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A Comprehensive Study on Green, Sustainable Approach to Form Polyphenols Like Catechin and Polysaccharides Like Dialdehyde Cellulose Conjugate to Enhance the Physical and Chemical Properties of Both Compounds – A Review

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Abstract

In the past few years, polyphenols and polysaccharides have attracted many researchers due to their special physical propertiesand extensive and highly rich biological activities. Polyphenols are also known as secondary metabolites andare naturally found in many autotrophic products like fruits, nuts, vegetables, legumes, seeds and more, especially in green tea (Camellia sinensis). China is the largest producer and the largest consumer of tea in the world, with India being the second. Whereas, polysaccharides are complex forms of carbohydrates composed of long chains of monosaccharide units linked together by glycosidic linkages. In which Dialdehyde cellulose is an emergent molecule and can be used as a backbone for the attachment of polyphenols to it, to overcome the limitations of auto-oxidation, less shelf life, molecular instability, etc. Synthesis of polyphenols and polysaccharides has been performed to improve the physical and chemical properties of both compounds with methods like Acid Catalysed Condensation Reaction, Free Radical grafting method, Carbodiimide coupling method and Enzyme catalysed methods. The successful synthesis of DAC and the formation of conjugate can be confirmed by the help of UV-Vis spectroscopic analysis, FTIR analysis, SEM analysis for microscopic morphology,and 2,2-Diphenyl-1-PicrylHydrazyl assay used to determine the radical scavenging activity of the compounds. This study aims in the review of green, sustainable, efficient methods for the synthesis of DAC and to put forth the efficiency of the two biomolecules which can help polysaccharides like Dialdehyde cellulose as not merely a carrier but an active scaffold which can amplify and prolongs the bioefficacy of catechin, thereby expanding the practical utility of both underutilised cellulose derivatives and green tea catechins for next generation active packaging, wound dressings and functional coatings.

Keywords: *polyphenols, polysaccharides, polyphenols- polysaccharides conjugate, functions, properties.*

Introduction

Polyphenols and Polysaccharides are naturally found bioactive compounds, with each having its own specific physicochemical properties as well as biological activities. Polyphenols are classified as phenolic compounds, structurally they contain at least one phenyl ring which is bonded to one or more hydroxyl groups. [1]. Polyphenols are of four major types based on the number of phenol rings, which are flavonoids, phenolic acids, stilbenes, and lignans. Among these four, Flavonoids are widely available in the ecosystem and are mainly plant-derived. Flavonoids like Catechin and Phenolic acids like Gallic acid and Ferulic acid have shown a wide spectrum of their functional role in recent studies, such as antioxidant activity, antimicrobial activity, anti-inflammatory properties, and UV-shielding properties. [2] [3] [4].

Among Polyphenols, catechins are a prominent group of plant-derived flavan-3-ols that have attracted sustained attention because of their broad nutritional, functional, and therapeutic relevance. They occur naturally in tea leaves, cocoa, grapes, apples, berries, and several medicinal plants, yet green tea (Camellia sinensis) remains the most widely investigated source due to its particularly high catechin content and well-documented bioactivity. Catechins are of different types with small differences in their hydroxylation pattern. Gallate substitution produces marked changes in radical-scavenging capacity, stability, and biological effect. This structural sensitivity is one of the reasons catechins continue to be a useful model for studying the relationship between chemical architecture and function. In recent years, catechins have attracted many researchers for their antioxidant properties

and their extraction methods which plays crucial role in their quality. Despite their features, polyphenols have certain limitations in their free, low molecular weight form, which are poor aqueous solubility, Chemical instability, Phase II Biotransformation, Low Gastrointestinal Absorption, and rapid excretion. [5] [6] [7] [8]. Whereas, Polysaccharides are polymers of carbohydrates compromising of atleast 20 monosaccharide units linked by glycosidic bonds on their C-terminal and N-terminal. [9] [10]. There are different types of polysaccharides differing mainly in their ring size, the anomeric configuration, the position of glycosidic linkage, sequence andbranching pattern. Natural polysaccharides are divided into 3 types: microbial polysaccharides, plant polysaccharides and animal polysaccharides. Plant polysaccharides are of two further types: lower plant polysaccharides and higher plant polysaccharides. In the organism's body, polysaccharides along with proteins and polynucleotides, play a very important role in maintaining different biological activities. Polysaccharides also have various physicochemical properties like antioxidant properties, antitumor propertiesand cholesterol lowering properties. [11] [12] [13]. Just like the polyphenols, polysaccharides also have a few limitations in their free molecular form, like low aqueous solubility and chemical instability. [14]. Polysaccharides like DAC, which is anthropogenic in nature, are modifications of cellulose. Among the cellulose modification techniques are esterification, etherification, and periodate oxidation. Periodate oxidation is one of the methods for introducing reactive functional groups into the cellulose structure. This action cleaves the carbon-carbon bond to create two aldehyde groups and selectively oxidises the vicinal hydroxyl groups at the C2 and C3 positions of the anhydroglucose units. This oxidation reaction yields a substance called Dialdehyde cellulose (DAC). The synthesis and characterisation of DAC are essential for understanding the oxidation conditions, aldehyde content, and material properties. DAC material is used in biomedical applications, including drug administration, tissue engineering, and wound dressings, as well as environmental applications such as wastewater treatment and heavy-metal adsorption, owing to its enhanced reactivity and functional diversity. DAC is increasingly explored for use in biodegradable composites, paper strengthening, coatings, and sustainable packaging materials [15] [16] [17]. Through recent studies, it has been demonstrated that the limitations of both biomolecules can be overcome by combining both biomolecules, which enhances their physical, chemical, and functional properties [18]. For instance, Awika et al., (2018), demonstrated reduction in the blood plasma and liver lipid levels, as a result of the combined consumption of both Polyphenols and Polysaccharides [19]. The interaction between the polyphenol and the polysaccharides can be of two types: either covalent bonding or non-covalent bonding, among which covalent bonding is more stable as compared to non-covalent bonding. [20].

Chemical-coupling, enzyme-mediated method, free radical grafting method, and acid-catalysed condensation reaction are a few different methods through which interaction between the two compounds can be achieved. In recent studies, many methods have been demonstrated, among which Laccase is the most widely used for grafting of polyphenols and polysaccharides. [21]. Another is Acid Catalysed Condensation reaction demonstrated by [22] in their study for the first time utilised an acid-catalysed condensation reaction for forming a starch aldehyde– Quercetin conjugate. [22]. By using the acid-catalysed condensation reaction to prepare polyphenols, this paper summarises the synthesis, structural analysis, bioactivities, and further potential applications of polyphenols-polysaccharides conjugates for future use.

1. Catechin, a polyphenol compound

1.1 Introduction to catechin

Catechins belong to the flavonoid family and are commonly discussed as flavan-3-ols. The principal molecules occurring in tea and other plant materials are catechin, epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), and epigallocatechingallate (EGCG). Although they share a common core structure, their biological behaviour differs substantially because the number and position of hydroxyl groups, the stereochemistry of the C ring, and the presence or absence of a gallate ester alter their reactivity. In practice, this means that catechins should not be treated as a single uniform entity but rather as a family of closely related compounds with distinct analytical and biological profiles. Green tea remains the richest and best-characterized source of catechins. Early phytochemical studies and later chromatographic.

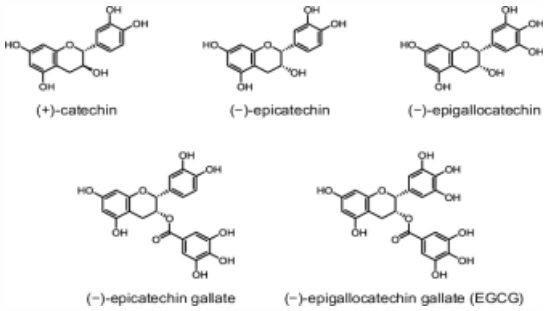

Figure 1: Chemical structures of major catechins (catechin, EC, EGC, ECG, and EGCG)

investigations established that catechins form the dominant polyphenolic fraction of the beverage and contribute strongly to its sensory and health-related qualities. [23]Among the individual derivatives, EGCG is typically the most abundant and also the most active in many antioxidant assays, while EC and catechin are generally less potent. This pattern is not accidental: it reflects the influence of hydroxyl density and gallate esterification on electron donation, metal chelation, and radical stabilization.

