# Characteristics of the Structure and Selected Biological Activities of Polysaccharides Isolated from Fedora 17 Hemp (*Cannabis sativa*)

by

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A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

Fall 2024

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## DEDICATION

This thesis is dedicated to all the educators that have fostered my love of science learning

Also, to Madison Warneck, for her endless love and encouragement throughout this journey.

Finally, to Dr. Kline and Dr. Farone, whose guidance and insight have been invaluable in the completion of this work.

Thank you for being my foundation and source of strength.

## ABSTRACT

This study focuses on characterizing the structural properties and biological activities of polysaccharides isolated from the Fedora 17 hemp (*Cannabis sativa L.*). Hemp polysaccharides are an underexplored resource with significant potential in industries such as biotechnology, agriculture, and medicine due to their antioxidant, anti-inflammatory, and immunomodulatory properties. Techniques, including ion-exchange chromatography (IEX), size-exclusion chromatography (SEC), and Fourier-transform infrared spectroscopy (FTIR), were employed to elucidate the molecular weight distribution and structural complexity of the polysaccharides. Additionally, antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DDPH) assay. The findings contribute to a broader understanding of hemp polysaccharides' potential applications in the development of sustainable and bioactive products.

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## Terms and Definitions

Chromatography: A laboratory technique used for separating a mixture into its individual components, applied in this study to analyze polysaccharides and monosaccharides in hemp.

Dextran: A standard polysaccharide used in the calibration of size-exclusion chromatography experiments to determine the molecular weights of unknown polysaccharides.

Fourier-Transform Infrared Spectroscopy (FTIR): A method for obtaining the infrared spectrum of absorption or emission of a solid, liquid, or gas, used to analyze the functional groups in polysaccharides.

Glycosidic Linkages: The bonds that connect monosaccharides in polysaccharides, which were analyzed in this study to understand the structural arrangement of hemp-derived polysaccharides.

Hemp (Cannabis sativa L.): A versatile crop with low tetrahydrocannabinol (THC) content (less than 0.3 wt.%), used for industrial applications such as textiles and biofuels. This study focuses on polysaccharides isolated from Fedora 17 Hemp.

High-Performance Liquid Chromatography (HPLC): A specific chromatography technique employed for the separation, identification, and quantification of polysaccharides using a refractive index detector in this study.

Ion-Exchange Chromatography (IEX): A technique used to separate ions and polar molecules based on their affinity to the ion exchanger, employed in the purification of polysaccharides in this study.

Monosaccharides: The simplest form of carbohydrates, consisting of a single sugar molecule with a keto group bonded to multiple hydroxyl groups. Examples include glucose, galactose, and mannose.

Phenol-Sulfuric Acid Assay: A chemical method used to quantify carbohydrates by measuring the absorbance of the sample solution at a specific wavelength (490 nm).

Polysaccharides: Complex carbohydrates composed of more than ten monosaccharide units linked together by glycosidic bonds. They are significant for energy storage (e.g., starch, glycogen), structural integrity (e.g., cellulose), and as essential components of cell membranes and extracellular matrices.

Size-Exclusion Chromatography (SEC): A form of chromatography used to separate molecules based on their size, applied here to determine the molecular weight of polysaccharides.

## List of Abbreviations

1. **THC**: Tetrahydrocannabinol
2. **HPLC**: High-Performance Liquid Chromatography
3. **HPSEC**: High-Performance Size-Exclusion Liquid Chromatography
4. **SEC**: Size-Exclusion Chromatography
5. **IEX**: Ion-Exchange Chromatography
6. **FTIR**: Fourier-Transform Infrared Spectroscopy
7. **DLS**: Dynamic Light Scattering
8. **DDPA**: 2,2-Diphenyl-1-picrylhydrazyl (used in antioxidant assays)
9. **Da**: Dalton (unit of molecular weight)
10. **mL**: Milliliters
11. **nm**: Nanometers
12. **v/v**: Volume/Volume ratio
13. **°C**: Degrees Celsius

# CHAPTER I

## 1.1 Introduction

Hemp (*Cannabis sativa L.*), a versatile and industrially significant crop, has been cultivated for thousands of years and is recognized for its broad range of applications, from textiles to biofuel production (**Bradley 2017, Dyer 2019**). While most research has been focused on cannabinoids, such as cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC) content, hemp's polysaccharides represent an underexplored area with immense potential. Polysaccharides, complex carbohydrates made up of more than ten monosaccharide units, play vital roles in plant structure and physiological function. These molecules are critical in various biological processes, such as energy storage (e.g., starch and glycogen), structural integrity (e.g., cellulose), and as essential components of cell membranes. In addition, polysaccharides have been shown to have a variety of medicinal properties, including antioxidant, anti-inflammatory, anti-tumor among others (**Liu 2015, Xue 2020**).

Industrial hemp, *Cannabis sativa* contains less than 0.3 wt.% THC, differs from medical and recreational marijuana (*Cannabis sativa*) and is primarily grown for its fibers, seeds, and oils (**FDA 2024**). After the extraction of cannabinoids and other small molecules, large quantities of polysaccharides remain in hemp residues. These compounds are often overlooked as a valuable resource. Polysaccharides offer several potential advantages, including their use in biotechnology, agriculture, and medicine, due

to their antioxidant, anti-inflammatory, and immunomodulatory properties. The characterization and utilization of these polysaccharides can unlock new possibilities for sustainable and innovative applications.

