities and surface areas are fabricated using electrospinning (Sahi et al. 2021; Greiner and Wendorff 2007; Konuk Ege et al. 2021). When a significantly high voltage is administered to a liquid droplet, the liquid's body becomes charged, electrostatic repulsion overcomes surface tension, and the droplet is expanded to form a Taylor cone, a key point of liquid stream ejection (Figure 1.13).

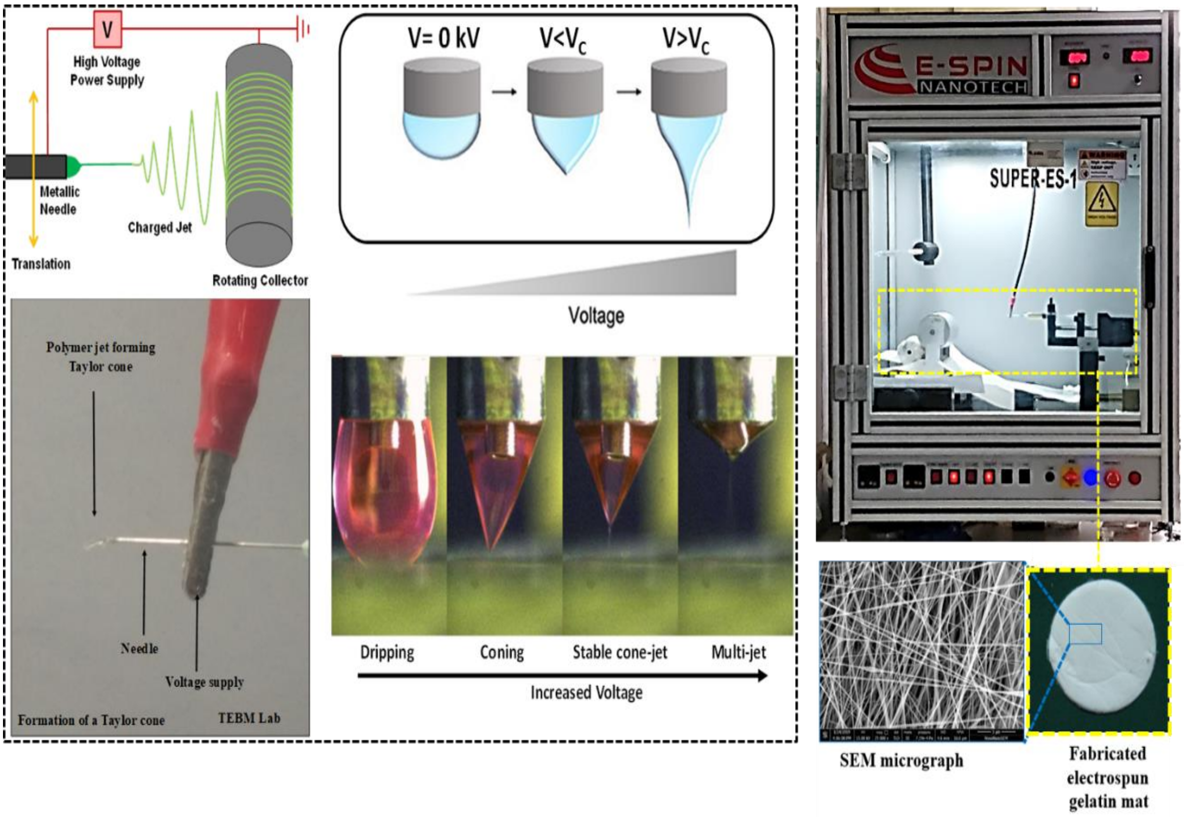


Figure 1.13: Diagrammatic representation of electrospinning unit setup, Taylor cone formation and nanofibrous mat (Sahi et al. 2021; Mulholland 2020). Images of the instruments have been obtained from the Tissue Engineering and Biomicrofluidics Laboratory, School of Biomedical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi.