1.2. Extraction of Catechins: Conventional and Advanced Approaches

A practical review of catechin research has to begin with extraction, because extraction determines not

only yield but also the quality of the bioactive fraction. Conventional techniques such as

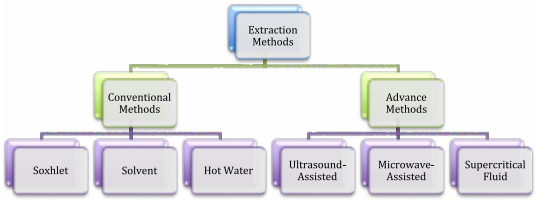

Figure 2: Classification of Extraction Methods Used for Catechin Recovery from Green Tea

Hot water extraction, solvent extraction, and ethanol-based extraction remain widely used because they are simple, low-cost, and accessible. At the same time, recent literature increasingly compares these older approaches with greener or faster methods such as ultrasound-assisted extraction, microwave-assisted extraction, and supercritical-fluid-based processing. Cioanca et al. clearly framed this transition by discussing the strengths and weaknesses of each method, with special attention to the trade-off between efficiency and operational simplicity. The early literature on catechin extraction relied mainly on solvent-based procedures. [23], showed that catechins constitute approximately 30–42% of the dry weight of green tea solids and demonstrated that extraction efficiency is strongly dependent on solvent polarity and processing conditions. This work laid the foundation for subsequent studies aimed at optimizing catechin recovery from tea matrices. [23] [24]. Later, Balentine, (1997), examined the chemistry of tea flavonoids and reported that aqueous mixtures of methanol, ethanol, and acetone extracted catechins more effectively than pure solvents. The authors attributed this improvement to enhanced solubilization of polar polyphenolic compounds, emphasizing the critical role of solvent composition during extraction. Further optimization studies conducted by Khokhar and Magnusdottir (2002) confirmed that temperature and extraction time have a direct effect on catechin yield, but also demonstrated that prolonged or overly harsh treatment can reduce the stability of sensitive fractions [25] Among conventional methods, Soxhlet extraction remains attractive because of its simplicity, low equipment demand, and suitability for laboratory-scale work. The main limitation is efficiency: mass transfer is slow, large solvent volumes are often required, and the process can be time-consuming. Even so, when the aim is to obtain a preliminary extract or to work with heat-sensitive material, these techniques still offer a practical starting point. Hot water extraction represents a more food-friendly variation of this idea and is especially relevant for tea beverages and nutraceutical preparations. [26] and Chan et al. (2009) both showed that aqueous extraction can recover considerable amounts of catechins while preserving useful antioxidant activity, provided the heating conditions are kept within a suitable range [26]. Soxhlet extraction has traditionally been used when higher extraction efficiency is needed. By continuously recycling hot solvent through the sample, it improves contact between solvent and plant tissue and often produces a more exhaustive recovery than simple maceration. The trade-off is thermal exposure. Because catechins, particularly EGCG and EGC, are sensitive to oxidation and heat, excessive Soxhlet duration may reduce the quality of the extract even when total recovery appears high. For that reason, Soxhlet is best viewed as a robust laboratory method rather than the most delicate one [27]. A useful way to compare the conventional methods is to think in terms of balance. Solvent is gentle but slow; hot water extraction is practical and food-compatible; Soxhlet is efficient but energetically heavier; and all of them depend strongly on solvent composition and operating time. [28] and [29] reinforced this point by showing that the plant matrix, pH, solid-to-liquid ratio, and extraction stage can all alter the amount of catechin ultimately recovered. Nikhili, (2009), demonstrated that interactions between catechins and cellular matrices significantly affect compound release during extraction. Their work suggested that extraction performance is governed not only by solvent properties but also by structural barriers within plant tissues. This finding contributed to the development of improved extraction strategies aimed at enhancing mass transfer and catechin recovery. A comprehensive optimization study was subsequently performed by, Vuong, (2011), who examined six operational variables influencing aqueous extraction. The authors reported that extraction at 80°C for 30 minutes under acidic conditions produced maximal catechin recovery. [29]. Furthermore, they demonstrated that extraction efficiency could be improved while simultaneously reducing water consumption through multi-stage extraction procedures. Their findings remain among the most frequently cited guidelines for green tea catechin extraction. In other words, extraction is not only a matter of choosing a solvent, but also of controlling the entire interaction between solvent, matrix, and temperature. The move toward advanced techniques emerged from the desire to increase yield while reducing solvent use and processing time. Ultrasound-assisted extraction (UAE) [30]uses acoustic cavitation to disrupt cell walls and accelerate mass transfer. Microwave-assisted extraction (MAE) [31], relies on rapid dielectric heating, which raises internal pressure within plant cells and encourages compound release. Supercritical fluid extraction (SFE) , generally using carbon dioxide with a polar modifier, offers a cleaner and more selective route, although it requires higher capital investment. Xu, (2017), compared several of these approaches and concluded that advanced systems often improve efficiency, but only when operating conditions are optimized for the target compound and matrix. [33]. Recent investigations have demonstrated that UAE can significantly enhance catechin recovery while reducing extraction time and solvent consumption. Rashidinenad, (2024), compared hot water extraction, ethanol extraction, and ultrasound-assisted extraction for the recovery of catechins from green tea waste. Using HPLC analysis, the authors observed that epicatechin was the dominant catechin fraction recovered across all extraction methods.

Their findings indicated that ultrasound treatment accelerated extraction kinetics and improved antioxidant recovery while maintaining catechin integrity. However, hot water extraction remained attractive because of its simplicity, low cost, and environmental compatibility. The growing popularity of UAE can be attributed to its ability to improve extraction efficiency without exposing catechins to prolonged thermal stress. Consequently, ultrasound-assisted extraction is increasingly regarded as a promising green technology for industrial catechin production. Because these methods differ in mechanism, they should not be described as simple replacements for one another. UAE is attractive when shorter extraction time and moderate equipment cost are important. MAE is useful when rapid heating can be controlled carefully to avoid degradation. SFE is appealing where solvent minimization and cleaner extract profiles are priorities. Their value becomes most evident when the final application requires a well-defined catechin profile rather than just a crude polyphenolic fraction.

Table 1: Comparative Analysis of Conventional and Advanced Catechin Extraction Methods.

Extraction Method	Principle	Solvent System	Extraction Time	Advantages	Limitations	Key Findings
Ultrasound-Assisted Extraction (UAE)	Acoustic cavitation disrupts plant cell walls and enhances solvent penetration.	Water, ethanol, hydroalcoholic mixtures	5–60 min	Reduced extraction time; lower solvent consumption; high extraction yield	Reduced extraction efficiency with highly viscous solvents	UAE significantly enhanced extraction efficiency while preserving antioxidant activity.
Microwave-Assisted Extraction (MAE)	Rapid microwave heating causes cell rupture and accelerates diffusion of bioactive compounds.	Water, ethanol, and other polar solvents	1–30 min	Rapid extraction; reduced solvent requirement; high efficiency	Risk of thermal degradation if extraction conditions are not optimized	Higher extraction efficiency with minimal solvent consumption and improved recovery of heat-stable phytochemicals.
Supercritical Fluid Extraction (SFE)	Supercritical CO_2 penetrates plant tissues under high pressure, selectively extracting target compounds.	Supercritical CO_2 with ethanol as a co-solvent	30–180 min	Solvent-free extracts; environmentally friendly; high product purity	High initial equipment and operational costs	Produced high-purity bioactive compounds with minimal solvent residues and excellent extract quality.

1.3 Analytical Characterization of Catechins

Once catechins have been extracted, they must be identified and quantified with reliable analytical tools. This is necessary not only for chemical confirmation, but also for comparing yield across methods and for understanding how processing influences composition. Because the major catechins are structurally similar, simple visual or colorimetric approaches are not sufficient when precise differentiation is required.