In the realm of polysaccharide research, understanding the molecular structure, size distribution, and biological activities of hemp-derived polysaccharides is crucial. Previous studies have shown that variations in extraction methods and the purification of polysaccharide fractions can significantly impact the structural integrity and functionality of these molecules (**Shi, 2016**). High-performance size-exclusion liquid chromatography (HPSEC) and other analytical techniques are essential for elucidating the molecular weight distribution and structural complexity of polysaccharides.

This study aims to characterize the structural properties and biological activities of polysaccharides isolated from the hemp variety known as Fedora 17. By analyzing the molecular weight distribution, glycosidic linkages, and potential antioxidant properties of these polysaccharides, this research seeks to bridge existing knowledge gaps and contribute to the growing body of literature on hemp-derived polysaccharides. The findings of this study will provide valuable insights into the potential applications of hemp polysaccharides in various industries, particularly in the development of sustainable and bioactive products.

The key objectives of this research are:

1. To characterize the molecular weight distribution of polysaccharides isolated from *Fedora 17* using size-exclusion chromatography (SEC) and dextran standards.

2. To analyze the structural complexity of the polysaccharides through glycosidic linkage studies and spectroscopic techniques.

3. To evaluate the antioxidant properties of the isolated polysaccharide fractions using a 2,2-diphenyl-1-picrylhydrazyl (DDPH) assay.

By achieving these objectives, this study will contribute to a deeper understanding of the structural and functional attributes of hemp polysaccharides, ultimately providing a foundation for future research and practical applications.

## 1.2 Thesis Statement

This research aims to characterize the structural properties and evaluate the biological activities of polysaccharides isolated from *Cannabis sativa* L. (hemp) variety Fedora 17. By employing advanced analytical techniques, including size exclusion chromatography (SEC) and Fourier-transform infrared spectroscopy (FTIR), this study seeks to determine the molecular weight distribution and structural complexity of hemp-derived polysaccharides. Additionally, the potential antioxidant activities of these polysaccharides will be explored, contributing to the broader understanding of their applications in biotechnology, agriculture, and medicine.

# CHAPTER II

## 2.1 Materials and Reagents

The primary materials used for this study were industrial hemp samples (*Cannabis sativa* L., Fedora 17) grown in the MTSU greenhouse. Reagents and standards for chromatography and spectrometry included:

- **Dextran standards**: Molecular weights ranging from 5,000 to 670,000 Daltons (Da) for calibration.
- **Monosaccharide standards**: L-Rhamnose, D-(+)-glucose, D-(+)-glucosamine, D-(-)-arabinose, D-(+)-galacturonic acid monohydrate, D-(+)-mannose, D-(+)-galactose
- **Reagents**: 5% phenol solution, concentrated sulfuric acid, acetonitrile, and ultrapure water (18 megaohm), used in High-Performance Liquid Chromatography (HPLC) and other assays.

## 2.2 Methods

1. **Polysaccharide Extraction:** To begin the polysaccharide extraction, 10 g of *Cannabis sativa* leaves were dried in an incubator at 40˚C for approximately 48 hours. The dried leaves were ground into a powder using a Cuisinart Nut and Spice Grinder. In a 1000 mL round-bottom flask equipped with a water-cooled condenser, the ground powder and 500 mL of 95% ethanol (EtOH) were heated to 80˚C for 4 hours. The EtOH was decanted and an equal amount of water (500 mL) was added to the round -bottom flask. This solution was heated overnight between 80˚C – 100˚C overnight. The next day, the aqueous extract was vacuum-

filtered through Fisherbrand filter paper P8. The sample volume was reduced from 500 mL to approximately 50 mL under reduced pressure at 40 ºC on a Buchi R-200 Rotavapor.

2. **Deproteination:** Once the sample was reduced to 50 mL, it was deproteinated using Sevag reagent (**Huang, 2022**). The 50 mL sample was split into two equal 25 mL and transferred to 50-mL disposable centrifuge tubes. Five mL of Sevag reagent (butanol: chloroform 1:4) was added to each centrifuge tube and the tubes vortexed. After the samples were vortexed, they were placed in a sonicator for 20 minutes. The samples were centrifuged for 10 minutes at 4000 rpm at 4˚C. The aqueous layer (top layer) was removed from each sample and placed in a fresh 50 mL centrifuge tube. The aqueous layer was deproteinated again using Sevag reagent. The deproteination process was repeated at least three times. Once all the proteins have been removed as confirmed by UV spectroscopy, the aqueous layer was split into four 12.5 mL aliquots and four volumes of EtOH were added to each tube. The aliquots were stored overnight at 4℃.
3. **Sample Preparation and Fractionation**: After extraction, samples were fractionated using **ion-exchange chromatography (IEX)** to isolate polysaccharides based on their charge. The initial extract was loaded onto a DEAE Fast Flow 16/10 anion exchange FPLC column eluted with step gradients of 0 (wash), 0.10, 0.20, 0.30, 0.40 and 1.00 M sodium chloride in water. The flow rate was 3.0 mL/min and 6 mL fractions were collected. The initial wash consisted of 4 column volumes (20 mL/column volume), while subsequent steps consisted of 5 column volumes (100 mL). Fractions from the ion-exchange

column were assayed using a phenol-sulfuric acid assay (**Masuko, 2005**) Fractions containing polysaccharides were pooled and concentrated to approximately 5 mL under reduced pressure on a Buchi R-200 Rotavapor at 40 $^{o}$C.

