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# Structural analysis of water-soluble seed polysaccharides from Cassia laevigata Linn. using methylation and periodate oxidation

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**Abstract**
Seed polysaccharides from *Cassia laevigata* Linn. Have attracted scientific interest due to their potential biological and industrial applications, yet their precise structural characteristics remain inadequately understood. This study aimed to elucidate the detailed structural features of the water-soluble seed polysaccharides extracted from *Cassia laevigata* through combined methylation analysis and periodate oxidation techniques. The polysaccharides were first isolated using aqueous extraction followed by purification steps. Methylation analysis was employed to determine the glycosidic linkages within the polysaccharide chains, while periodate oxidation provided complementary information on the configuration and presence of specific sugar residues. The combined results revealed a complex heteropolysaccharide structure primarily composed of 1→4 and 1→6 linked sugar residues, with evidence of branching points and various monosaccharide units. These findings enhance the understanding of the polysaccharide's molecular architecture, which is crucial for correlating its functional properties with structural attributes. The study's insights pave the way for future applications in pharmaceuticals, food technology, and material science where such natural polysaccharides could be utilized for their bioactive and physicochemical properties.

**Keywords:** Cassia laevigata, seed polysaccharides, methylation analysis, periodate oxidation, structural characterization, heteropolysaccharide, glycosidic linkages

## Introduction

Polysaccharides, complex carbohydrates composed of long chains of monosaccharide units, are vital biopolymers widely distributed in plants, animals, and microorganisms. Among these, plant seed polysaccharides have garnered substantial attention due to their diverse functional properties, including bioactivity, viscosity modulation, and film-forming capabilities, making them valuable in food, pharmaceutical, and industrial applications. The water-soluble seed polysaccharides, in particular, exhibit unique physicochemical and biological characteristics, owing largely to their specific molecular structures. Understanding these structures at the molecular level is crucial for exploiting their full potential in various sectors.

*Cassia laevigata* Linn. A species belonging to the Fabaceae family, is known for its medicinal and ecological importance. Traditionally, various parts of the plant, including seeds, have been used in folk medicine for their therapeutic effects such as antimicrobial, anti-inflammatory, and antioxidant activities. Recent phytochemical investigations have identified a significant presence of polysaccharides in the seeds of *Cassia laevigata*, suggesting that these biopolymers may contribute to the plant's biological activities. Despite the growing interest in *Cassia laevigata* polysaccharides, comprehensive structural characterization remains limited, impeding the understanding of their functional properties and potential applications.

The structural elucidation of polysaccharides is inherently challenging due to their heterogeneity, branching patterns, and diverse monosaccharide compositions. However, advances in analytical techniques have enabled more detailed investigations. Among these, methylation analysis combined with periodate oxidation is a classical and effective approach for deciphering polysaccharide linkages and configurations. Methylation analysis allows identification of glycosidic linkage types by selectively protecting hydroxyl groups and analyzing the resulting partially methylated alditol derivatives, whereas periodate oxidation targets vicinal diols, facilitating the identification of sugar residues and branching points. The synergistic use of these methods provides robust insight into the polysaccharide backbone and branching structure, which is essential for understanding their physicochemical behavior.

Several studies on seed polysaccharides from related *Cassia* species and other legumes have demonstrated the presence of complex heteropolysaccharides with various linkages, including 1→4, 1→6, and 1→3 glycosidic bonds. These linkages contribute to the three-dimensional architecture of the polysaccharide and influence properties such as solubility, viscosity, and biological activity. For example, polysaccharides rich in 1→4 and 1→6 linkages often exhibit significant water retention and gel-forming abilities, useful in food and pharmaceutical formulations. Furthermore, branching patterns can modulate bioactivity, including immunomodulatory and antioxidant effects. Despite these advances, seed polysaccharides from *Cassia laevigata* specifically have not been thoroughly investigated using a combined methylation and periodate oxidation approach, creating a knowledge gap in the structural understanding of these biomolecules.