As a result, chromatographic and spectroscopic techniques have become essential in catechin research. High-performance liquid chromatography (HPLC) remains the benchmark method for routine catechin analysis. Dallugre, (2000), demonstrated that reversed-phase HPLC can separate the principal tea catechins with high reproducibility and good sensitivity. Subsequent work using HPLC-diode array detection (HPLC-DAD) further strengthened this approach because it allows both retention-time comparison and spectral verification [35]. Jakabova, (2024), showed that validated HPLC-DAD methods are especially useful for the simultaneous determination of several catechin derivatives in a single run, making them suitable for quality control and comparative studies [36]. When structural confirmation is needed, LC-MS adds a much higher level of selectivity. Zeeb, (2000), used LC–APCI–MS to identify multiple catechin-related compounds and demonstrated that mass-based detection is particularly valuable when chromatographic separation alone cannot fully resolve closely related molecules. In practical terms, HPLC provides the quantitative backbone, while LC-MS provides confidence in identity. Together, they represent the most powerful analytical combination for complex extracts [37]. Spectroscopic methods remain useful because they are faster and require less sample preparation. UV-visible spectroscopy provides a quick first indication of phenolic content, while FTIR identifies characteristic functional groups such as hydroxyl and aromatic moieties. Robb et al., (2002), showed that FTIR can complement chromatography by confirming structural features in catechin-rich samples [38]. At the same time, non-destructive approaches are becoming more attractive. Giorgini et al., used ATR-FTIR combined with PCA to distinguish tea samples and infusion profiles. The method does not replace HPLC for exact quantification, but it is highly useful for rapid fingerprinting and sample differentiation [39].

1.4. Biological Relevance and Applications

The significance of catechins extends far beyond their extraction and characterization. Their diverse biological activities have attracted considerable attention in the fields of nutrition, medicine, and functional food development. Although catechins are widely recognized for their antioxidant properties, their biological effects involve much more than direct free radical scavenging. These flavan-3-ols modulate cellular signalling pathways, regulate inflammatory responses, influence mitochondrial function, and contribute to the maintenance of metabolic homeostasis. Consequently, catechins have emerged as promising candidates for applications ranging from disease prevention and therapy to food preservation and performance enhancement.

1.4.1 Role of Catechins in Neurodegenerative Disorders

Neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD), are characterized by progressive neuronal loss driven by oxidative stress, mitochondrial dysfunction, protein aggregation, and chronic neuroinflammation. As current treatments primarily alleviate symptoms rather than prevent disease progression, naturally occurring neuroprotective compounds have gained significant interest. Catechins, particularly epigallocatechingallate (EGCG), exert neuroprotective effects through multiple mechanisms.

They scavenge reactive oxygen species (ROS), enhance antioxidant defenses, inhibit β-amyloid and α-synuclein aggregation, preserve mitochondrial function, and suppress inflammatory signaling pathways. Joshi, (2021), highlighted the ability of catechins to reduce oxidative damage and protein aggregation associated with Alzheimer's and Parkinson's disease. [40]. Andrade et al., further demonstrated that N-acetyl cysteine (NAC)-catechin hybrid compounds may provide enhanced neuroprotection by simultaneously reducing oxidative stress, inhibiting β-amyloid aggregation, and improving neuronal antioxidant capacity for the treatment of Alzheimer's disease Similarly, Sebastiani, (2021), reported that catechins improve mitochondrial homeostasis and neuronal survival in several neurological disorder. [41]. Collectively, these findings support the potential of catechins as multi-target neuroprotective agents.

1.4.2 Role of Catechins in Cancer Prevention and Therapy

Cancer is characterized by uncontrolled cellular proliferation, resistance to apoptosis, angiogenesis, and metastatic progression. Limitations associated with conventional therapies have encouraged the exploration of naturally occurring compounds capable of targeting multiple oncogenic pathways. Catechins, particularly EGCG, exhibit significant anticancer activity by modulating pathways involved in cell proliferation, apoptosis, angiogenesis, and inflammation. Farhan (2022) reported that catechins regulate PI3K/Akt, MAPK, and NF-κB signaling pathways, thereby suppressing tumor growth and progression. [32]. Amatroodi, (2020), demonstrated that EGCG induces cell-cycle arrest, promotes apoptosis, and inhibits VEGF-mediated angiogenesis. [42]. Studies focusing on breast cancer further revealed that catechin derivatives suppress tumor proliferation, invasion, and metastasis while enhancing the efficacy of conventional therapies. [43]. More recently, TsouhFokou et al. (2025) highlighted advances in catechin nano formulations and delivery systems designed to improve bioavailability and clinical effectiveness. [44]. These findings emphasize the potential of catechins as promising adjuncts in cancer prevention and treatment.

1.4.3 Role of Catechins in Cardiovascular and Metabolic Disorders

Cardiovascular diseases and metabolic disorders remain major global health concerns. Oxidative stress, chronic inflammation, endothelial dysfunction, and impaired lipid metabolism contribute significantly to the development of atherosclerosis, hypertension, dyslipidemia, and diabetes. Catechins exhibit cardioprotective and metabolic benefits through antioxidant, anti-inflammatory, and lipid-regulating activities. Bharadwaj and Khanna reported that green tea catechins improve cardiovascular health by reducing oxidative stress and lipid abnormalities. [45]. Sheng et al. (2023), further demonstrated that catechins attenuate atherosclerotic progression by activating endogenous antioxidant systems and suppressing vascular inflammation. [47] showed that catechins interact directly with cholesterol molecules, supporting their role in cholesterol regulation using DFT studies. [47] highlighted the ability of catechins to improve insulin sensitivity, regulate glucose metabolism, and reduce diabetes-associated complications. These combined effects underscore the therapeutic potential of catechins in cardiovascular and metabolic health [48].

1.4.4 Role of Catechins in Mitochondrial Protection

Mitochondrial dysfunction contributes to numerous chronic diseases by impairing ATP production and increasing oxidative stress. Consequently, strategies aimed at preserving mitochondrial integrity have become increasingly important. Catechins provide mitochondrial protection through their antioxidant and regulatory activities. They reduce ROS generation, maintain mitochondrial DNA integrity, regulate calcium homeostasis, and promote mitochondrial biogenesis. Chen, (2022), reported that catechins improve oxidative phosphorylation, enhance ATP synthesis, and reduce apoptosis associated with mitochondrial dysfunction. These findings suggest that catechins may play an important role in maintaining cellular energy metabolism and preventing mitochondrial damage in chronic diseases [49].

1.4.5 Role of Catechins in the Regulation of Systemic Inflammation

Chronic systemic inflammation is a common pathological feature of cardiovascular diseases, diabetes, obesity, cancer, and neurodegenerative disorders. Persistent inflammatory responses contribute to tissue injury and disease progression. Catechins possess potent anti-inflammatory properties through their ability to modulate multiple signalling pathways. They suppress NF-κB and MAPK activation while enhancing antioxidant defences via Nrf2 signalling. [50]. demonstrated that catechins effectively reduce inflammatory cytokine production and oxidative stress, thereby limiting chronic inflammatory damage. Their dual antioxidant and anti-inflammatory actions highlight the potential of catechins as therapeutic agents for inflammation-associated disorders.

1.4.6 Role of Catechins in the Treatment of Bacterial Infections

The emergence of antibiotic-resistant bacteria has intensified the search for alternative antimicrobial agents. Catechins have attracted attention because of their broad-spectrum antibacterial activity. These compounds exert antimicrobial effects by disrupting bacterial cell membranes, inhibiting biofilm formation, suppressing virulence factors, and enhancing antibiotic susceptibility. [51] reported that EGCG exhibits significant antibacterial activity against several clinically relevant pathogens and may act synergistically with conventional antibiotics. Such findings suggest that catechins could serve as valuable adjunctive agents in the management of bacterial infections.

1.4.7 Role of Catechins in Enhancing Skeletal Muscle Performance

Age-related muscle loss, exercise-induced fatigue, and skeletal muscle disorders negatively affect physical performance and quality of life. Oxidative stress and impaired mitochondrial function are important contributors to muscle deterioration. Catechins improve skeletal muscle performance by enhancing mitochondrial efficiency, promoting fatty acid oxidation, and reducing oxidative damage. [52] reported that catechins improve exercise capacity, preserve muscle mass, and attenuate muscle atrophy. These findings indicate that catechins may be useful nutritional interventions for maintaining muscle health and supporting physical performance.