1.8.2.5 Three-Dimensional (3D) bioprinting

3D bioprinting is an advanced technique for fabricating three-dimensional tissue engineering scaffolds. It involves the precision layer-by-layer deposition of bio-inks, which are cell-laden materials or biomaterials, to create complex and functional scaffolds. This method employs computer-aided design (CAD) models to direct the printing process; enabling precise control over scaffold geometry and architecture. Living cells, such as stem cells or specialized cell types, can be combined with hydrogels, polymers, or decellularized extracellular matrix (dECM) components to form bio-inks. The bioprinter dispenses bio-ink through a nozzle or print head, and the material is deposited under precise control to form the desired scaffold structure. After printing, the scaffold can undergo additional processing stages, such as crosslinking or maturation, to improve its mechanical properties and functionality. Bioprinting in three dimensions enables the fabrication of complex tissue structures, such as blood vessels, cartilage, and organs, by precisely placing cells and biomaterials in predetermined spatial arrangements. Based on individual anatomical data, patient-specific scaffolds can be designed and printed using this method, which offers the possibility for personalized medication. Additionally, it allows for patient-specific customization and the incorporation of bioactive molecules (Xie et al. 2020; Luo, Wei, and Huang 2019; Ozbolat, Peng, and Ozbolat 2016)One of the primary limitations is the restricted range of available biomaterials suitable for 3D printing. Although numerous polymers have been employed, the choice is still limited compared to traditional fabrication methods (Ozbolat, Peng, and Ozbolat 2016; Agarwal et al. 2020; Banerjee et al. 2022; Datta et al. 2020). This method is also limited by potential delamination, reduced mechanical strength, slow fabrication speed and limited resolution.

1.9 Foremost characterization methods

To validate the scaffold as a potential material, FTIR, mechanical testing, biocompatibility assessments, and critical feature analysis were performed as discussed below:

Fourier Transform Infrared (FTIR) spectroscopy is essential for identifying chemical compounds in various materials and products. FTIR testing provides valuable insights into the molecular composition of samples, making it a versatile analytical technique used across different industries. FTIR analysis involves sending infrared radiation through a sample, where molecules absorb the radiation and convert it into rotational or vibrational energy. FTIR spectroscopy operates on the fundamental principle that, compared to a monochromatic light beam, it employs a broadband infrared light source encompassing a broad spectrum of infrared frequencies. By passing this broad spectrum of light through a Michelson interferometer, the mirror modulates the various infrared frequencies at varying rates. Following this, the sample is subjected to absorption at particular infrared frequencies that correspond to the molecular vibrations of the sample, as the modulated beam interacts with it. An interferogram is produced when the detector computes the total light intensity for every position of the mirror. The interferogram is subsequently subjected to a Fourier transform by a computer in order to generate a frequency domain spectrum that illustrates the absorption at every infrared frequency.

Biocompatibility evaluation for scaffolds in tissue engineering involves assessing safety, immunological response, inflammatory reactions, tissue integration and biodegradability. By conducting comprehensive biocompatibility testing, researchers can ensure that the scaffolds used in tissue engineering applications are safe, effective and compatible with biological systems; facilitating successful tissue regeneration and repair (Fu et al., 2022; Ivanov et al., 2019; Zhang et al., 2020). Various types of biocompatibility testing are essential to ensure the safety and effectiveness of tissue-engineered constructs when interacting with biological systems. These tests include assessing the toxicity of the tissue-engineered construct, determining if the materials used in the scaffold have any harmful effects on cells; thus ensuring the construct's safety for cellular interactions. Additionally, it involves evaluating the degradation properties of the scaffold over time, however, it is critical for understanding how the scaffold degrades in the body and whether the degradation by-products are biocompatible and do not harm surrounding tissues.

To effectively assess the mechanical properties of a luffa composite scaffold, several mechanical tests are recommended. Compression testing is crucial for determining the compressive strength and behavior of the scaffold under a compressive load, providing insights into its structural integrity and stability, which are vital for material selection and design considerations across various industries. Compression testing and tensile testing play a vital role in assessing the mechanical properties of materials utilized in bone tissue engineering applications. Bone tissue experiences both compressive and tensile forces within the body. It is crucial for bones to have the strength to endure the forces exerted on them during various physical activities such as walking, running and supporting weight. Bones are also subject to various forces, such as those generated by muscle contractions and bending movements. The structure of bone is highly intricate, with unique properties that allow it to withstand both compression and tension. Using implant materials that do not closely match the mechanical behaviour of bone may result in stress shielding, bone resorption, and ultimately implant failure. Tests such as bending or shear may only offer limited insights into particular behaviours. While it is essential to conduct comprehensive mechanical characterizations, including tensile, compression, bending, shear, torsion, impact, fatigue and hardness tests in order to accurately replicate the bone architecture, these characterizations are not mandatory for the successful development of bone implants. Nevertheless, in contrast, compressive and tensile testing offer a comprehensive evaluation of the material's capacity to endure the intricate mechanical stresses encountered by bone within the human body; thereby facilitating the selection of suitable implant materials. However, bending testing yields data pertaining to flexural modulus, bending rigidity and bending strength. Shear testing is utilized to measure shear strength and shear modulus, whereas torsion testing is employed to evaluate the bone's resistance to rotational forces without encountering failure. Impact testing evaluates the capacity of a bone to withstand fracture and absorb energy in the face of dynamic or abrupt loading conditions. The resistance of a bone to cyclic stresses or recurrent loading is assessed through fatigue testing. Moreover, Compression and tensile testing adhere to widely recognized ASTM or ISO standards; ensuring consistent evaluation across various materials and studies. Overall, compression and tensile testing play a vital role in bone tissue engineering applications. These tests directly evaluate the material's capacity to endure the intricate mechanical forces that bones encounter in the body. Additionally, they offer comprehensive data that are essential for choosing suitable implant materials. The consistent format of these tests also guarantees the dependability and ability to compare the results.