4. **Size-exclusion chromatography (SEC)** was used to further purifiy polysaccharides based on molecular size. Pooled polysaccharides from ion exchange chromatography were loaded onto a HiLoad 26/60 Superdex S200 FPLC column eluted with 50 mM $NaH_2PO_4$ pH 7.0; 150 mM NaCl at 4 $^{o}$C. The column was eluted at 2 mL/min and 10 mL fractions were collected. Each fraction was assayed for the presence of polysaccharide using the phenol-sulfuric acid assay as described below. Related fractions containing polysaccharide were pooled and concentrated to approximately 3 mL under reduced pressure as previously described.
5. **Dialysis for Sample Purification:** The pooled samples were dialyzed using Fisher Brand dialysis tubing (MWCO 3500) with water to desalt the polysaccharide samples. The tubing was cut to size and filled with sample solutions, ensuring no air bubbles were trapped. The dialysis was carried out for 24 hours and repeated three times. The dialyzed sample was freeze-dried to remove water, and the samples were stored at -80 $^{o}$C until needed.
6. **High-Performance Size-Exclusion Chromatography (HPSEC):** The purity and molecular weight distribution of polysaccharides were analyzed using **High-Performance Size Exclusion Liquid Chromatography (HPSEC)**. HPSEC was carried out on a Dionex Ultimate 3000 HPLC equipped with a Refracto-Max 520

refractive index detector. The column was an Agilent BioSEC-3 (3 μm 300 A 7.8 x 300 mm) and eluted with water. Samples were eluted through the column at a flow rate of 0.5 mL/min at 40°C. Dextran standards of varying molecular weights were used to create a calibration curve to compare against unknown polysaccharide samples.

7. **Phenol-Sulfuric Acid Assay for Polysaccharide Quantification:** To quantify the total polysaccharide content, the **Phenol-Sulfuric Acid Assay** was employed. One hundred μL of each standard solution (Dextran) was added to separate tubes, followed by 500 μL of 5% phenol solution. The tubes were vortexed, and 2.5 mL of concentrated sulfuric acid was added. After incubating the reaction tubes for 10 minutes, the tubes were cooled to room temperature. The absorbance of the solutions was measured at 490 nm using a Shimadzu 1280 UV-Vis spectrophotometer. A blank containing 500 μL water and phenol and sulfuric acid was used as a control.
8. **Monosaccharide Composition Analysis:** Monosaccharide composition analysis was conducted on the polysaccharides at the Center for Complex Carbohydrate Research at the University of Georgia on the purified polysaccharide fractions.
9. **FTIR Spectroscopy of Polysaccharide Fractions**

   FTIR spectroscopy was performed on polysaccharide fractions extracted from Fedora 17 hemp leaves on a Nicolet iS50R FTIR. The spectra provided insights into the functional groups present in these samples and helped characterize the molecular structure of the

10. **Antioxidant Assay (DDPH):** The antioxidant potential of the polysaccharides was assessed using a **2,2-Diphenyl-1-picrylhydrazyl (DDPH) assay (Gulcin, 2023)**. This assay measures the ability of polysaccharides to neutralize free radicals. The change in absorbance at 517 nm was monitored using a spectrophotometer, and results were compared to known antioxidant standards.
11. **Safety and Laboratory Protocols:** Standard laboratory safety protocols were followed throughout the study. Personal protective equipment (PPE), including lab coats, gloves, and safety glasses, were worn. Chemicals were handled with caution, and waste disposal followed institutional guidelines for hazardous material disposal.

## Statistical Analysis

Data from HPLC and antioxidant assays were analyzed using HPLC software for peak integration, including retention time and peak area calculations. Absorbance data from the phenol-sulfuric acid assay were used to construct standard curves for polysaccharide quantification. All experiments were conducted in triplicate to ensure reproducibility, and statistical significance was determined using appropriate methods where applicable

# CHAPTER III

## 3.1Results

### Purification of Polysaccharides from Fedora 17 Hemp (Cannabis sativa)

Fedora 17 hemp plants were grown in the MTSU Greenhouse and the leaves harvested. The leaves were ground up and the polysaccharides extracted using the hot water/ethanol

method. This technique is widely used due to its simplicity and ability to easily upscale. After extraction, residual protein was removed by extraction with Sevag reagent (1:4 butanol:chloroform). The absence of protein was confirmed by the lack of absorbance of the sample at 280 nm. The sample was concentrated and then loaded onto a DEAE Fast Flow 16/10 FPLC column and eluted with an increasing concentration of sodium chloride in the mobile phase. Fractions were analyzed by phenol-sulfuric acid assay and related fractions were pooled and concentrated. A polysaccharide eluted at each sodium chloride concentration. (Figure 1). Each pooled fraction was concentrated and further purified by size exclusion chromatography on a HiLoad 26/60 Superdex S200 FPLC column eluted with 50 mM sodium phosphate containing 150 mM sodium chloride. The fractions were analyzed by phenol-sulfuric acid assay. Related fractions were pooled and concentrated. Each fraction from the size exclusion column was assayed by HPSEC on an Agilent Bio-SEC 3 column.