1.4.8 Role of Catechins in the Food Industry

Growing consumer preference for natural food additives has increased interest in plant-derived antioxidants. Food spoilage, lipid oxidation, and quality deterioration remain major challenges in food processing and storage. Catechins are widely used as natural antioxidants and preservatives due to their ability to inhibit lipid oxidation and improve product stability. [53] reviewed the isolation and application of green tea catechins in food systems and concluded that they effectively enhance shelf life, preserve sensory quality, and increase the functional value of food products. These characteristics make catechins attractive ingredients for the development of functional and health-promoting foods.

Table 2: Summary of studies on the biological applications of catechins.

Journal	Application
Journal of the American College of Nutrition	Neurodegenerative disorders
Journal of Alzheimer's Disease	Alzheimer's disease and multi-target neuroprotection
Nutrients	Neurological and neurodegenerative disorders
International Journal of Molecular Sciences	Cancer prevention and therapy
Molecules	EGCG-based anticancer mechanisms
Food and Health	Breast cancer
Food Science and Nutrition	Clinical cancer applications
Chinese Journal of Natural Medicines	Cardiovascular disorders
Frontiers in Pharmacology	Atherosclerosis
Open Chemistry	Cholesterol regulation
Nutrients	Diabetes and metabolic disorders
International Journal of Molecular Sciences	Mitochondrial protection
Food Science and Biotechnology	Systemic inflammation
Pathogens	Bacterial infections
Critical Reviews in Food Science and Nutrition	Skeletal muscle performance
Food Reviews International	Food industry applications

2. Dialdehyde cellulose (DAC), a modified polysaccharide compound

2.1 Cellulose

Plant cell wall are primarily composed of cellulose which is a naturally occurring high-molecular-weight polysaccharide (polymer). It is the most prevalent organic polymer on Earth, also cellulose is essential for providing plants with mechanical strength and rigidity. Cellulose is composed of β-D-glucose repeating units connected by β(1→4) glycosidic links. It forms long, linear chains. The molecular formula of cellulose is $(C_6H_{10}O_5)_n$, where "n" shows the number of glucose units, which can range from several hundred to several thousand [54]. The structure of cellulose is linear, unbranchedand its each glucose unit is rotated 180° relative to its neighbouring unit, allowing the chains to align closely. This arrangement helps to form hydrogen bonds; within the same chain, molecules aggregate into tightly packed bundles called microfibrils of high tensile strength and structural stability [55]. Cellulose contains three hydroxyl (–OH) groups per glucose unit, located at the C2, C3, and C6 positions. These functional groups are responsible for hydrogen bonding and acts as a reactive site for chemical modification. As a carbohydrate, cellulose is insoluble in water due to its strong intermolecular interactions or Vanderwals forces and high crystallinity. It consists crystalline regions, which are highly ordered and rigid, and amorphous regions, which are less ordered and most chemically reactive [56]. Cellulose is particularly found in plant materials such as wood, cotton, and agricultural fibers. Cotton is almost pure cellulose, whereas wood contains about 40–50% cellulose along with other components like lignin and hemicellulose. Cellulose is considered as an environment friendly material because of its abundance and biodegradability [54]. Acetobacter, Gluconobacter, Komagataeibacter, Rhizobium, Agrobacterium, and Sarcina are among the genera of bacteria that can synthesise cellulose in addition to plant-derived cellulose from wood, cotton, and bamboo; this is known as bacterial cellulose (BC) or microbial cellulose (MC) [57]. It has various industrial applications. It is used in paper and pulp industry, textile manufacturing (cotton and rayon), the food industry as dietary fiber, and in pharmaceuticals as a binder and stabilizer. Native cellulose has certain limitations, such as low solubility and limited chemical reactivity, which restrict its use in advanced applications [54]. To overcome these limitations, cellulose can be chemically modified to produce derivatives such as cellulose acetate, carboxy methyl cellulose, and dialdehyde cellulose. The modifications improve its solubility, reactivity, and functional properties, making it suitable for applications in biomedical engineering, packaging, and environmental technologies [58]. Cellulose is a structurally important, renewable, and versatile biopolymer that has a unique chemical structure and properties, making it essential in both natural systems and industrial applications [54] [59].

2.2 Structure and properties

Cellulose is a colorless, odorless, and tasteless natural polymer with strong affinity for water, making it hydrophilic. It typically exhibits a contact angle in the range of 20–30°, indicating its ability to interact with moisture. It is also a chiral compound and is biodegradable in nature. Under extreme conditions, such as high temperature, cellulose can undergo thermal transitions, and studies have shown that it melts at around 467 °C under controlled experimental conditions. Cellulose can be chemically hydrolyzed into glucose units when treated with concentrated acids at elevated temperatures [60] [61]. β(1→4)-glycosidic linkages bind repeated units of D-glucose together to form cellulose's structural components. α(1→4)-glycosidic linkages are used to join starch and glycogen. Cellulose does not create a coiled or branched structure in the β-linkage, but rather a straight, unbranched chain.

The molecule has a stiff, rod-like structure due to the glucose units' stretched conformation. Extensive hydrogen bonds are formed between adjacent chains as well as within the same chain by the many hydroxyl groups on the glucose units. The chains are held strongly together by hydrogen bonds, forming highly structured structures called microfibrils that give cellulose its remarkable tensile strength [62] [63]. These cellulose microfibrils are embedded in a matrix of lignin and other polysaccharides within plant cell walls. Plant tissues benefit from this arrangement's mechanical strength and structural stability. Cellulose fibres in wood have a similar role to steel bars in reinforced concrete, while lignin serves as a binding agent to keep the fibres together. Modern imaging methods like fluorescence microscopy have been employed to explore these processes in living cells since the mechanical behaviour of cellulose in plant cell walls is directly linked to cell growth and expansion [64] [65]. Among natural sources, cotton fibers consist of one of the purest forms of cellulose. It contains more than 90% of this polymer. Compared to starch, cellulose exhibits a much higher degree of crystallinity. Starch can easily transition from crystalline to amorphous form upon heating in water; cellulose requires significantly higher temperature and pressure to undergo such transformation, reflecting its strong structural organization [62] [66]. There are various crystalline forms of cellulose, such as cellulose I, II, III, and IV. Depending on its biological source, native cellulose (cellulose I) can be either Iα or Iβ. Higher plants primarily contain the Iβ form, while bacteria and algae create cellulose that is rich in the Iα form. Cellulose II, which is more stable than cellulose I, is a type of regenerated cellulose that is present in processed fibres. The irreversible transformation of cellulose I into cellulose II shows variations in their thermodynamic stability. Certain chemical treatments can produce further forms, such as cellulose III and IV [67] [68]. In nature, cellulose is rarely found in a pure form and is usually associated with other components such as hemicellulose, lignin, and pectin. However, bacterial cellulose is relatively pure and exhibits higher water retention and mechanical strength due to its longer polymer chains [69] [70]. Cellulose is made up of fibrils with both crystalline and amorphous parts at the microscopic level. These fibrils can be broken down into smaller structures like cellulose nanofibrils and nanocrystals via mechanical, chemical, or enzymatic treatments. The special qualities of these nanocellulose materials, including their large surface area, superior mechanical strength, and capacity to create cutting-edge materials like hydrogels, aerogels, and nano composites, have drawn a lot of attention. They are useful in contemporary material science and nanotechnology applications because they stabilise emulsions and create liquid crystalline phases [17] [71].

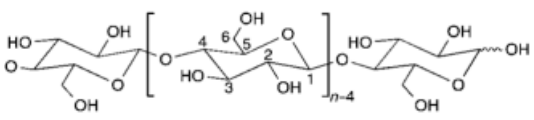

Figure 3: Chemical Structure of Cellulose [60]

2.3 Limitations of Native Cellulose

Native cellulose has a number of intrinsic drawbacks that limit its direct implementation in cutting-edge applications, despite its abundance, biodegradability, renewability, and superior mechanical qualities. Its highly organised molecular structure, numerous intermolecular interactions, and small number of functional groups available for chemical reactions are the main causes of these restrictions. As a result, chemical modification is frequently necessary to improve its performance and increase its industrial usability [72] [59]. The high crystallinity of cellulose is one of its main drawbacks. Extensive intra- and intermolecular hydrogen bonding as well as other non-covalent interactions stabilise the highly structured crystalline regions that make up cellulose chains. Although its densely packed structure offers mechanical strength, it also makes chemical reagents and solvents less accessible. Because of this, many reactions only take place at the cellulose fibre surface, which reduces the effectiveness of chemical modification procedures [60] [63] [62]. Its poor solubility in water and a large proportion of commonly used organic solvents is another major limitation. Due to its strong intermolecular connections, high crystallinity, and dense hydrogen-bonding network, cellulose is remarkably resistant to degradation. In accordance with studies, only a few specific solvent systems, including ionic liquids, N-methylmorpholine-N-oxide (NMMO), and some alkaline solvent systems, can dissolve cellulose. Only cellulose with comparatively low crystallinity and degree of polymerisation is dispersed with even conventional aqueous NaOH systems, and the solubility achieved is usually limited [73] [74] [75].