1.10 Research objectives

This thesis aims to develop luffa-based porous composite scaffolds using LC as a prime material for tissue and oil absorption applications. To accomplish this, advanced techniques such as freeze-drying and the hand lay-up approach were employed to fabricate the scaffold, aiming to mimic the cytocompatibility of the scaffolds and facilitate oil-water separation. Through this research, we have successfully fabricated scaffolds that can address the requirements of both tissue and oil absorption applications. Due to its unique properties, LC has emerged as a highly promising candidate for scaffold preparation. In this study, we utilized various forms of LC by extracting and incorporating them into scaffold fabrication. We aim to enhance scaffold stability and integrity through chemical modifications and crosslinking techniques; enabling them to sustain cellular growth and water treatment applications. The in vitro characterization of these scaffolds revealed outstanding biocompatibility of these composite scaffolds, which has demonstrated superior cell performance in terms of cellular growth and proliferation. Additionally, the mechanical properties assessment demonstrated excellent strength in the fabricated scaffolds. LC has not been extensively explored to a great extent. We strongly believe it holds immense potential as a promising candidate for further investigations and advancements in various fields.

The research objectives have been sub-divided into the following sections:

1. Fabrication and in vitro characterization of luffa-based composite scaffolds incorporated with gelatin, hydroxyapatite, and psyllium husk for bone tissue engineering.

   1.1. Optimization of polymeric solutions to achieve the ideal composition for scaffold fabrication.
   1.2. Physicochemical characterization of the resulting porous scaffolds.
   1.3. Evaluation of thermal stability and degradation behavior of the fabricated scaffolds.
   1.4. Performance of compression tests to analyze the load-bearing capacity of the scaffolds.
   1.5. Assessment of cellular biocompatibility to evaluate how well the scaffolds support cellular growth and proliferation.
2. In vivo characterization of a luffa-based composite scaffold upon subcutaneous implantation in Rats
   2.1. Scaffold fabrication techniques and implantation in the rat model.
   2.2. Assessment of biochemical analysis, i.e., liver, kidney, and heart functions in the rats
   2.3. Histopathological studies of the rat tissues after implanting the fabricated scaffolds.
3. Cellulose-based Luffa cylindrica (mat, flakes, and powder) reinforced polydimethylsiloxane (PDMS) composites for oil and organic solvent absorption.
   3.1. Fabrication of the composite scaffolds using a hand-lay technique incorporating various forms of Luffa cylindrica with PDMS.
   3.2. Calculations of surface roughness to determine the texture and the roughness of the composite scaffolds.
   3.3. The thermal stability and degradation behavior of the fabricated scaffolds to understand their performance under different temperature conditions.
   3.4. Absorption studies to assess the ability of the composite scaffolds to absorb oil, phosphate-buffered saline (PBS), and organic solvents.
   3.5. Investigation of the potential of the composite scaffolds for oil-water separation.

1.11 Thesis outline

The thesis is structured into five chapters. **Chapter 1** offers a comprehensive overview of tissue engineering scaffolds and their crucial role in regenerating damaged tissue. It emphasizes the essential properties required to develop ideal scaffolds that can substitute complex living tissues. Additionally, this chapter briefly describes the enabling technologies employed in fabricating three-dimensional (3D) scaffolds for tissue engineering applications. It also explores the advantages conferred by natural fibers in scaffold design, highlighting their unique properties and benefits. Furthermore, a detailed literature review is presented on luffa cylindrica, encompassing its chemical composition, bioactive compounds and properties. The diverse range of applications of luffa-based scaffolds is also discussed.

