**Separation of Lignin from Cannabis Sativa L Biomass by an Environmentally Safe Alkaline Method and its Structural Analysis**

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**Abstract. This scientific article describes the chemical-alkaline method for extracting lignin from Cannabis Sativa L. The plant stem was selected as the object in the study, and sodium hydroxide (NaOH) solution was used to extract lignin. A systematic analysis was conducted on the effects of the processing parameters: concentration (1–7%), process temperature (60–140°C) and duration (30–140 minutes) on lignin release and the degree of cellulose polymerization. The experimental results showed a decrease in the lignin content from 9.6% to 1.1%, which confirms the effect of the hydrolytic and saponification process of the NaOH solution. It was also found that high temperature and long processing time negatively affected the degree of cellulose polymerization, and optimal results were achieved with 3% alkali concentration, 100°C temperature and 60 minutes of processing. FTIR (Fourier Transform Infrared Spectroscopy) analysis was used to determine the release of lignin from the plant stem. In the infrared spectrum of the original plant, lignin-specific vibrations such as phenolic –OH, aromatic C=C, and ether C–O–C groups were detected, and the presence of G/S (guaiacyl/syringyl) units was proven. The decrease in the intensity of the FTIR peaks after extraction chemically confirmed the decrease in the lignin content. This study demonstrates the possibility of extracting lignin from Cannabis sativa L in an environmentally safe, effective and economically viable way.**

**Keywords: Cannabis Sativa L, lignin, FTIR spectroscopy, alkali, fiber**

**INTRODUCTION**

**The development of polyols (i.e., alcohols with multiple hydroxyl groups) from renewable natural sources, mainly vegetable oils (e.g., soybean, flax, sunflower, Cannabis Sativa L) or cellulose and other biomass-based materials, is of great interest to researchers, especially since many laws now require the use of "green" chemicals to reduce environmental pollution [1]. To achieve this requirement, environmentally friendly, sustainable raw materials are needed that have little or no negative impact on the economy and food security. A raw material that can fully meet this requirement is lignocellulosic materials [2].**

The Cannabis sativa L plant has served as a valuable natural resource for humans since ancient times, finding application in areas such as fiber production, medicine, and various technical fields. Its fibers are particularly appreciated in industries like construction and textiles due to their notable strength, low weight, and durability. Among the key factors influencing fiber quality and application range are their physicochemical properties—most importantly, the lignin content [3].

Lignin is a critical structural component of the plant cell wall. It enhances the mechanical strength of fibers and plays a protective role by resisting microbial degradation and decay. Measuring lignin levels is essential for optimizing fiber processing techniques, improving product quality, and developing innovative treatment methods.

From a morphological standpoint, cellulose is composed of both crystalline and amorphous regions. The crystalline phase makes up the majority of cellulose, while the amorphous regions are less ordered and form a smaller portion of the structure. **Unlike glucose in other glucan (a polymer consisting of carbohydrates, the basis of which is glucose), the disaccharide cellobiose is involved as a repeating unit in the cellulose chain.**

**Cellulose molecules contain internal and external hydrogen bonds, which firmly connect the glucose units to each other. Chemically, it is a straight-chain homopolymer composed of D-glucopyranose units linked by 1,4-glucosidic bonds.**

Cellulose is composed mainly of carbon (44%), oxygen (49%), and hydrogen (7%), and is represented by the general formula (C₆H₁₀O₅)ₙ. Here, n is the degree of polymerization, which indicates the number of glucose units in the cellulose chain and can range from hundreds to thousands. For example, the degree of polymerization in cellulose in wood can reach 10,000, and in natural cotton - up to 15,000.

The main components of biomass are: hemicellulose, lignin, and polysaccharides formed by their cross-linking.

The second rich component of the industry lignocellulosic biomass is hemicellulose which constitutes approximately 25 to 35 percent of the dry mass of the biomass. It is much more reactive to heats and chemicals, as opposed to cellulose, because of their unstructured manner of arrangement at the molecular-level. Its major significance lies within the structure of plant cell walls, which basically acts as an interconnective medium between cellulose microfibrils and makes associations with lignin molecules and delivers security as well as strength to the whole plant tissue [4, 5].