Low chemical reactivity is another characteristic of native cellulose. Despite having three hydroxyl groups at the C2, C3, and C6 locations in each anhydroglucose unit, many of these groups are involved in hydrogen bonding and are therefore not easily accessible for chemical reactions. Because of this limited accessibility, derivatisation reactions are less effective and may require pretreatment or dissolution before modification [60] [62] [76]. Cellulose cannot be melted and reshaped like other synthetic polymers, given that it does not exhibit thermoplastic properties. Cellulose resists thermal degradation rather than melting when heated, which restricts its ability to be processed using standard polymer-processing techniques as extrusion and injection moulding. [77] [78]. It also has limited usefulness. Other reactive functional groups needed for specific applications, including drug delivery, biomedical engineering, adsorption, and smart materials, are absent from native cellulose, which primarily consists of hydroxyl groups. As a result, it is frequently required to add new functional groups through chemical modification in order to improve performance and impart desirable qualities [76] [68]. Numerous studies on cellulose modification have been spurred by these inherent constraints. Because it adds reactive aldehyde groups, decreases crystallinity, improves accessibility, and greatly boosts the chemical reactivity of cellulose for advanced applications, periodate oxidation is one of the most significant methods for producing

dialdehyde cellulose (DAC) [79] [80] [81].

2.4 chemical modification of cellulose

Cellulose, despite being an abundant, renewable, and biodegradable polymer, exhibits certain inherent limitations such as poor solubility in common solvents, lack of thermoplasticity, and relatively low chemical reactivity due to its highly ordered crystalline structure and extensive hydrogen bonding network. These limitations significantly restrict its direct utilization in advanced material applications. Consequently, chemical modification of cellulose has emerged as an essential strategy to tailor its physicochemical properties and expand its industrial applicability. The presence of three reactive hydroxyl (–OH) groups per the anhydroglucose unit, localised at the C2, C3, and C6 positions, is the vital basis for cellulose modification. New functional groups can be added to the cellulose backbone by using these hydroxyl groups as active sites for different chemical synthesis processes. Properties like solubility, hydrophilicity, mechanical strength, thermal stability, and chemical reactivity can all be changed by controlled modification, creating a variety of cellulose derivatives with distinct purposes. Among the most extensively studied modification pathways are esterification, etherification, oxidation, and graft polymerization. Esterification involves the reaction of cellulose hydroxyl groups with organic or inorganic acids, resulting in derivatives such as cellulose acetate, which exhibit improved solubility and thermoplastic behavior. Etherification reactions, on the other hand, introduce alkyl or hydroxyalkyl groups into the cellulose structure, producing derivatives such as carboxymethyl cellulose (CMC), methyl cellulose, and hydroxyethyl cellulose. These derivatives are widely used due to their water solubility and functional properties, including thickening, stabilizing, and emulsifying capabilities [82]. In addition to substitution reactions, graft polymerization represents a significant approach for modifying cellulose. In this method, synthetic polymer chains are covalently attached onto the cellulose backbone, resulting in hybrid materials that combine the properties of natural and synthetic polymers. Advanced grafting techniques have enabled better control over molecular architecture, leading to enhanced physical and chemical properties, such as improved compatibility, mechanical strength, and responsiveness to environmental *stimuli*.

Oxidation is another important modification route, particularly relevant to the formation of dialdehyde cellulose (DAC), which is central to the present study. In periodate oxidation, the vicinal hydroxyl groups at the C2 and C3 positions of cellulose are selectively cleaved, resulting in the formation of two aldehyde groups per oxidized unit. This reaction not only disrupts the crystalline structure of cellulose but also introduces highly reactive aldehyde functionalities, significantly increasing its chemical reactivity. The resulting DAC exhibits enhanced swelling properties, increased accessibility, and the ability to form cross-linked networks through reactions such as Schiff base formation.

These characteristics make DAC particularly suitable for applications in biomedical materials, wastewater treatment, and functional polymers. Recent research has emphasized that chemical modification of cellulose does not merely improve existing properties but fundamentally transforms cellulose into a versatile platform for advanced material design. Modified celluloses can be engineered for specific applications, including hydrogels, nanocomposites, drug delivery systems, and environmentally responsive materials. Furthermore, modern approaches focus on sustainable and green chemistry routes, aiming to minimize the use of hazardous reagents while maximizing efficiency and functionality [83]. Thus, chemical modification serves as a critical bridge between native cellulose and high-performance functional materials. By introducing specific functional groups and altering the structural organization of cellulose, it becomes possible to overcome its intrinsic limitations and unlock its full potential. In this context, dialdehyde cellulose represents a key modified derivative, where controlled oxidation enhances reactivity and enables diverse applications.

2.5Structural Changes in Cellulose After Periodate Oxidation

Periodate oxidation induces significant structural changes in cellulose by selectively oxidizing the vicinal hydroxyl groups at the C2 and C3 positions of the anhydroglucose unit (AGU). These changes alter the molecular structure, crystallinity, chemical reactivity, and physical properties of cellulose, resulting in the formation of dialdehyde cellulose (DAC). One of the most important structural modifications is the cleavage of the C2–C3 carbon–carbon bond within the glucose ring. During oxidation, sodium periodate attacks the vicinal hydroxyl groups at these positions, causing the opening of the pyranose ring structure and generating two aldehyde groups per oxidized anhydroglucose unit. This transformation changes the original cyclic glucose structure into a more flexible and reactive open-chain form [79]. Another major consequence of oxidation is the reduction in cellulose crystallinity. Native cellulose possesses highly ordered crystalline regions stabilized by extensive intra- and intermolecular hydrogen bonding. Periodate oxidation disrupts this ordered arrangement by breaking the C2–C3 bond and altering the molecular geometry of the cellulose chains. As the degree of oxidation increases, the crystalline regions are progressively converted into amorphous regions, resulting in decreased crystallinity and increased accessibility of the cellulose structure [79]; [84]. Oxidation also causes changes in the hydrogen-bonding network of cellulose. The conversion of hydroxyl groups into aldehyde groups reduces the number of hydroxyl functionalities available for hydrogen bonding. Consequently, the strong intermolecular interactions responsible for the rigidity and compact structure of native cellulose are weakened. This structural loosening contributes to enhanced swelling behavior and improved accessibility of chemical reagents to the cellulose matrix [85].

Furthermore, the introduction of aldehyde groups significantly increases the chemical reactivity of cellulose. These aldehyde functionalities can participate in Schiff-base reactions, cross-linking processes, and conjugation with various molecules, making DAC a versatile intermediate for the preparation of functional materials. The aldehyde groups may also exist in hydrated, hemiacetal, or hemialdal forms, further influencing the physicochemical behavior of DAC [80]. The structural changes associated with periodate oxidation also affect the physical properties of cellulose, including its porosity, water absorption capacity, flexibility, and surface characteristics. These modifications enhance the suitability of DAC for applications in hydrogels, biomedical scaffolds, drug delivery systems, and environmental remediation materials.

2.6 Properties of Dialdehyde Cellulose (DAC)

Dialdehyde cellulose (DAC) possesses unique physicochemical and chemical properties due to the introduction of aldehyde groups into the cellulose structure through periodate oxidation. The conversion of hydroxyl groups at the C2 and C3 positions into aldehyde functionalities significantly alters the structure, reactivity, and performance of cellulose.

1. Presence of Reactive Aldehyde Groups

The most important characteristic of DAC is the presence of two aldehyde groups per oxidized anhydro glucose unit. These aldehyde groups act as highly reactive sites that can participate in Schiff-base reactions, cross-linking, grafting, and conjugation with biomolecules. This enhanced reactivity makes DAC a valuable intermediate for the synthesis of advanced functional materials [79].