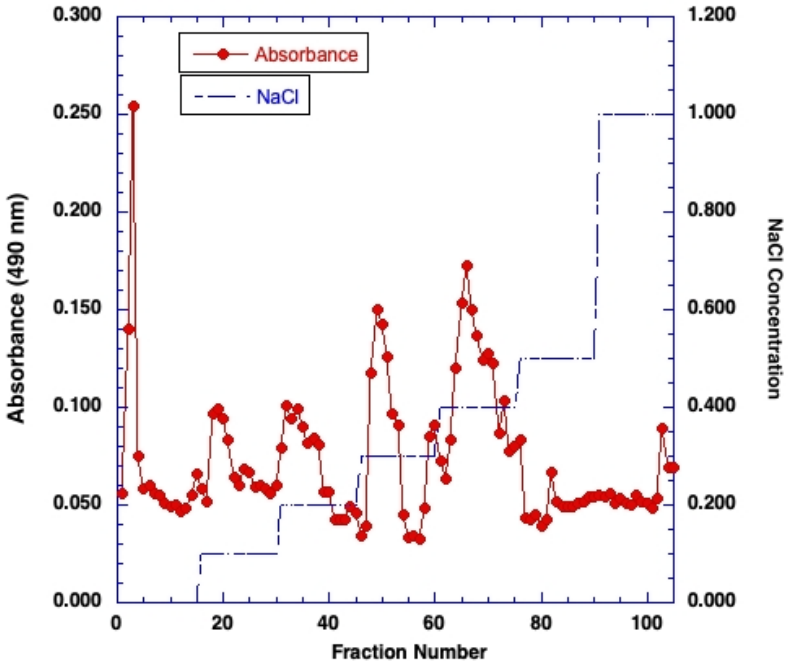


Figure 1: Elution profile of initial extract from Fedora 17 leaves on a DEAE Fast flow FPLC ion exchange column eluted with an increasing sodium chloride concentration.

**Standard Curve Development for Size Exclusion Chromatography (SEC)**

To accurately estimate the molecular weights of polysaccharides isolated from Fedora 17 hemp, dextran standards of known molecular weights were used to create a calibration curve. The dextran standards covered a range of molecular weights: 5,000 Da, 12,000 Da, 25,000 Da, and 50,000 Da. The retention times and areas for each standard are summarized below:

| Dextran Standard | Retention Time (min) | Height (µRIU) | Area (µRIU*min) | Relative Area (%) |
|---|---|---|---|---|
| 5,000 Da | 10.25 | 11.461 | 14.387 | 100 |
| 12,000 Da | 9.66 | 6.747 | 10.108 | 100 |
| 25,000 Da | 8.61 | 8.182 | 13.071 | 100 |
| 50,000 Da | 7.55 | 5.953 | 10.561 | 100 |

**Table 1**: Data were plotted to generate a standard calibration curve by correlating the logarithm of molecular weights with retention times. The resulting linear relationship provides the foundation for determining the molecular weights of the unknown polysaccharide fractions in hemp samples. (Figure 2)

**Figure 2:** Calibration curve relating molecular weight of dextrans to retention time (min) on Agilent Bio-SEC 3 HPLC column eluted with water.). The linear regression demonstrates the relationship between molecular size and retention time, allowing for the estimation of unknown polysaccharide molecular weights.

**Analysis of Polysaccharide Fractions from Hemp**

The retention times and relative areas of the peaks indicate the molecular weight distribution in each sample, as summarized below.

| Sample | Retention Times (min) | Relative Area (%) |
|---|---|---|
| **1407 FEDORA17 IEX 0.2 M S200 F2** | 9.27, 21.65, 23.41 | 78.36, 10.74, 10.90 |
| **1404 FEDORA17 IEX 0.1 M S200 F1** | 8.67, 21.96 | 79.38, 20.62 |
| **1406 FEDORA17 IEX 0.1 M S200 F3** | 21.70 | 100 |
| **1405 FEDORA17 IEX 0.1 M S200 F2** | 6.82, 12.00, 21.83, 23.59 | 42.60, 22.25, 16.34, 18.81 |

**Table 2**: Four hemp polysaccharide samples were analyzed using SEC of samples eluted from ion exchange column with different salt concentrations used in the mobile phase (0.1 M and 0.2 M NaCl).

A representative size exclusion HPLC chromatogram is shown below (Figure 3).