Hemicellulose is distinctly different from cellulose by a chemical perspective. It does not have a linear and crystalline structure like cellulose and instead occurs in a disordered, amorphous form. Its chemical composition is xylan, galactomannan, arabinoxylan, glucomannan, xyloglucan, glucuronoxylan, and several acetylated polysaccharides. Hemicellulose molecules are usually linked together by β-(1,4)-glycosidic bonds, and rarely by β-(1,3)-glycosidic bonds. Xylans are found in most plant species and are composed of chains of β-D-xylopyranose units, with arabinose, glucuronic acid or its 4-O-methyl ester, acetate, ferulic, and p-coumaric acids attached to these chains. The composition of hemicellulose varies depending on the raw material, that is, the type of plant, with conifers usually containing xylans, while broadleaf trees mainly contain glucomannans.

The third major component of biomass is lignin, a complex, irregular, and loosely branched polymer composed of phenylpropane compounds. Lignin acts as a “cellular glue” in plant structures, making the cell wall resistant to external influences, giving strength and rigidity to the fibers, and also protecting the plant from insects, pathogens, and oxidants [6].

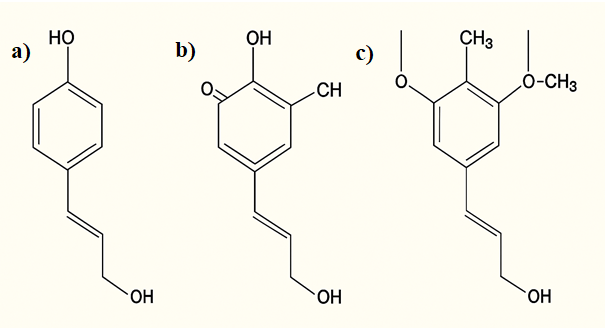
The main monomers of lignin are: -coumaryl alcohol, -coniferyl alcohol and -sinapy alcohol, and these monomers form the following structural units: p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively [7].

The composition of lignin also varies depending on the plant species as follows:

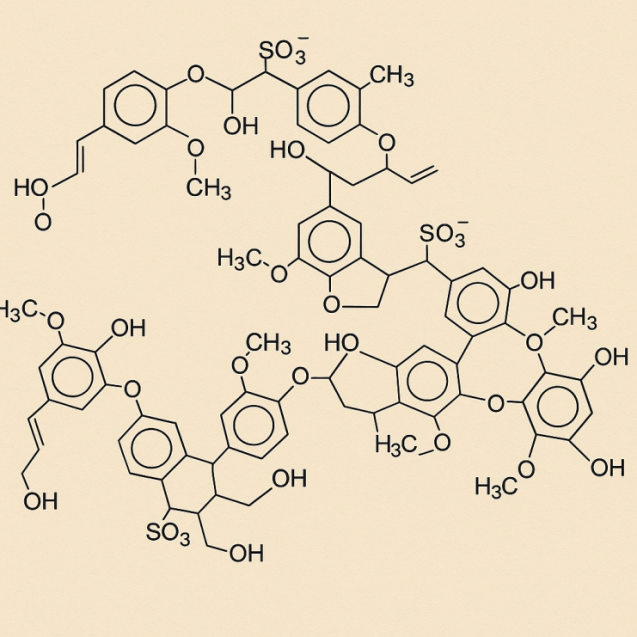
* Conifers predominantly produce guaiacyl-type (G) lignin;
* Dicots (polysperms) typically contain a mix of guaiacyl and syringyl (G–S) units;
* Monocots (monosperms) feature a more diverse G–S–H pattern, incorporating all three monolignol-derived units [8].

There are various chemical and hydrogen bonds between cellulose, hemicellulose and lignin, and cellulose fibers are surrounded by lignin and hemicellulose, which are mainly connected to each other through hydrogen bonds. There are also chemical bonds between hemicellulose and lignin, which are formed through galactose and arabinose molecules [9, 10].

Scientists [11] note that lignin is a biopolymer with a highly branched and structurally complex structure, the chemical composition of which varies depending on the plant. It is one of the most abundant natural polymers in plant biomass, its structure is amorphous and disordered, and it is composed of phenylpropanoid monomers. These monomers are asymmetrically linked to each other in a three-dimensional network structure through C–C (carbon–carbon) and C–O (carbon–oxygen) bonds. Lignin is composed of three main phenylpropane compounds, namely p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol (Figure 1), which are called “monolignols,” and these monomers combine to form the basic structure of lignin.