2. Reduced Crystallinity

Periodate oxidation disrupts the ordered arrangement of cellulose chains by cleaving the C2–C3 bond and opening the glucose ring. As a result, the crystallinity of cellulose decreases and the proportion of amorphous regions increases. The reduction in crystallinity improves the accessibility of reagents and enhances the reactivity of the material [79].

3. Increased Chemical Reactivity

Compared with native cellulose, DAC exhibits significantly higher chemical reactivity because of its aldehyde functionalities and increased amorphous character. The aldehyde groups can readily react with amino, hydrazide, and other nucleophilic groups, facilitating further chemical modifications [86].

4. Enhanced Swelling and Water Absorption

The disruption of the crystalline structure and hydrogen-bonding network increases the accessibility of water molecules to the cellulose matrix. Consequently, DAC generally exhibits greater swelling capacity and water absorption than native cellulose, which is beneficial for hydrogel and biomedical applications [87].

5. Biodegradability and Biocompatibility

Like cellulose, DAC is derived from renewable natural resources and remains biodegradable. Many studies have also demonstrated its suitability for biomedical applications because of its biocompatibility and low toxicity, particularly when used in tissue engineering and drug delivery systems [88].

6. Cross-Linking Ability

The aldehyde groups in DAC can react with amino-containing compounds to form Schiff bases (imine bonds). This property enables DAC to act as a natural cross-linking agent for proteins, chitosan, gelatin, collagen, and other biopolymers, leading to the formation of stable three-dimensional networks [89].

7. Improved Adsorption Capacity

The introduction of aldehyde groups and the increase in amorphous regions enhance the surface activity of DAC. As a result, DAC-based materials often exhibit improved adsorption capacities for dyes, heavy metals, and other pollutants, making them useful in environmental remediation applications [16].

8. Thermal Properties

Periodate oxidation generally decreases the thermal stability of cellulose because the oxidation process disrupts the ordered crystalline structure and introduces reactive aldehyde groups. Therefore, DAC usually decomposes at slightly lower temperatures than native cellulose. [90]

9. Film-Forming and Hydrogel-Forming Ability

Due to its reactive aldehyde groups and ability to undergo cross-linking reactions, DAC can be used to prepare films, membranes, hydrogels, and composite materials with desirable mechanical and functional properties [91].

2.7 Characterization of Dialdehyde cellulose

The introduction of aldehyde groups considerably alters the physicochemical properties of cellulose. These aldehyde functionalities enhance the chemical reactivity of cellulose and facilitate further modifications through Schiff base formation, cross-linking reactions, and grafting [92] [87]. To confirm successful oxidation and evaluate the properties of DAC, characterisation is required. To determine the degree of oxidation and the number of aldehyde groups added to the cellulose structure, aldehyde content analysis is frequently performed. The synthesis of aldehyde functionalities is confirmed, and distinctive functional groups are identified using Fourier Transform Infrared Spectroscopy (FTIR). Surface morphological changes are revealed using scanning electron microscopy (SEM).UV–Visible Spectroscopy (UV–Vis) is employed to assess oxidation-related changes and support the confirmation of chemical modification. Additional analytical techniques such as X-ray Diffraction (XRD), Thermogravimetric Analysis (TGA), and Nuclear Magnetic Resonance (NMR) may also be used for detailed structural and thermal characterization [90] [79].

2.8 Applications of Dialdehyde Cellulose (DAC)

1. Biomedical Applications

The presence of highly reactive aldehyde groups makes DAC particularly valuable in biomedical engineering. These aldehyde groups readily react with amino-containing biomolecules, allowing the formation of biocompatible and biodegradable materials for healthcare applications [15] [87] [89].

1.1 Drug Delivery Systems

DAC is widely used in controlled drug delivery systems because it can form hydrogels and polymeric networks capable of encapsulating therapeutic agents. The aldehyde groups facilitate cross-linking with biopolymers such as chitosan and gelatin, enabling sustained and targeted drug release. DAC-based hydrogels have been investigated for transdermal drug delivery and wound-healing patches.

1.2 Tissue Engineering

DAC serves as a scaffold material for tissue engineering because of its biocompatibility, biodegradability, and ability to form three-dimensional porous structures. Through Schiff-base reactions with proteins such as collagen and gelatin, DAC creates matrices that support cell adhesion, proliferation, and tissue regeneration.

1.3 Wound Dressings

DAC-based hydrogels and films possess excellent moisture-retention properties and can maintain a favorable environment for wound healing. Their ability to incorporate antimicrobial agents further improves wound management and reduces infection risk.

1.4 Antimicrobial Materials

The aldehyde functionalities of DAC can immobilize antimicrobial compounds or metal nanoparticles, producing antibacterial materials for biomedical coatings, dressings, and packaging applications.

1.5 Enzyme and Protein Immobilization

DAC provides reactive aldehyde sites that form covalent bonds with amino groups in proteins and enzymes. This property is useful in biosensors, biocatalysis, and biomedical diagnostics.

1.6 Biomedical Hydrogels and Sponges

DAC is extensively used in the preparation of injectable hydrogels, porous sponges, and soft biomaterials because of its excellent cross-linking ability and low toxicity. These materials are being explored for regenerative medicine and tissue repair.

2. Environmental Applications

DAC has gained attention as an environmentally sustainable material for pollution control due to its high reactivity, porosity, and adsorption capability. [16] [17] [93]

2.1 Water Purification

DAC-based membranes, aerogels, and adsorbents are employed in water treatment systems for removing contaminants. The increased surface activity and modifiable aldehyde groups improve pollutant adsorption efficiency.

2.2 Heavy Metal Adsorption

DAC and DAC-derived materials have demonstrated strong adsorption capacities toward toxic metal ions such as lead, cadmium, chromium, and copper. The aldehyde groups can be further functionalized to improve metal-binding efficiency.

2.3 Dye Removal from Wastewater

The porous structure and reactive functional groups of DAC-based materials facilitate the removal of synthetic dyes and organic pollutants from textile and industrial wastewater.

2.4 Oil–Water Separation

DAC-derived aerogels and porous materials can be modified to selectively absorb oils and organic solvents, making them useful for oil-spill cleanup and industrial wastewater treatment.

2.5 Environmental Remediation

DAC serves as a platform for producing advanced adsorbents, filtration membranes, and aerogels designed for environmental cleanup and pollutant recovery.

2.6 Sustainable Filtration Materials

Because DAC originates from renewable cellulose resources, it contributes to the development of biodegradable filtration systems that reduce dependence on synthetic polymers.

3. Industrial Applications

The industrial importance of DAC stems from its high chemical reactivity, cross-linking capability, and renewable origin. [92] [94] [60]

3.1 Paper Strengthening

DAC improves paper strength through inter-fiber cross-linking. The aldehyde groups form covalent bonds with cellulose fibers, resulting in enhanced wet strength, durability, and dimensional stability.

3.2 Textile Finishing

DAC is used as a bio-based finishing agent for textiles. It improves wrinkle resistance, dimensional stability, and dye retention while reducing reliance on synthetic chemical finishes.

3.3 Biodegradable Packaging Materials

DAC-based films and coatings are being developed as environmentally friendly alternatives to petroleum-based plastics. Their biodegradability and ability to form strong polymer networks make them attractive for sustainable packaging.

3.4 Bio-Based Adhesives

DAC can function as a renewable adhesive because its aldehyde groups readily react with hydroxyl and amino groups in natural materials. Studies have demonstrated its effectiveness as a wood adhesive with excellent bonding performance.

3.5 Polymer Composites
DAC is incorporated into polymer matrices to improve mechanical strength, water resistance, and structural stability. It serves as a reinforcing component in sustainable composite materials.

3.6 Functional Coatings and Films
The high reactivity of DAC enables the production of protective coatings, barrier films, and antimicrobial surfaces for industrial applications.

3.7 Smart and Functional Materials
DAC is increasingly utilized in advanced materials such as responsive hydrogels, sensors, Nano composites, and stimuli-responsive systems because of its ability to undergo further chemical modification.

2.9 Advantages of Dialdehyde Cellulose (DAC)
Dialdehyde cellulose (DAC) offers several important advantages over native cellulose because periodate oxidation introduces reactive aldehyde groups while largely preserving the cellulose polymer backbone. These advantages explain why DAC is widely investigated for biomedical, environmental, and industrial application.