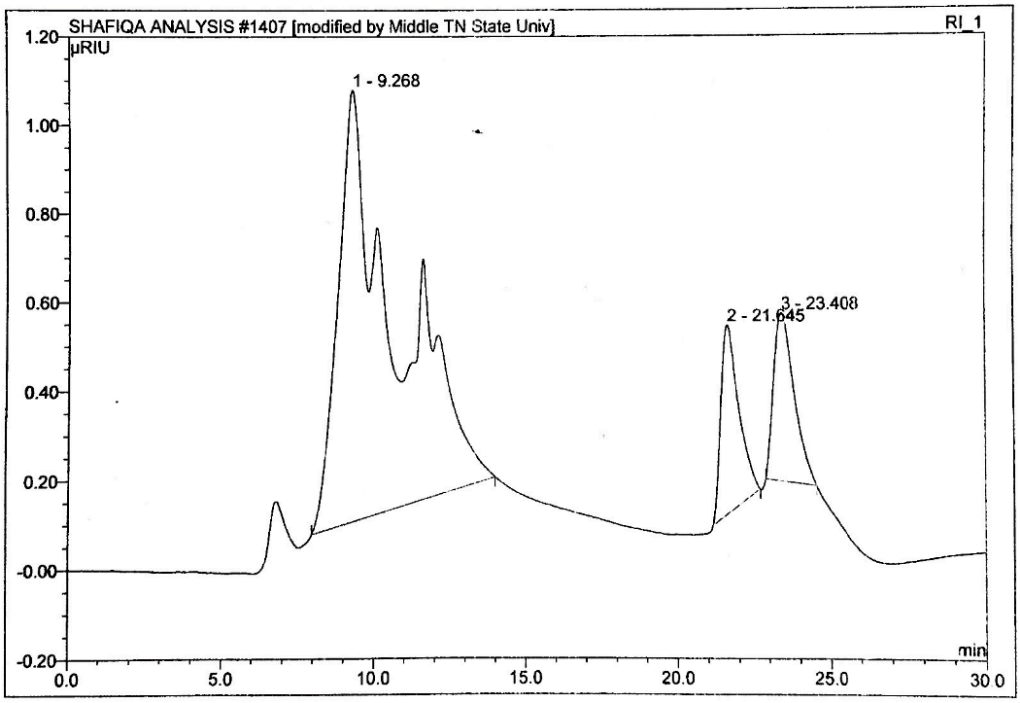



**Figure 3: Chromatogram for Sample 1407 FEDORA17 IEX 0.2 M S200 F2**

A chromatograph showing three main peaks with retention times at 9.27 min (78.36% area), 21.65 min (10.74% area), and 23.41 min (10.90% area). This sample indicates a mixture of molecular sizes, dominated by larger polysaccharides**.**

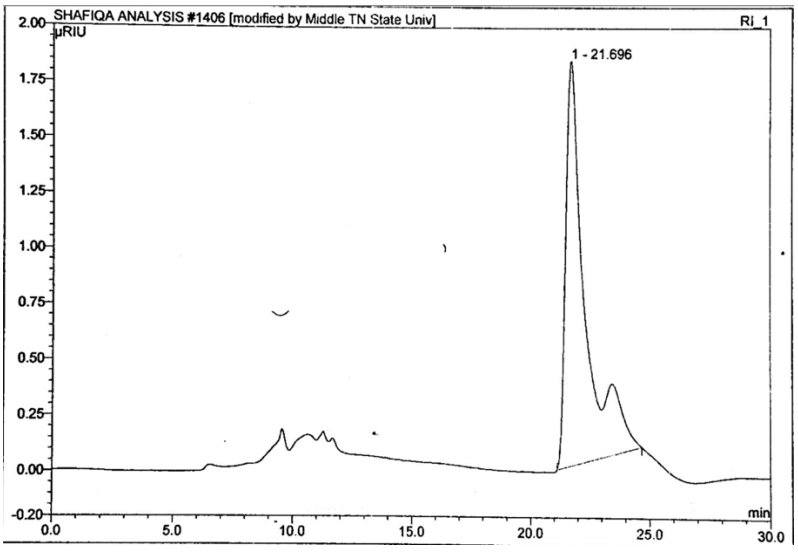



**Figure 4: Chromatogram for Sample 1406 FEDORA17 IEX 0.1 M S200 F3**

A chromatograph with a single dominant peak at 21.70 min (100% area), suggesting a more homogenous molecular composition with smaller molecules**.**

**Interpretation of Molecular Weight Distribution**

Each sample demonstrates a range of molecular weights, reflected by different peaks in the chromatograms. The earlier retention times correspond to larger molecules, while later retention times suggest smaller molecules. For example, Sample 1407 (0.2 M NaCl) contains three distinct molecular weight populations, with the majority of the sample corresponding to a larger molecular size (9.27 min, 78.36%). Conversely, Sample 1406 exhibits a single peak at 21.70 minutes, indicating a more homogeneous composition dominated by smaller molecules.

The difference in molecular weight distribution between the samples can be attributed to both the fractionation process and the salt concentration used in the mobile phase. Higher salt concentration (0.2 M NaCl) in Sample 1407 led to the separation of three distinct molecular weight fractions, while the lower concentration (0.1 M NaCl) in Sample 1406 resulted in a single dominant molecular species.