**FIGURE 1.** Empirical formulas of phenylpropane compounds:a) p-coumaryl alcohol; b) sinapyl alcohol; c) coniferyl alcohol.



**FIGURE 2.** Complex structural model of a putative lignosulfonate in coniferous wood

The monomer composition of lignin in different plant species differs, as well as the ways in which they are interconnected. This further complicates the overall structure of the lignin molecule. The most widely used type of lignin in industry is lignosulfonate, the structure of which is presented in Figure 2.

It should be noted that the study of lignin in its pure form in its natural state is a very difficult task. Because after lignin is extracted, its molecules break down into fragments with different structures or form covalent bonds with carbohydrate compounds. This leads to a change in the three-dimensional network structure of lignin.

Nevertheless, many studies conducted in recent years have made it possible to determine the presence of different types of covalent bonds based on different samples of lignin [12].

An analysis of scientific research in recent years has shown that the processes of lignin separation and its various extraction methods have been covered in detail in many studies [13]. In addition, the possibilities of using lignin-based biomaterials in the production of electroactive devices and specialty chemicals have also been studied in depth [14]. Studies have revealed the prospects for using porous carbon materials derived from lignin in a wide range of energy storage technologies, including batteries, fuels, solar cells, and supercapacitors [15]. However, there are a number of problems associated with the purity and structural composition of lignin-based materials in the production of lignin-based materials.

The Global Chemicals Outlook II report published by the United Nations noted that the scientific community is increasingly focusing on “green” chemicals. Therefore, scientists have recommended using lignin as one of the main energy sources in the paper industry, namely as a fuel for heating steam boilers in the cooking process [16].

In addition, lignosulfonates, which are formed during the sulfide cooking process of lignin, are widely used as plasticizers in the cement and concrete industries. This indicates that the scope of lignin is expanding. However, in recent years, as a result of the decrease in the number of sulfide processing plants, the volume of lignosulfonate production has decreased.

Nevertheless, lignin is of great interest in the fields of modern chemistry and materials science. In particular, its conversion into high-value products for the production of phenols, activated carbon and carbon fibers is considered a promising direction. This further increases the relevance of lignin as an environmentally friendly alternative raw material [16].

**METHODOLOGICAL PART**

The object of this research work is the plant Cannabis sativa L. Among the various methods used to extract lignin from plant biomass, the chemical-alkaline method stands out as one of the simplest and most economical methods. This process is based on reagents such as sodium hydroxide (NaOH), potassium hydroxide (KOH) or ammonium hydroxide (NH₄OH) to hydrolyze and dissolve lignin from the plant.

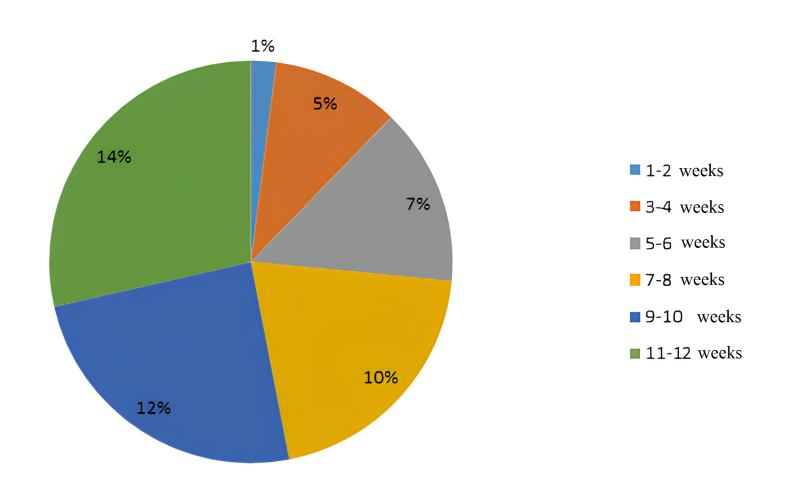
Extraction of lignin from the plant is carried out in laboratory conditions at a temperature of 70 to 140 ° C, a pH of 10-11, and a process duration of 1 to 4 hours.