Chapter 2 pertains to the fabrication of chemically crosslinked luffa-based composite scaffolds using the freeze-drying technique. The morphological analysis of the fabricated scaffolds is carried out using Scanning Electron Microscopy (SEM). A degradation study is conducted to assess the stability of the composite scaffold. Attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR) analysis is employed to investigate the molecular interactions within the formed structures. Differential scanning calorimetry (DSC) analysis was performed to investigate the thermal properties of the obtained scaffolds. Mechanical testing is conducted to assess the strength of scaffolds. Moreover, the potential of the scaffolds for tissue regeneration is evaluated through porosity and swelling studies, MTT (MTT(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay and long-term cell culture. A graphical representation of the findings in this chapter is represented in Figure 1.14.

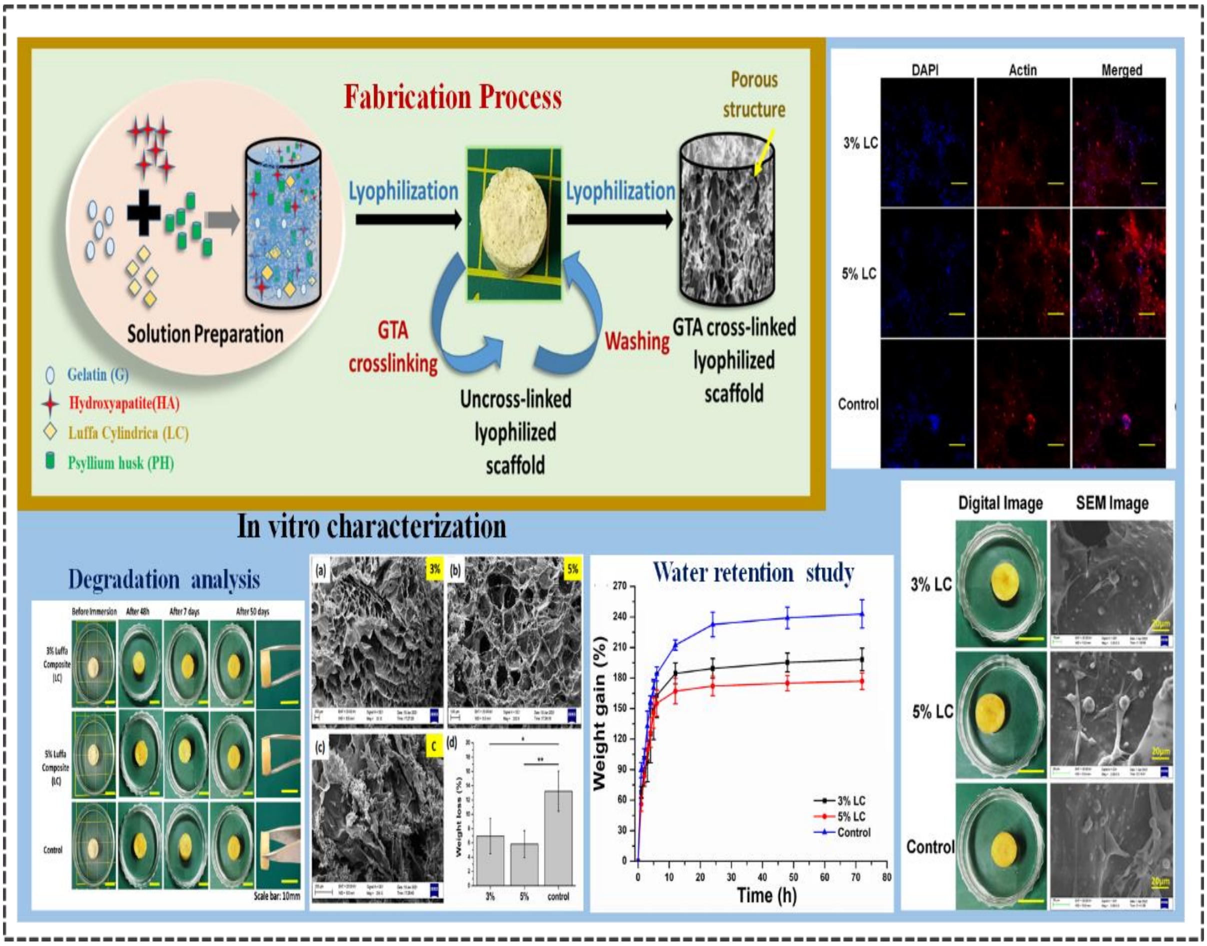


Figure 1.14: A graphical representation of chapter 2 (fabrication and invitro characterization of luffa-based composite scaffolds incorporated with gelatin, hydroxyapatite, and psyllium husk).