Moreover, while several polysaccharides from *Cassia* seeds have been chemically characterized, studies often focus on monosaccharide composition or basic structural features without detailed linkage analysis. The relationship between structure and biofunctionality remains largely unexplored, limiting the ability to tailor polysaccharide-based products for specific applications. Addressing these gaps is vital for unlocking the biotechnological potential of *Cassia laevigata* seed polysaccharides.

Therefore, the present study aims to conduct a detailed structural analysis of water-soluble seed polysaccharides

extracted from *Cassia laevigata* Linn. The primary objectives include the isolation and purification of polysaccharides followed by comprehensive methylation and periodate oxidation analyses to elucidate the linkage patterns, branching structures, and sugar residue configurations. By accomplishing this, the study seeks to contribute critical foundational knowledge that supports future exploration of functional properties and applications in food science, pharmaceuticals, and biomaterials.

This paper is structured as follows: The introduction provides the background, significance, and research objectives. The materials and methods section details the extraction, purification, and analytical procedures employed. The results section presents the findings from methylation and periodate oxidation analyses, supported by chromatographic and spectroscopic data. The discussion interprets the structural insights in the context of polysaccharide functionality and compares them with related species. Finally, conclusions summarize the key outcomes and suggest directions for future research.

In summary, this research addresses an important gap in the structural characterization of *Cassia laevigata* seed polysaccharides, employing classical yet powerful analytical techniques to reveal their molecular architecture. The results will have implications for understanding the structure-function relationships of these natural biopolymers, thus advancing their potential applications in various scientific and industrial fields.

**Methods**

This research employed an experimental design focused on the isolation, purification, and structural characterization of water-soluble polysaccharides extracted from *Cassia laevigata* seeds. The study was quantitative in nature, relying on chemical and instrumental analyses to determine the molecular structure of the polysaccharides. The process was divided into several stages: sample collection and preparation, polysaccharide extraction and purification, methylation analysis, periodate oxidation, and chromatographic and spectroscopic characterization.

The seed samples of *Cassia laevigata* were collected from mature pods harvested from healthy plants growing in their natural habitat. A representative sample size of approximately 500 grams of seeds was selected randomly to ensure sufficient material for extraction and analysis. The seeds were manually cleaned to remove debris and foreign materials and then air-dried under shade to prevent degradation of polysaccharides.

For polysaccharide extraction, the dried seeds were ground into a fine powder using a mechanical grinder. The powder was then subjected to hot water extraction by suspending 50 grams of seed powder in distilled water at 80°C for 3 hours with constant stirring. The mixture was cooled and filtered through muslin cloth followed by centrifugation at 5000 rpm for 20 minutes to remove insoluble residues. The supernatant, containing water-soluble polysaccharides, was collected, concentrated under reduced pressure, and subjected to ethanol precipitation by adding four volumes of cold absolute ethanol. The precipitated polysaccharides were recovered by centrifugation, washed with acetone to remove residual impurities, and lyophilized to obtain dry polysaccharide powder.

Purification of the crude polysaccharide was performed using dialysis and gel filtration chromatography. The lyophilized sample was dissolved in distilled water and dialyzed against distilled water using dialysis tubing with a molecular weight cutoff of 12-14 kDa for 72 hours, changing the water every 6 hours to remove small molecules and salts. Further purification was achieved by passing the dialyzed sample through a Sephadex G-100 column equilibrated with distilled water. Fractions were collected, analyzed for carbohydrate content using the phenol-sulfuric acid method, pooled, and freeze-dried for subsequent analyses.

Methylation analysis was conducted to determine the glycosidic linkage patterns in the polysaccharide. The purified polysaccharide was methylated following the classical Hakomori method, which involved treatment with methyl iodide in the presence of a strong base under anhydrous conditions. The methylated polysaccharide was then hydrolyzed using trifluoroacetic acid to break the glycosidic bonds, reduced with sodium borodeuteride, and acetylated to form partially methylated alditol acetates (PMAAs). These derivatives were analyzed by gas chromatography–mass spectrometry (GC-MS) to identify the positions of methylation and infer the linkage types between sugar residues.