Table 2: Properties and Advantages of Dialdehyde Cellulose (DAC)

Property / Advantage	Description / Benefit
High chemical reactivity	Aldehyde groups readily undergo Schiff-base formation, cross-linking, grafting, and conjugation reactions, enabling easy functionalization.
Efficient cross-linking ability	DAC can covalently react with amino-containing polymers (e.g., gelatin, collagen, chitosan, and proteins) to produce stable hydrogels, films, and scaffolds.
Improved accessibility	Oxidation decreases cellulose crystallinity and increases amorphous regions, allowing solvents and reagents to penetrate the material more effectively.
Enhanced swelling and water uptake	Disruption of the hydrogen-bonding network generally increases swelling capacity, making DAC suitable for hydrogels and wound dressing applications.
Biodegradability and renewable origin	DAC is produced from cellulose, an abundant renewable biomass resource, making it an environmentally sustainable alternative to petroleum-based polymers.
Biocompatibility	Properly prepared and purified DAC-based materials exhibit good biocompatibility with biological systems, supporting applications in tissue engineering and drug delivery.
Tunable functionality	The degree of oxidation (dialdehyde content) can be controlled by adjusting reaction conditions, enabling customization of reactivity, swelling behavior, and cross-linking density.
Excellent platform for composite materials	Reactive aldehyde groups facilitate interactions with polymers, proteins, nanoparticles, and other fillers, enabling the fabrication of advanced composites and functional coatings.
Effective adsorption platform	DAC can be further functionalized with chelating or amine groups, improving adsorption efficiency for dyes, heavy metals, and other environmental pollutants.
Versatility across applications	DAC is applicable in hydrogels, wound dressings, tissue engineering scaffolds, drug delivery systems, adsorbents, biodegradable packaging, adhesives, coatings, and functional films.

3. Synthesis of Polyphenols – Polysaccharides Conjugates and Their Biological Activities

3.1 Methods of Conjugate Formation

1. Free-radical grafting method– This method consists of a redox initiator pair, usually ascorbic acid and hydrogen peroxide, to produce hydroxyl radicals(OH); these radicals extract hydrogen atom from the polysaccharide backbone, like -CH and -OH groups on chitosan and also it can be from the C6 hydroxy methyl group of cellulose, creating macro-radicals along the chain. In the presence of gallic acid, the polysaccharide macro-radical attaches to anaromatic ring, forming a stable carbon–carbon covalent bond between the polyphenol and the saccharide backbone [95].

2. Carbodiimide-Mediated Coupling –When the polyphenol consists of a carboxylic acid moiety and the polysaccharide contains primary amines or abundant hydroxyls,the1-ethyl-3-(3-dimethylaminopropyl) carbodiimide / N-hydroxysuccinimide (EDC/NHS) system forms a well-defined amide (with $-NH_2$) or ester (with –OH) linkage. Now the mechanism further proceeds with the activation of the carboxyl group to an O-acylisourea intermediate, which is converted into a more stable NHS-ester,and is finally attached by the polysaccharide nucleophile. [96] demonstrated the amide bond formation between the carboxyl group of ferulic acid and the C2-amine of chitosan. However, this method is less preferred as compared to the free radical grafting method due to its higher cost and higher toxicity.

3. Enzyme-Catalysed Conjugation- [97] in his study, treated chitosan with catechinand with gallic acid in the presence of laccase from Trametesversicolor and confirmed it by Uv vis spectroscopic analysis and FTIR analysis the successful conjugation between chitosan with catechin and gallic acid.

4. Acid-catalysed condensation reaction – This method was first performed by Yong et al ., (2020) for the synthesis of starch aldehyde Quercetin conjugate. The starch aldehyde gets protonated and is followed by nucleophilic attack by quercetin by C6 and C8 carbon attaches to Quercetin, subsequently followed by another attack of Quercetin with loss of H_2O. This method was more efficient, simpleand safer as compared to free radical grafting method.

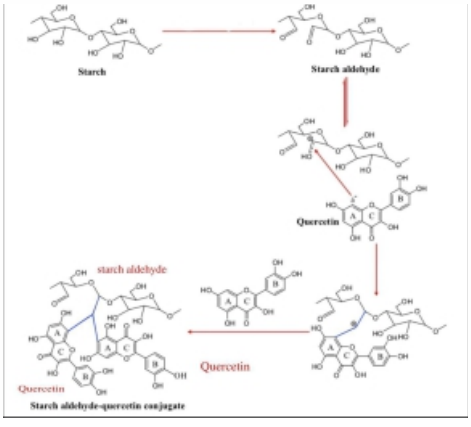

Figure 4: Synthesis of Starch Aldehyde-Quercetin conjugate by Acid Catalysed Condensation reaction [22].

3.2 Structural characterization

1. Uv Vis spectroscopic analysis- UV-Vis determines the covalent attachment of polyphenols by tracing the alteration in polyphenols electronic absorption. Free polyphenols exhibit characteristics of π-π* and n-π* bands. After forming the conjugate, the conjugate retains these bands, but often displays a bathochromic shift and broadening due to new covalent bonding, where no polyphenols leaches out. For instance, felluric acid grafted with carboxylic curdain displayed peaks at 270 nm to 320 nm. Similarly, gallic acid displayed peak absorbance at 270 nm to 300 nm. However, researchers also found that few conjugate-like banana condensed tannin inulin (BCT g inulin) showed peaks at 200 -270 nm, this was caused by active groups of BCT, which decreased the π-π^* electronic transition energy gap [98].

2. FTIR Spectroscopy – This method is used to analyse the composition of new covalent bonds and functional groups of unknown organic substances. Usually, new bands are formed in covalently linked conjugates. For instance, new characteristic absorption bands appeared at about 1514 cm^{-1} in the IR spectra of ferulic acid-grafted curdlan conjugates (Cur-g-FA) in comparison with those of curdain, displaying successful conjugate formation between felluruc acid and curdain. [99]. [22] presented FTIR results on dialdehyde starch with four different types of catechin, where each displayed their own specific features, confirming successful conjugate formation.

3. SEM analysis-SEM analysis is used for the observation of microscopic morphological characteristics of a substance and field-emission scanning electron microscope (FE-SEM) can obtain highly three-dimensional data on the surface microstructure of the sample. Currently, SEM and FE-SEM have been used to observe the surface morphology of polyphenol-polysaccharides [100] [101] [102]. Studies have shown that the polysaccharides have smooth surface, while the conjugate leads to rough, uneven, andwrinkled surfaces [103] [47] [18].

3.3 Biological activities of polyphenols polysaccharides conjugate

1. Antioxidant activity

The antioxidant properties of catechins linked with different polysaccharides were evaluated by detecting multiple indicators over recent years. The radical-scavenging capacity of a conjugate is the most immediate functional test, typically measured by DPPH, ABTS, or FRAP assays. [104] documented biological activity of polyphenol polysaccharides, as their ability to scavenge reactive oxygen and nitrogen species and to interrupt free-radical chain reactions. He documented the unsubstituted B-ring catechol and galloyl ester moieties of the grafted catechin, which readily donate hydrogen atoms or single electrons to stabilise radicals. The Schiff base linkage involves the A-ring at C6 or C8, these critical antioxidant functionalities remain fully accessible. [103] reported in catechin, Tremellafuciformis polysaccharide (TPS) and its conjugate, where the DPPH radical scavenging activity of TPS and catechin-g-TPS was enhanced in a concentration-dependent manner. Where he presented the antioxidant properties of the copolymer were enhanced after forming the conjugate The A and B rings of catechin contain many electron-donating groups that strongly capture free radicals,and when catechin was linked to TPS, the antioxidant properties of TPD increased significantly. [105]. Similarly, other researchers also found that the DPPH radical scavenging activity of many polysaccharides such as chitosan, dextran, starch, chitin/glucan complex, and carboxy methyl starch was improved by conjugating with quercetin [106].

2. Antimicrobial activity

Studies have demonstrated in polyphenol–chitosan conjugates that the catechol and galloyl moieties of catechin can inhibit fungal growth by disrupting ergosterol biosynthesis and chelating essential metal ions. The same functional groups are present in DAC–catechin conjugates, and it is therefore expected that these conjugates possess antifungal activity against common spoilage fungi such as Aspergillus and Penicillium species [107]. Caffeic acid and gallic acid when grafted onto chitosan fibres by using laccase retained more than 90% of their original antibacterial activity against *S. aureus* and *E. coli* after multiple laundering cycles. [108]. [109] in their study, reported enhanced antimicrobial activity of catechin-g-CS conjugates compared to unmodified Cs.