Chapter 3 of the thesis presents the investigation of the biocompatibility of fabricated luffa-based composite scaffolds described in Chapter 2. The primary focus of this chapter was to conduct in vivo studies by implanting the scaffolds in rat animals. Histological and biochemical analyses were performed on various organs, including the kidney, liver, and heart. The key findings of this chapter are visually represented in Figure 1.15.

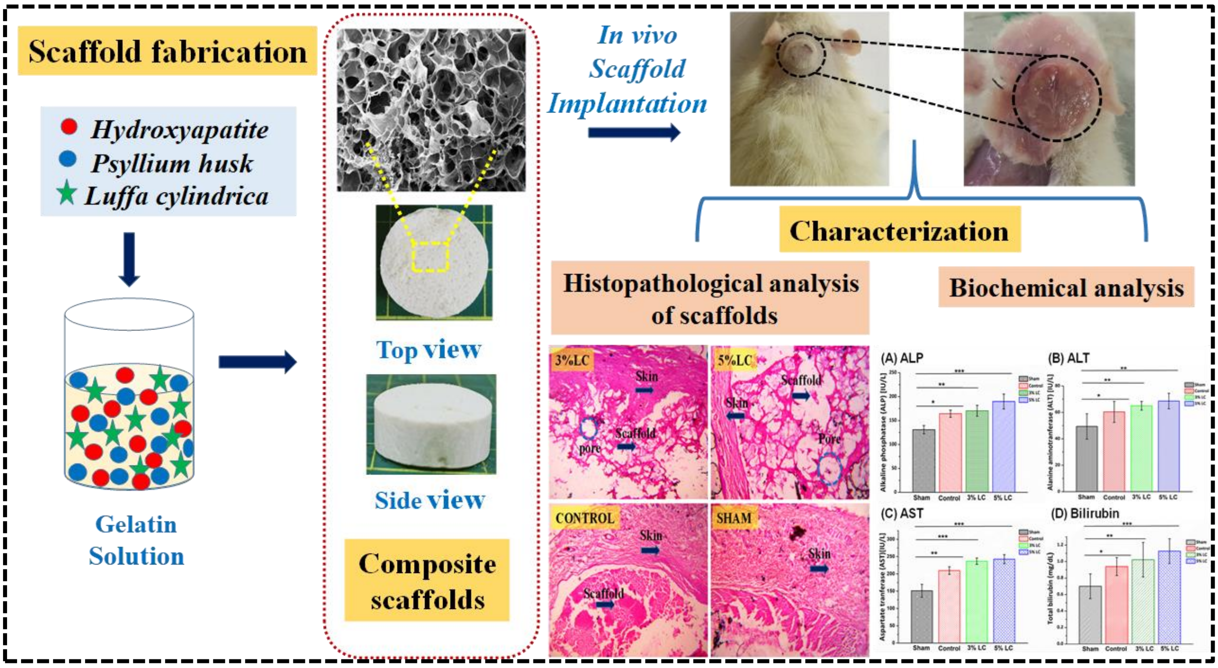


Figure 1.15: A graphical representation of chapter 3 (In Vivo Characterization of Luffa-based Composite Scaffolds upon Subcutaneous Implantation in Rats.

In **Chapter 4,** luffa-PDMS composite scaffolds were fabricated using a hand lay-up approach, incorporating various forms of luffa and PDMS. Extensive characterizations were conducted, including assessments of surface roughness, porosity, surface wettability, oil and PBS absorption, degradation, thermal stability, oil spillage and mechanical properties. The presence of luffa in the scaffolds demonstrated exceptional oleophilicity, while PDMS exhibited excellent swelling capabilities in organic solvents. The findings suggest that luffa-PDMS-based scaffolds hold great potential as a desired material for a wide range of environmental remediation applications.