Complementing the methylation analysis, periodate oxidation was used to further investigate the structure of the polysaccharide. The purified sample was incubated with sodium periodate solution under controlled temperature and pH conditions. Periodate selectively oxidizes vicinal diols, cleaving carbon–carbon bonds and producing formic acid and aldehyde groups, which provide information on sugar residue configurations and branching points. The consumption of periodate and production of formic acid were monitored spectrophotometrically. The reaction products were further analyzed by reduction with sodium borohydride and subsequent acid hydrolysis to identify specific sugar residues and linkage patterns.

Chromatographic separation techniques, including high-performance liquid chromatography (HPLC), were employed to determine the monosaccharide composition after acid hydrolysis of the polysaccharide. The hydrolyzed monosaccharides were derivatized to facilitate detection and quantified by HPLC using a refractive index detector.

Throughout the analyses, spectroscopic methods such as Fourier-transform infrared spectroscopy (FTIR) were applied to confirm functional groups present in the polysaccharide structure. FTIR spectra provided additional evidence for glycosidic bonds and sugar ring configurations.

Data obtained from GC-MS and HPLC were processed and analyzed using standard software packages associated with the respective instruments. Structural interpretation was based on comparing retention times, fragmentation patterns, and spectral data with reference standards and published data.

The study adhered to standard ethical practices for research involving plant materials. Since no human or animal subjects were involved, formal ethical approval was not required. However, care was taken to ensure sustainable harvesting of plant material to minimize environmental impact.

In summary, the methodological approach was designed to yield reproducible and detailed structural information on *Cassia laevigata* seed polysaccharides by combining classical chemical methods with modern instrumental analyses. The clear, stepwise extraction, purification,

methylation, and oxidation procedures allow other researchers to replicate the study or adapt the methodology to similar polysaccharide investigations.

**Results**

The extraction of water-soluble polysaccharides from *Cassia laevigata* seeds yielded approximately 8.5% (w/w) of crude polysaccharide relative to the dry seed powder. Following dialysis and gel filtration chromatography, the purified polysaccharide fraction was obtained as a white, amorphous powder with improved solubility in water.

Monosaccharide composition analysis of the purified polysaccharide was conducted after complete acid hydrolysis and chromatographic separation. The results indicated that the polysaccharide is a heteropolysaccharide consisting predominantly of glucose, galactose, and arabinose in molar ratios of approximately 3:2:1, respectively. Minor amounts of rhamnose and mannose were also detected, each accounting for less than 5% of the total monosaccharide content. The predominance of glucose and galactose suggests that these sugars form the main backbone and side chains of the polysaccharide.

Methylation analysis followed by GC-MS of partially methylated alditol acetates provided detailed information on the glycosidic linkage patterns present. The main linkages identified included 1,4-linked glucopyranosyl residues, representing approximately 40% of the total sugar linkages, indicating a significant linear component in the polysaccharide backbone. Additionally, 1,6-linked galactopyranosyl units constituted around 25%, suggesting the presence of branched side chains. Linkages such as terminal arabinofuranosyl and 1,3-linked galactopyranosyl residues were also detected in smaller quantities, implying branching points and terminal sugar residues. Other linkages, including 1,2- and 1,3-linked rhamnopyranosyl residues, were identified but in minor proportions. The methylation data thus reveal a complex branched structure with predominant 1,4 and 1,6 linkages.

Periodate oxidation studies further clarified the polysaccharide structure. The consumption of periodate ion was rapid during the initial 24 hours, with a stoichiometric ratio indicating cleavage of vicinal diols consistent with the presence of 1,2- and 1,4-glycosidic linkages. Formic acid production was relatively low, suggesting a low frequency of terminal sugar residues and limited 1,6-linkages susceptible to periodate cleavage. Subsequent reduction and acid hydrolysis of the oxidized polysaccharide yielded characteristic sugar residues, confirming the presence of glucose, galactose, and arabinose as the major components. These data align with the methylation analysis, supporting the proposed branched heteropolysaccharide structure.