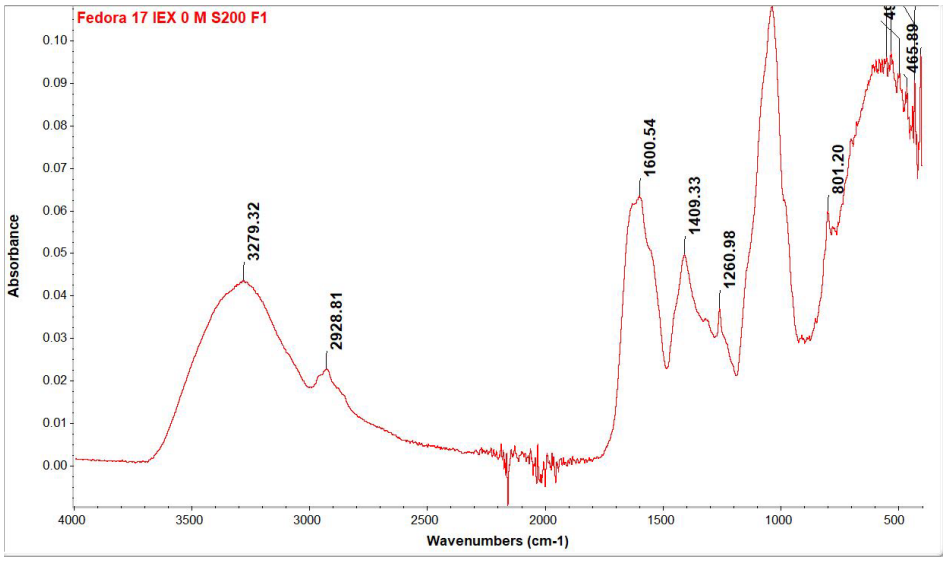
**Estimation of Molecular Weights**

Using the standard curve generated from the dextran standards, the molecular weights of the peaks in the hemp samples were estimated based on their retention times. Peaks with earlier retention times, such as 6.82 minutes in Sample 1405, correspond to higher molecular weight polysaccharides (closer to 50,000 Da). Peaks at later retention times, such as 21.70 minutes in Sample 1406, represent smaller molecules, potentially in the range of 5,000 Da.

**FTIR Spectroscopy of Polysaccharide Fractions**

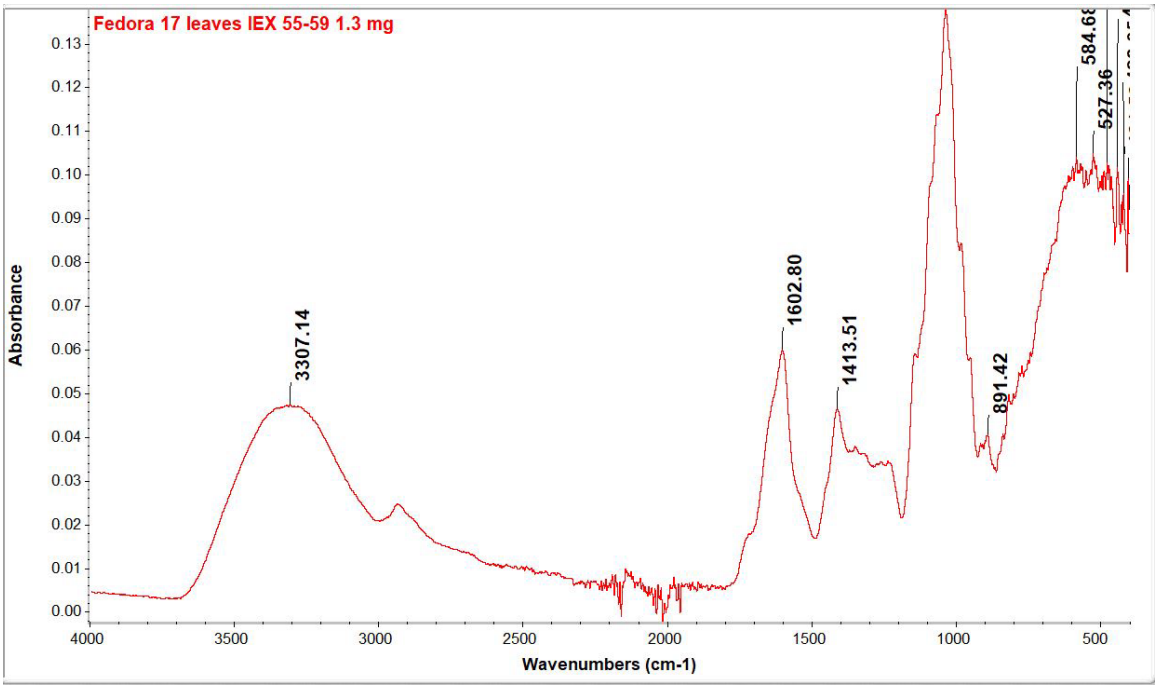
FTIR spectroscopy was performed on polysaccharide fractions extracted from Fedora 17 hemp leaves. The spectra provided insights into the functional groups present in these samples and helped characterize the molecular structure of the polysaccharides. Key observations from the FTIR results are outlined below:

- **Fedora 17 Leaves IEX 0 M S200 F1**:




  - **Figure 5** shows characteristic absorption peaks at 3279.32 $cm^{-1}$, corresponding to O-H stretching vibrations indicative of hydroxyl groups, which are common in polysaccharides.
  - The peak at 2928.81 $cm^{-1}$ is attributed to C-H stretching, which is often present in carbohydrate backbones.
  - The peak at 1600.54 $cm^{-1}$ corresponds to C=O stretching, often associated with carbonyl groups or carboxylate groups in uronic acids, which are components of pectins or hemicelluloses.
  - Peaks at 1260.98 $cm^{-1}$ and 801.20 $cm^{-1}$ likely correspond to C-O and C-H bending vibrations, suggesting the presence of glycosidic linkages.