One of the main advantages of the alkaline extraction process is that it easily dissolves lignin, is environmentally friendly and has easily controlled process conditions, therefore, in this research work, the alkaline method was selected as the most suitable method for extracting lignin from Cannabis sativa L.

**RESULTS AND DISCUSSION**

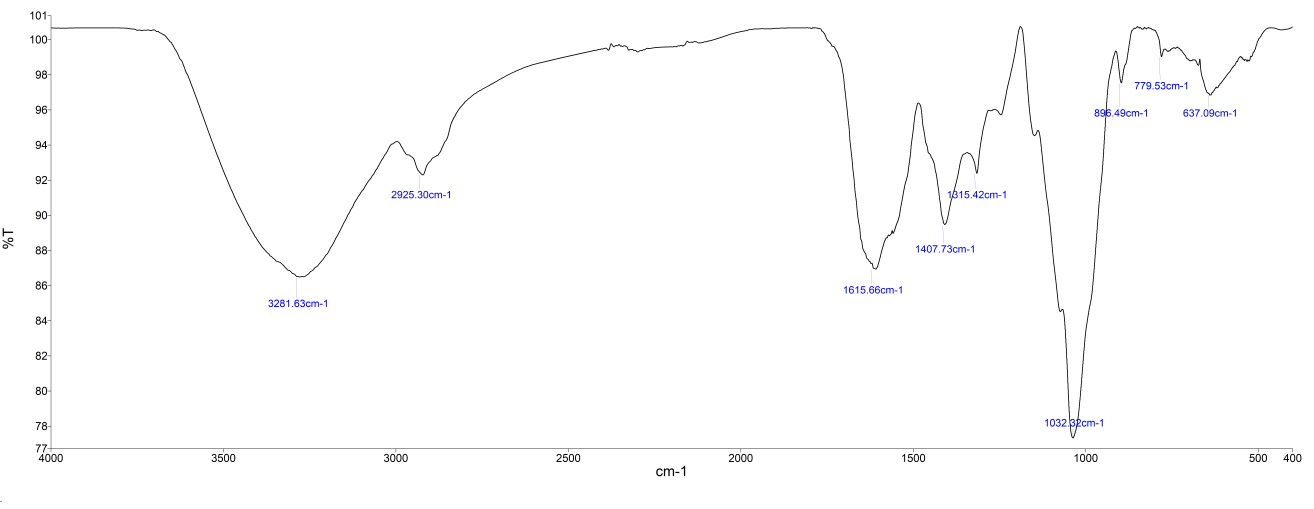
Cannabis Sativa L plant is widely studied as a fiber plant, and determining the amount of lignin in its components is important in assessing the quality of the fiber. Different anatomical parts of the plant - leaves, stems and roots - have significant differences in lignocellulose content.

The relative amount of lignin in the stems is high, which is especially evident in the inner woody (xylem) layers. Scientific literature shows that the average lignin content in the entire stem mass is 14% [17, 18]. However, the lignin content of the bast fibers (fibers located in the tertiary cell layer of the plant stem) that make up the outer part of the stem is much lower, usually around 5–9%. These bast fibers are considered the most suitable component for industrial fiber production because they have a high cellulose content (≈85%) [19]. The lignin content of the leaves is relatively low, with this figure in most plants being in the range of 2–5%. Although direct lignin content for Cannabis Sativa leaves is rarely reported, the presence of phenolic compounds related to lignin, in particular lignanamides, has been reported in their composition. This indicates that the leaves are lignocellulosic. The presence of lignin in the roots indicates the presence of phenolic structures, in particular in the form of lignanamides, and lignin compounds in the root tissues. This fact also indicates that the roots are a component rich in phenolics [20, 21].