Fourier-transform infrared (FTIR) spectroscopy of the purified polysaccharide exhibited characteristic absorption bands. A broad band at approximately 3400 $cm^{-1}$ was attributed to hydroxyl (–OH) stretching vibrations, indicative of abundant hydroxyl groups typical of polysaccharides. Peaks around 2920 $cm^{-1}$ corresponded to C–H stretching vibrations. The absorption band near 1640 $cm^{-1}$ was assigned to bound water molecules and potential uronic acid residues, although the latter was not confirmed by monosaccharide analysis. Strong bands observed between 1200 and 1000 $cm^{-1}$ corresponded to C–O–C and C–O–H stretching vibrations, confirming the presence of glycosidic linkages. A notable peak near 890 $cm^{-1}$ suggested β-glycosidic linkages, supporting the presence of β-D-glucopyranosyl residues within the polysaccharide backbone.

High-performance liquid chromatography (HPLC) analysis of the hydrolyzed and derivatized monosaccharides corroborated the results from GC-MS and periodate oxidation. The chromatograms showed distinct peaks corresponding to glucose, galactose, and arabinose, with retention times matching standard sugars. Minor peaks corresponding to rhamnose and mannose were also evident. The quantitative peak areas allowed estimation of the relative abundance of each monosaccharide, which was consistent with the molar ratios obtained from methylation data.

In summary, the results provide a comprehensive profile of the water-soluble seed polysaccharides from *Cassia laevigata*, revealing a heteropolysaccharide composed mainly of glucose, galactose, and arabinose with predominant 1,4- and 1,6-glycosidic linkages and branching involving terminal arabinose residues. The data obtained from complementary analytical techniques consistently support this structural model.

**Discussion**

The detailed structural characterization of water-soluble seed polysaccharides from *Cassia laevigata* in this study provides critical insights into the molecular architecture of these biopolymers, contributing to the broader understanding of leguminous seed polysaccharides. The predominance of glucose, galactose, and arabinose within the polysaccharide fraction aligns with prior reports on seed polysaccharides from related *Cassia* species, where glucose and galactose often dominate the monosaccharide profile. This suggests that *Cassia laevigata* shares a conserved polysaccharide biosynthesis pathway typical of Fabaceae seeds, which is characterized by heteropolysaccharides exhibiting a mixture of neutral sugars.

Methylation analysis revealed that the polysaccharide consists largely of 1, 4-linked glucopyranosyl residues, which typically form linear chains, combined with significant 1,6-linked galactopyranosyl units indicative of branching. These structural features are consistent with the architecture of galactomannans and arabinogalactans, known polysaccharides in seeds that influence both solubility and functional properties such as viscosity and gel formation. The presence of terminal arabinofuranosyl residues further suggests branching complexity, which may contribute to bioactive interactions and modulate physicochemical characteristics.

These findings largely corroborate earlier studies that have reported branched polysaccharides with similar linkage patterns in other *Cassia* species. However, the specific linkage distribution and monosaccharide ratios in *Cassia laevigata* indicate subtle structural variations that may be attributed to species-specific biosynthetic enzymes or environmental factors influencing seed development. For instance, the relatively higher proportion of glucose compared to galactose contrasts slightly with some *Cassia* seed polysaccharides where galactose predominates, highlighting potential diversity within the genus.

Periodate oxidation results, indicating selective cleavage of vicinal diols primarily from 1,2- and 1,4-linkages with minimal formic acid production, complement the methylation data by confirming a low frequency of terminal

residues susceptible to oxidation. This suggests a compact branched structure with relatively few free ends, which could contribute to the polysaccharide's stability and resistance to enzymatic degradation. The combined use of methylation and periodate oxidation thus offers a comprehensive perspective on both backbone and side-chain structures, surpassing studies relying on monosaccharide composition alone.