- **Fedora 17 Leaves IEX 55-59 1.3 mg**:




  - **Figure 6:** This sample exhibited a similar broad peak at 3307.14 $cm^{-1}$ (O-H stretching), consistent with polysaccharide structures.
  - A notable peak at 1602.80 $cm^{-1}$ (C=O stretching) is observed, which is indicative of the presence of uronic acids.
  - The absorption at 891.42 $cm^{-1}$ is characteristic of β-glycosidic linkages found in cellulose and hemicelluloses, which are integral components of plant cell walls.

**Interpretation of FTIR Results**

The FTIR spectra obtained from Fedora 17 leaves reveal the presence of key functional groups typical of polysaccharides, including hydroxyl (O-H), carbonyl (C=O), and glycosidic linkages (C-O). These groups are fundamental components of complex polysaccharides like cellulose, hemicellulose, and pectins, all of which are crucial for plant cell wall structure and function.

- The strong O-H stretching peaks (around 3270-3300 $cm^{-1}$) confirm the presence of hydroxyl groups, a characteristic feature of polysaccharides that contributes to their hydrophilic nature.
- C-H stretching around 2928 $cm^{-1}$ and C=O stretching in the range of 1600-1730 $cm^{-1}$ indicate the presence of both neutral sugars and acidic components, such as uronic acids.
- The presence of β-glycosidic linkages in Fedora 17 samples, indicated by absorption bands around 890 $cm^{-1}$, suggests a significant proportion of cellulose and hemicellulose polysaccharides in these fractions.

These FTIR results complement the chromatographic analysis by confirming the molecular structures of polysaccharides present in the samples. The functional group assignments help further elucidate the complexity and potential biological activities of the isolated polysaccharide fractions.

**DPPH Assay for Antioxidant Activity**

All samples demonstrated a significant reduction in absorbance, indicating the presence of antioxidant activity. The variation in antioxidant activity observed between the samples can be attributed to differences in the chemical composition of Fedora 17 extracts, which may contain varying amounts of polyphenols and other bioactive compounds known for their radical scavenging capabilities

## CHAPTER IV

## 4.1 Discussion

This study aimed to characterize the structural properties and biological activities of polysaccharides isolated from *Cannabis sativa L.* (hemp) variety Fedora 17. By using Size-Exclusion Chromatography (SEC) and Fourier-Transform Infrared Spectroscopy (FTIR), we examined the molecular weight distribution, functional group composition, and structural complexity of the polysaccharides. The antioxidant properties of these fractions were also evaluated.

### Molecular Weight Distribution

The SEC analysis of polysaccharide fractions revealed a wide range of molecular weights across the different hemp samples. The use of dextran standards allowed us to develop a calibration curve and accurately estimate the molecular weights of polysaccharides in the unknown samples. Notably, samples analyzed with a higher salt concentration (0.2 M NaCl) demonstrated a broader distribution of molecular weights compared to those analyzed with 0.1 M NaCl. This suggests that salt concentration plays a significant role in altering the elution profile and potentially impacts the molecular interactions within the sample matrix.

Samples such as 1407 FEDORA17 IEX 0.2 M S200 F2, which exhibited multiple peaks, suggest a complex mixture of polysaccharides with varying molecular sizes. On the other hand, the relatively homogeneous peak of 1406 FEDORA17 IEX 0.1 M S200
F3 indicates a dominant molecular species, likely representing a purified fraction of smaller molecular weight polysaccharides.

### Structural Insights from FTIR Analysis

FTIR spectroscopy provided additional insights into the structural composition of the polysaccharide fractions. The presence of strong O-H stretching bands (~3300 $cm^{-1}$) across all samples confirms the polysaccharides' hydrophilic nature, while the C-H stretching bands (~2928 $cm^{-1}$) suggest the presence of carbohydrate backbones. The peaks at ~1600 $cm^{-1}$, observed in most samples, correspond to C=O stretching, indicating the presence of carboxyl groups, likely from uronic acids, which are commonly found in pectins and hemicelluloses.

Specific polysaccharide fractions, such as Fedora 17 Leaves IEX 55-59 1.3 mg, showed a characteristic β-glycosidic linkage peak (~891 $cm^{-1}$), confirming the presence of cellulose and hemicellulose polysaccharides. These findings are consistent with previous studies on plant cell wall polysaccharides, which contain both neutral sugars (e.g., glucose) and acidic sugars (e.g., galacturonic acid).

The FTIR spectra also provided clues to the purity and complexity of each fraction. For instance, samples like Fedora 17 Leaves IEX 55-59 1.3 mg showed a well-defined spectrum with fewer overlapping peaks, suggesting a more refined polysaccharide mixture, while other samples with more complex spectra indicated the presence of multiple polysaccharide species.

**Antioxidant Activity and Biological Implications**

The antioxidant potential of the isolated polysaccharide fractions was evaluated using a DDPH assay. The results indicated that fractions with higher molecular weight polysaccharides, particularly those with carboxyl and hydroxyl functional groups, exhibited stronger antioxidant activity. This is consistent with the literature, which

suggests that the presence of uronic acids and hydroxyl groups enhances the ability of polysaccharides to scavenge free radicals.