While some research papers also reported that the antimicrobial activity of the polyphenol-polysaccharide conjugates was slightly lower because of differences in strains, pH, and degree of substitution [110] [111].

3. Anti-inflammatory properties

[112] demonstrated that EGCG inhibits the activation of nuclear factor-kappa B (NF-κB)and reduces the expression of pro-inflammatory cytokines such as tumour necrosis Factor-alpha (TNF-α) and interleukin-6 (IL-6), and down-regulates inducible enzymes like Cyclooxygenase-2 (CO_x-2) and inducible nitric oxide synthas [112].

4. Anticancer properties

[113] demonstrated *in vitro* and experimental studies on the Anticarcinogenic potential of tea polyphenols through direct binding to carcinogens, inducing Phase II enzymes such as UDP-glucuronosyltransferase and inhibition of heterocyclic amine formation. The mechanism includes catechin-mediated apoptosis and cell cycle arrest, inhibition of the transcription factors NF-κB and AP-1, and suppression of protein tyrosine kinase activity and c-jun mRNA expression. Several studies have shown EGCG to have an effect on tumor suppressor miRNA. [114] [115]. Polyphenols can also target oncogenic transcription factors. Thus, various polyphenols play key roles ranging from STAT signalling through estrogen receptor inhibition to NF-kB and HIF-1 modulation in the down regulation of cancer-affected cells.

Epigallocatechin-3-gallate (EGCG), however, has been considerably demonstrated its chemo- preventive features in cancer stem cells. EGCG has demonstrated the ability to inhibit nasopharyngeal carcinoma (NPC) CSCs through attenuation of STAT3and NF-κB p65 activity(Lin, 2014; Li,2015). In further studies, EGCG with Quercetin and Curcumin have led to the inhibition of $CD44^{+}/CD133^{+}$ prostate cells by inhibiting Vimentin, Slug, Snail, and nuclear β-catenin expression and LEF-1/TCF responsive reporter activity of cancer stem cells migration andinvasion. With Curcumin, it has presented inhibition of STAT3 phosphorylation and retention of STAT3-NFkB interaction, which inhibits the formation of spheres in breast tumor cells. Treatment with EGCG has demonstrated inhibition of the expression of stem cell markers i.e., Oct 4, Sox2, Nanog, and CD44, resulting in suppression of head and neck squamous cell carcinoma. [116] [117] [118]

3.4 Application of Polyphenol Polysaccharide conjugate

Naturally, polyphenols and polysaccharides compounds both possess exceptional therapeutic properties, but due to their own physical limitations, they are not able to perform at their full potential. However, in recent years the conjugate of two biomolecules has been able to overcome their limitations [119] [120].

1. Controlled drug delivery and release

Zhang et al., (2024), introduced phenyl boronic acid-modified hyaluronic acid developed as a Glucose-responsive polysaccharide carrier for diol-containing polyphenols, with boronate-ester bonds cleaving under elevated glucose conditions to achieve disease-responsive drug release. [121]. [18] reported hydrophilic polysaccharide conjugation improves dispersibility, protects the polyphenol from acidic gastric conditions, modulates the rate of enzymatic hydrolysis, and enables mucosal adhesion.

Polysaccharides have affinity for specific cell surface receptors, which are only expressed during tissue alteration or damage and when polyphenols like catechin are bound to chitosan or hyaluronic acid, this polymer binds to specific receptors like CD44 present on the cancer membrane and a muco-adhesive layer located in the intestinal lining. Now, this precise linkage triggers receptor-mediated endocytosis and entry of the nano-conjugate through the cell membrane into the matrix of the targeted cell. [122] [123]. In recent studies, polyphenols, when bound to polysaccharides, have demonstrated that they can travel through the bloodstream without leaking and can enter the tumor cells through the large endothelial gaps and may accumulate in tumor tissues, displaying highly effective passive targeting. [2] [123].

2. Mechanism of Biotransformation evasion of polyphenols polysaccharides conjugate

Polyphenols exhibit poor systemic bioavailability due to their biotransformation in enterocytes and in liver. Enzymes like UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and catechol-O-methyl transferase (COMT) convert aglycones into glucuronidated, sulfated, and methylated metabolites, which are often less active and readily excreted. This severely limits the concentration of intact polyphenol that reaches target organs. [124]. In the Chitosan-EGCG conjugate, the Chitosan masked the phenolic hydroxyl group of EGCG, providing stability and preventing in the phase II metabolite formation. [125]. A few studies have suggested that the conjugate of polyphenols and polysaccharides are present naturally, like ferulic acid bound to arabinoxylan chains in cereal bran by ester linkage. Upon consumption, these complexes are not liberated during small-intestinal digestion as human enzymes are not able to hydrolyse the covalent linkages. As a result, they completely bypass enterocyte phase II metabolism. The complex reaches the colon, where the microbiota express esterases and glycosidases that release the polyphenol aglycone. Now the resultant compound can act locally on the colonic epithelium or can be absorbed into the systemic circulation, transporting the polyphenol to the large intestine as its target organ. [126]

3. Enhancement in the shelf life of the compounds

Ferulic acid–chitosan conjugate exhibited significantly higher thermal stability than free ferulic acid and retained over 80 % of its DPPH radical-scavenging activity after prolonged heating, whereas the free phenolic acid lost nearly all activity under identical conditions, suggesting that grafting a polyphenol onto a polysaccharide restricts the molecular mobility and sterically protects the sensitive hydroxyl groups from autoxidation. [126]. Similarly, in recent studies performed by Tasnim et al., (2025), presented enhancement in the antioxidant, antimicrobial, and shelf life of food materials. [127] [128] presented tea polyphenols incorporated with pectin-based films and being synergistically reinforced by graphene oxide to exhibit enhanced antioxidant propertiesand sustained release of polyphenol from the biopolymer matrix. [129] demonstrated that various phenolic compounds incorporated into polysaccharide-based films and coatings significantly enhance the product shelf life by mitigating quality degradation due to oxidation and microbial growth and presented chitosan-flavonoid films containing polysaccharide-flavonoid conjugates developed by grafting flavonoids onto starch aldehyde or chitosan, which displayed better swelling ability, stability, and antioxidant and antibacterial activities compared to films containing unmodified flavonoids.

Conclusion

Polyphenols such as catechin have been a part of the human diet from the past to the present, either knowingly or unknowingly. However, their functional applications are limited due to inherent drawbacks such as rapid excretion, low gastrointestinal absorption, and poor aqueous solubility. Previous research has demonstrated that conjugation with high-molecular-weight carbohydrates can significantly enhance the therapeutic potential of catechin by improving its antioxidant, anticancer, and anti-inflammatory activities, as well as its thermal stability and shelf life.

The present study aims to develop a green, sustainable, efficient, and cost-effective approach for the synthesis of a polyphenol–polysaccharide conjugate to overcome the physicochemical limitations of catechin. It also highlights the potential of an underexplored biomolecule, dialdehyde cellulose (DAC), which possesses desirable properties such as excellent cross-linking ability, increased chemical reactivity, the presence of reactive aldehyde groups, thermal stability, and enhanced adsorption capacity. Furthermore, the study emphasizes the physicochemical characteristics of catechin, its biological and therapeutic activities, and its potential applications in the food industry. The formation of the DAC–catechin conjugate can be achieved through various conjugation methods, and its successful synthesis can be confirmed using UV–Visible spectroscopy, Scanning Electron Microscopy (SEM), the DPPH radical scavenging assay, Fourier Transform Infrared (FTIR) spectroscopy, and High-Performance Liquid Chromatography (HPLC). The resulting conjugate has potential applications in controlled drug delivery and active food packaging. However, due to the limited experimental evidence available, it is difficult to determine the detailed structural characteristics of DAC–catechin conjugates, including the exact binding sites and distribution pattern of catechin on the DAC backbone. This limitation hinders the establishment of a clear structure–activity relationship. Therefore, further in-depth investigations are required to elucidate the structural features and functional mechanisms of these conjugates.

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