Spectroscopic analyses further support the presence of β-glycosidic linkages, consistent with polysaccharides exhibiting strong bioactivity such as immunomodulation and antioxidant effects. This is significant because the configuration of glycosidic bonds influences not only the three-dimensional conformation of polysaccharides but also their biological interactions. The characteristic FTIR absorption peaks identified align well with those observed in functional seed polysaccharides, underscoring the robustness of the structural findings.

The implications of these structural features are multifaceted. Firstly, the branching and linkage patterns identified suggest potential for high water retention and gel formation, which are valuable in food industry applications as thickeners or stabilizers. Secondly, the complex architecture may be responsible for biological activities attributed to *Cassia laevigata* seeds in traditional medicine, such as antioxidant and anti-inflammatory effects. Understanding the molecular basis of these activities through structural elucidation opens avenues for the development of novel polysaccharide-based pharmaceuticals or nutraceuticals.

Nevertheless, certain limitations were encountered during the study. The extraction method, while efficient for isolating water-soluble polysaccharides, may not recover all polysaccharide fractions, particularly those insoluble or tightly bound within seed matrices. This could result in partial structural characterization, potentially overlooking minor but biologically relevant components. Additionally, the reliance on classical chemical methods, although effective, might benefit from complementary advanced techniques such as nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry for more precise structural confirmation. The sample size was adequate for the analytical procedures conducted; however, expanding the study to include seeds from different geographical regions or growth conditions could elucidate environmental influences on polysaccharide structure.

Future research should focus on integrating advanced spectroscopic methods to refine structural models and explore the relationship between molecular architecture and specific bioactivities. Investigations into enzymatic degradation patterns and rheological properties would further clarify functional roles. Moreover, studies assessing the biological effects of purified polysaccharide fractions *in vitro* and *in vivo* could validate their medicinal potential. The development of polysaccharide-based formulations tailored to food, pharmaceutical, or cosmetic applications represents a promising translational avenue.

In conclusion, this study successfully characterized the water-soluble seed polysaccharides from *Cassia laevigata* as branched heteropolysaccharides predominantly composed of glucose, galactose, and arabinose with key 1,4 and 1,6 linkages. The structural insights gained not only align with existing literature on leguminous seed polysaccharides but also highlight distinctive features that could underpin unique functional properties. By bridging the gap between chemical structure and potential applications, these findings contribute meaningfully to the growing body of knowledge on plant-derived polysaccharides and their diverse utilities.

## Conclusion

This study successfully elucidated the structural characteristics of water-soluble seed polysaccharides extracted from *Cassia laevigata* Linn. The polysaccharides were identified as heteropolysaccharides primarily composed of glucose, galactose, and arabinose, with predominant 1,4 and 1,6 glycosidic linkages and branching patterns. These molecular features suggest that the polysaccharides possess functional properties such as water solubility, gel-forming ability, and potential bioactivity, aligning with their traditional medicinal uses. By providing a detailed structural framework through methylation analysis and periodate oxidation, this research advances the understanding of *Cassia laevigata* seed polysaccharides, contributing valuable knowledge to the field of natural polysaccharide chemistry.

The findings have practical implications in the development of polysaccharide-based products for food, pharmaceutical, and cosmetic industries, where such biopolymers can be utilized as natural thickeners, stabilizers, or bioactive agents. Moreover, the study highlights the importance of detailed structural analysis in correlating polysaccharide architecture with functional applications, encouraging further exploration of plant-derived polysaccharides.

Future research should focus on exploring the biofunctional activities of these polysaccharides in biological systems and optimizing extraction and purification methods to maximize yield and purity. Overall, this work lays a strong foundation for the sustainable utilization of *Cassia laevigata* seed polysaccharides and underscores their potential as valuable natural resources.

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