**FIGURE 3.** Changes in the lignin content of the stem of Cannabis Sativa L depending on the maturation period

During the vegetative period of Cannabis Sativa L, lignin biosynthesis gradually becomes active (Figure 3). In the first weeks, especially in young tissues of 2–3 weeks, the lignin content is very low, around 3–5%, and during this period cellulose and pectin substances predominate. In the stage of active growth (4–6 weeks), the lignification process begins, and lignin accumulation increases sharply and reaches 10–15%. Especially close to flowering, lignin biosynthesis reaches its highest level, and as a result of the formation of secondary tissues in the plant stem, the lignin content increases to 20–25%. These changes ensure the mechanical strength of the Cannabis Sativa L plant and its preparation for physiological maturation. Also, the increasing lignin content during the growing season directly affects its fiber separation technology - during early harvest, fiber separation is easier due to the low lignin content, while during late harvest, on the contrary, chemical or enzymatic treatment is necessary to separate the fiber due to strong lignification.



**FIGURE 4.** Infrared spectrum of fiber from the stem of Cannabis Sativa L

In our studies, the FTIR (Fourier Transform Infrared Spectroscopy) method was used to determine the presence of lignin in a sample taken from the Cannabis Sativa L plant and to evaluate its functional groups (Figure 4). The spectral analysis was carried out in the range of 4000–400 cm⁻¹, and a number of vibrations characteristic of lignin were recorded.

The absorption region detected in the analysis at 3281.63 cm⁻¹ represents the vibrations of the –OH groups, which indicates the presence of phenolic and aliphatic alcohols. The absorption at 2925.50 cm⁻¹ in the figure belongs to aliphatic chains such as CH₂ and CH₃, indicating the presence of hydrocarbon fragments in the side groups of lignin.

Vibrations associated with aromatic rings were recorded around 1615.66 and 1515.42 cm⁻¹, which confirms the presence of aromatic structures characteristic of lignin. In particular, the peak at 1515.42 cm⁻¹ indicates the presence of the main structural units of lignin - guaiacil and sinapyl fractions. The vibration recorded at 1407.73 cm⁻¹ corresponds to the deformation vibrations of the -CH₂- methylene and -CH₃ methyl groups.

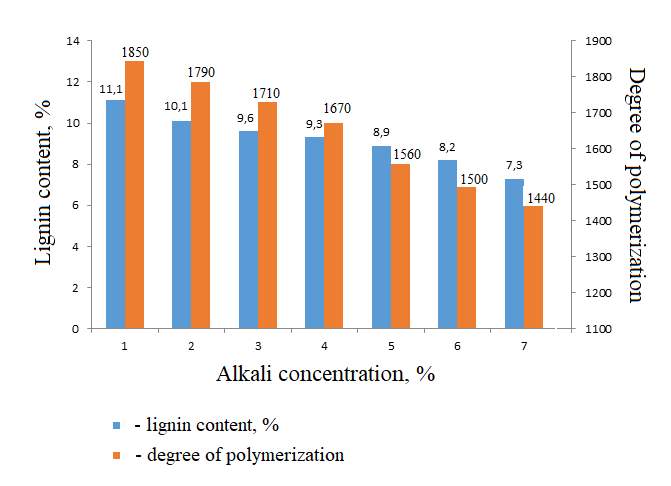
Also, the strong absorption detected at 1032.02 cm⁻¹ corresponds to the vibrations of the C–O carbon hydrogen bonds, which indicates the presence of phenolic and ether groups. The peaks observed at 779.53, 696.49 and 637.09 cm⁻¹ correspond to the low-frequency resonances of the vibrations of the aromatic rings. All these spectral data reliably substantiate the presence of lignin components in the analyzed sample.

Based on the above analysis, it can be said that the FTIR spectrum of lignin from Cannabis Sativa L indicates its phenylpropanoid-based structure, rich in hydroxyl, carbonyl and ether groups. This analysis shows that lignin consists of mixed compounds of the G/S (guaiacyl/syringyl) type.

The process concentration, process duration and temperature were studied for the extraction of lignin from Cannabis sativa L by chemical-alkaline method. In alkaline solution, hydrogen bonds between cellulose chains weaken and hydroxyl groups bind more strongly to alkali ions (OH⁻). As a result, cellulose macromolecules swell and the amount of amorphous phase increases. This process is expressed as follows:



In addition, in alkaline media, the morphological structure of cellulose becomes more amorphous, which makes it more susceptible to hydrolysis, enzymatic or chemical modification. In particular, in 17–18% NaOH solution, cellulose is converted to form II (alkali cellulose). From the data presented, it was determined to start the alkali treatment at low concentrations in the studies.