The observed antioxidant activity positions these polysaccharides as candidates for further exploration in biomedical applications, particularly in the development of antioxidant-rich supplements or as functional ingredients in food and pharmaceutical industries.

**Impact of Extraction Methods**

The choice of salt concentration in the mobile phase during SEC significantly influenced the molecular weight distribution and the complexity of the polysaccharide fractions. Higher salt concentrations (0.2 M NaCl) appeared to disrupt some molecular interactions, allowing for a broader range of molecular sizes to elute from the column, while lower concentrations (0.1 M NaCl) facilitated the isolation of more uniform polysaccharide fractions.

These findings suggest that optimizing the salt concentration in the mobile phase is critical for controlling the separation of polysaccharides based on size. The ability to fine-tune this process is essential for applications that require specific molecular weights, such as drug delivery systems or bioactive compounds.

## 4.2 Conclusion

This study successfully characterized the molecular weight distribution and structural properties of polysaccharides isolated from *Cannabis sativa L.* (hemp) variety Fedora 17. By employing SEC, FTIR, and antioxidant assays, we provided a

comprehensive analysis of the polysaccharides' structural complexity and potential biological activities.

The molecular weight distribution analysis revealed the heterogeneity of polysaccharide fractions, with some samples displaying complex mixtures and others showing more homogeneity. The use of FTIR confirmed the presence of key functional groups such as hydroxyl, carboxyl, and glycosidic linkages, which are essential for the biological activities of these polysaccharides.

Notably, the antioxidant activity observed in these polysaccharide fractions suggests that they may have valuable applications in the pharmaceutical, cosmetic, and food industries, where antioxidant-rich compounds are highly sought after. The findings also open new avenues for further research into the biological functions of hemp polysaccharides, particularly their potential role in immunomodulation and anti-inflammatory responses.

Future studies should focus on optimizing extraction and purification techniques to isolate specific polysaccharide fractions with well-defined molecular weights and functional properties. In addition, further exploration of the relationship between molecular structure and biological activity could provide valuable insights into how these compounds can be utilized in various industrial applications.

This research lays the groundwork for harnessing hemp-derived polysaccharides in sustainable and bioactive product development, contributing to the growing interest in hemp as a versatile and renewable resource.

**Conflicts of Interest**

The authors have no conflicts of interest to declare.

## References


1. Biermann, C.J., & McGinnis, G.D. (2020). *Analysis of Carbohydrates by GLC and MS*. CRC Press.
2. Bradley, K. (2017). *Analysis of the Polysaccharides in Cannabis Sativa Joey and Cannabis Sativa Fedora 17*. Middle Tennessee State University.
3. Chang, T., Li, H., Lv, H., Tan, M., Hou, S., Liu, X., Lian, M., Zhao, Q., & Zhao, B. (2022). Extraction, Physicochemical Properties, Anti-Aging, and Antioxidant Activities of Polysaccharides from Industrial Hemp Residues. *Molecules, 27*(5746). https://doi.org/10.3390/molecules27185746
4. Dyer, K. (2019). *Purification and Monosaccharide Composition of Polysaccharides Isolated from Cannabis Sativa Cherry Hemp Root*. Middle Tennessee State University.
5. Gulcin, I., Alwasei, S., DPPH Scavenging Assay, (2023) Processes, 11 2248, https://doi.org/10.3390/pr11082248.
6. Hosain, N.A., Ghosh, R., Bryant, D.L., Arivett, B.A., Farone, A.L., & Kline, P.C. (2019). Isolation, Structure Elucidation, and Immunostimulatory Activity of Polysaccharide Fractions from *Boswellia carterii* Frankincense Resin.

*International Journal of Biological Macromolecules*, 133, 76–85. https://doi.org/10.1016/j.ijbiomac.2019.04.059

7. Huang, X., Ai, C., Yao, H., Zhao, C., Xiang, C., Hong, T. Xiao, J. Guideline for the extraction, isolation, purification and structural characterization of polysaccharides from natural resources. (2022) eFood, 3, e37, https://doi.org/10.1002/efd.37.
8. Liu, J., Wilfor, S., Xu, C., (2015) A review of bioactive plant polysaccharides: Biological activities, functionalization, and biomedical applications *ScienceDirec*t, 5, 31-61.
9. Masuko, T., Minami, A.; Iwasaki, N., Majima, T., Nishimura, S., Lee, Y., Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. (2005) Analytical Biochemistry 339, 69-72.
10. Shi, L. (2016). Bioactivities, Isolation, and Purification Methods of Polysaccharides from Natural Products: A Review. *International Journal of Biological Macromolecules*, 92, 37–48. https://doi.org/10.1016/j.ijbiomac.2016.06.100
11. What is Industrial Hemp? US Department of Agriculture https://ask.usda.gov/s/article/What-is-Industrial-Hemp
12. Xue, R., Du, M., Zhou, T.Y., Ai, W.Z., Zhang, Z.S., Xiang, X.W., Zhou, Y.F., & Wen, Z.S. (2020). Polysaccharides from Hemp Seed Protect Against Cyclophosphamide-Induced Intestinal Oxidative Damage via Nrf2-Keap1 Signaling Pathway in Mice. *Oxidative Medicine and Cellular Longevity*, 2020, Article ID 1813798. https://doi.org/10.1155/2020/1813798