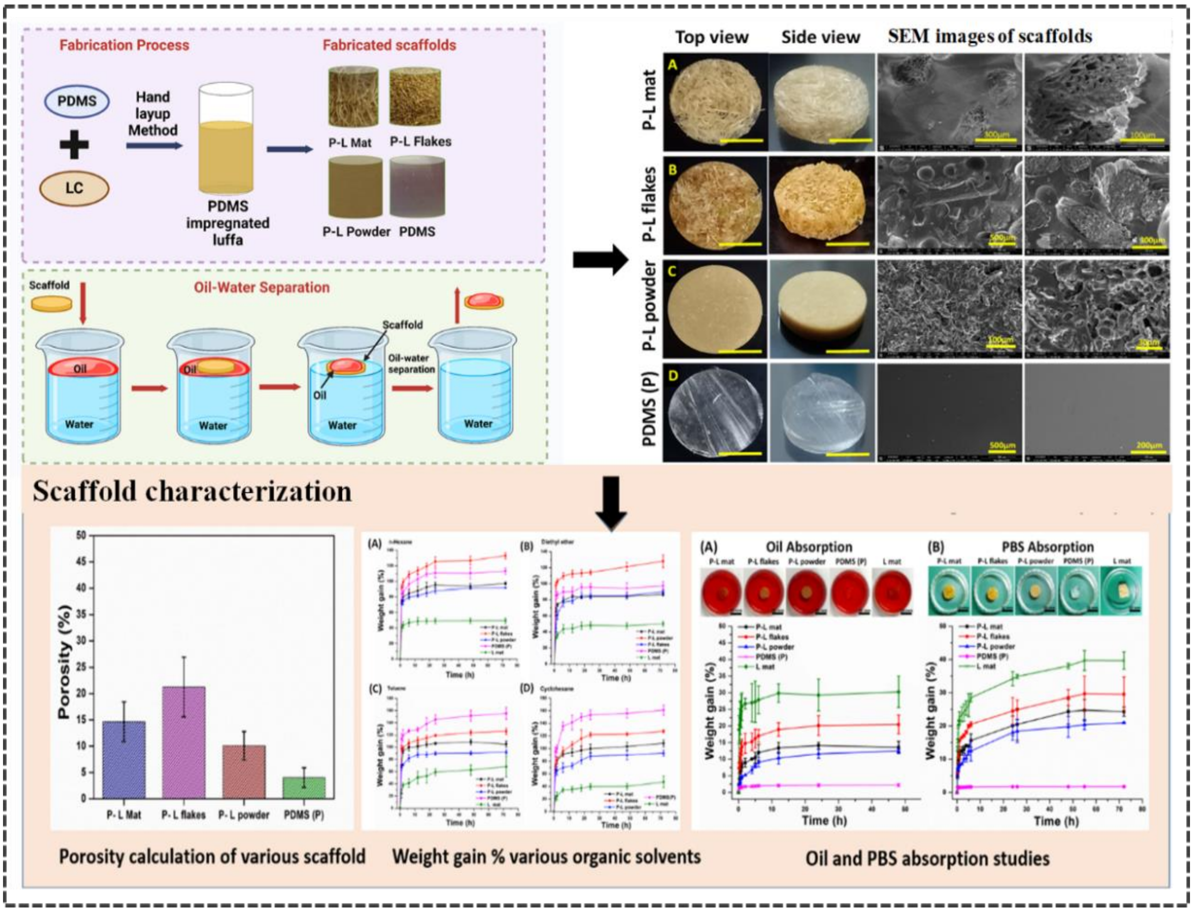


Figure 1.16: A graphical representation of chapter 4 (cellulose-based luffa cylindrica (mat, flakes, and powder) reinforced polydimethylsiloxane composites for oil and organic solvent absorption).

Chapter 5 concludes the thesis, summarizing the main findings and discussing the study's implications for the future. The investigations conducted throughout this thesis demonstrate compelling evidence that the fabricated scaffolds possess significant potential to be used in tissue engineering and oil sorption applications.

1.12 Translational potential of the thesis

The thesis aims to explore the potential applications of Luffa cylindrica (LC) and investigate the physico-chemical and mechanical characteristics of scaffolds fabricated using advanced technologies for biomedical and environmental purposes. The current research demonstrates the development and fabrication of various three-dimensional structures using the freeze-drying technique for tissue engineering applications. In another approach, we utilized hand lay-up technique to develop scaffolds by using luffa and polydmethylsiloxane (PDMS) that exhibited the potential application in managing of environmental oil spill challenges.

The use of the fabricated LC/HA/G/PH based scaffolds is primarily focused on bone tissue engineering. The composite scaffolds incorporate luffa cylindrica (LC), gelatin (G) hydroxyapatite (HA) and psyllium husk (PH). Due to the notable characteristics, including high mechanical strength, cost-effectiveness, eco-friendly, abundant availability, hydrophilicity and oleophilic properties, LC has been utilized in diverse fields such as packaging, automobiles, and water treatment (Anastopoulos and Pashalidis 2020; Psarra and Papanicolaou 2021). Considering these factors, LC emerges as an excellent choice for scaffold fabrication. Gelatin possesses the cell-adhesive RGD sequence, resembling the composition of natural extracellular matrix proteins. This similarity facilitates cell adhesion and spreading on the scaffold surface; creating a favorable environment for cell growth and tissue regeneration. The inclusion of hydroxyapatite enhances the properties of the scaffolds by improving bioactivity and supports bone regeneration by promoting osteoconductivity. The psyllium husk within the scaffolds potentially improves the mechanical strength, porosity and biocompatibility. The in vitro studies of luffa-based composite scaffolds demonstrate significant levels of cellular compatibility and proliferation of osteoblast-like cells, and a high degree of porosity which is essential for facilitating cell infiltration, nutrient exchange, and tissue ingrowth; thus promoting bone tissue regeneration and enabling suitable mechanical strength necessary for the structural support of bone tissue. LC composite holds significant translational potential due to its diverse ethnobotanical and phytochemical composition including components like oleanolic acid, carotenoids, and chlorophyll, which contributes to its medicinal properties used in traditional medicine systems. Its pharmacological actions have shown promising antioxidant and antimicrobial properties.

Furthermore, an extensive study was conducted on the utilization of different luffa-based scaffolds infused with PDMS with an objective of removing oil from water to address the immediate environmental concern of oil spills in marine ecosystems. The results indicate that P-L flakes (luffa flakes impregnated in PDMS) exhibit a significantly higher level of oil absorption capacity compared to other forms of luffa-PDMS scaffolds; enabling them to absorb a significant amount of oil and toxic solvents, most likely due to high microcapillary properties of luffa flakes. Additionally, the hydrophobic characteristics of PDMS enable buoyant forces to overcome the weight of the luffa-PDMS composite membrane, preventing it from sinking.

Furthermore, luffa material has been an extensively explored by several other researchers in biomedical and therapeutic applications. Luffa sponge has been explored as a scaffold for the culture of human hepatocyte cell lines; indicating its potential in supporting cell growth and function in a three-dimensional environment (Chen et al. 2003). The influence of chemically modified luffa on the preparation of nanofibers has been explored; emphasizing the versatility and adaptability of luffa-based materials for various biomedical applications (Mary Stella and Vijayalakshmi 2019). In the field of biomedicine, luffa has shown promising therapeutic potential due to its various biological and therapeutic activities. Research has shown that luffa acutangula possesses a wide range of beneficial properties, including hepatoprotective, antidiabetic, antiulcer, anticancer, CNS depressant, fungistatic, analgesic, antimicrobial and immunomodulatory effects. Luffa contains a wide range of bioactive compounds that give it a multitude of pharmacological activities. This makes it an important resource for drug discovery and development in the biomedical field (Shendge and Belemkar 2018). The biocompatibility and biomechanical characteristics of luffa-based scaffolds combined with hydroxyapatite and poly-L-lactic acid have also been studied; indicating that these scaffolds are promising options for cartilage tissue engineering (Cecen et al. 2016). According to the literature, various natural materials such as cotton fibers, coconut husk, treated bark and kapok fibers have been reported to clean up oil spills from water. However, their low absorption capacity, poor oil/water selectivity, sinking after sorption and limited reusability impeded their practical applicability (Sayed and Zayed 2006; Ali et al. 2011; Wang et al. 2017; Futalan et al. 2022; Lv et al.). Based on the abovementioned information, luffa-based scaffolds and products are anticipated to contribute towards bridging the gap between fundamental research and real-world medical interventions; offering promising prospects for improving healthcare outcomes through advanced biomaterial technologies. In addition to biomedical and tissue engineering applications, luffa-based scaffolds exhibits potential for various translational research applications such as environmental remediation, wastewater treatment and pollutant filtration applications. The versatility and customizable nature of these scaffolds renders them suitable for addressing diverse environmental challenges.

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