**FIGURE 5.** Effect of alkali concentration on fiber quality parameters

It can be seen from the figure that the results of the process carried out at an alkali (NaOH) concentration in the range of 3-5% show an increase in the efficiency of lignin extraction from Cannabis Sativa L stems. According to it, when the alkali concentration increased from 3% to 5%, the lignin content decreased from 9.6% to 8.9%, respectively. This indicates an increase in the hydrolytic and saponification effect of the NaOH solution on the lignin fraction.

In this case, changes in the range of 5-7% especially provided intensive lignin separation: a sharp decrease was observed from 8.9% (point 5) to 7.3% (point 7). These results indicate the decomposition of aromatic structures (benzene rings) at high alkali concentrations.

However, the results recorded on the degree of polymerization indicate that alkali also has an adverse effect on cellulose. In the figure, this indicator is 1850 at 1% NaOH, and decreases to 1440 at 7% NaOH. This decrease is associated with the reactions of β-elimination (the formation of a double bond between two atoms (C=C)) and depolymerization (the breakdown of the polymer chain)), which lead to the shortening of the cellulose chain under alkaline conditions.

This means that as the alkali concentration increases, although lignin separation is achieved, the molecular integrity of cellulose decreases. Therefore, the optimal processing conditions can be considered a 3% alkali concentration, since in this range the lignin separation is high (9.6%), but the degree of polymerization value is relatively maintained (1710).

In the next stages, the effect of process temperature and duration on the lignin separation efficiency and the structural stability of cellulose was studied.

**TABLE 1.** Effect of process duration on fiber quality parameters

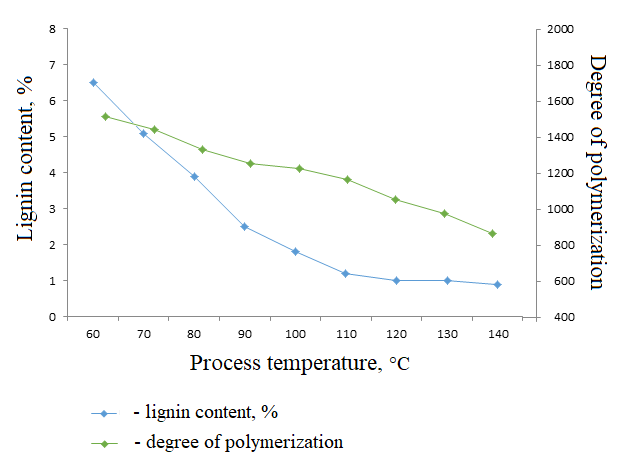
|  |  |  |
| --- | --- | --- |
| Process duration, min | Lignin content, % | Degree of polymerization |
| 30 | 9,6 | 1710 |
| 40 | 8,2 | 1680 |
| 50 | 7,4 | 1605 |
| 60 | 6,5 | 1550 |
| 70 | 5,9 | 1470 |
| 80 | 5,1 | 1400 |
| 90 | 4,8 | 1250 |
| 100 | 4,5 | 1200 |

The relationship between the efficiency of lignin extraction from the stem of Cannabis sativa L and the structural integrity of cellulose with the change in the process duration was analyzed. Changes in the lignin content and the degree of polymerization were observed under the conditions of boiling duration from 30 to 100 minutes.

According to the results, the lignin content decreased consistently with increasing process duration. For example, if the lignin content was 9.6% at 30 minutes, then at 100 minutes this indicator dropped to 4.5%. This indicates that the time factor has a significant effect on lignin separation and that as a result of the long process duration, a large part of the lignin undergoes hydrolysis.

At the same time, the degree of polymerization of cellulose showed the opposite trend. That is, as the boiling duration increased, the degree of polymerization decreased. At 30 minutes, this indicator was 1710, and at 100 minutes it decreased to 1200. This indicates that depolymerization of cellulose chains occurs during the process.

The optimal equilibrium was observed at a processing interval of 60 minutes: during this interval, the lignin content decreased significantly (by 6.5%), while the degree of polymerization remained around 1550. These conditions allow for efficient separation of lignin while maintaining the qualitative and stable properties of the fibrous material.



**FIGURE 6.** Effect of process temperature on fiber quality parameters

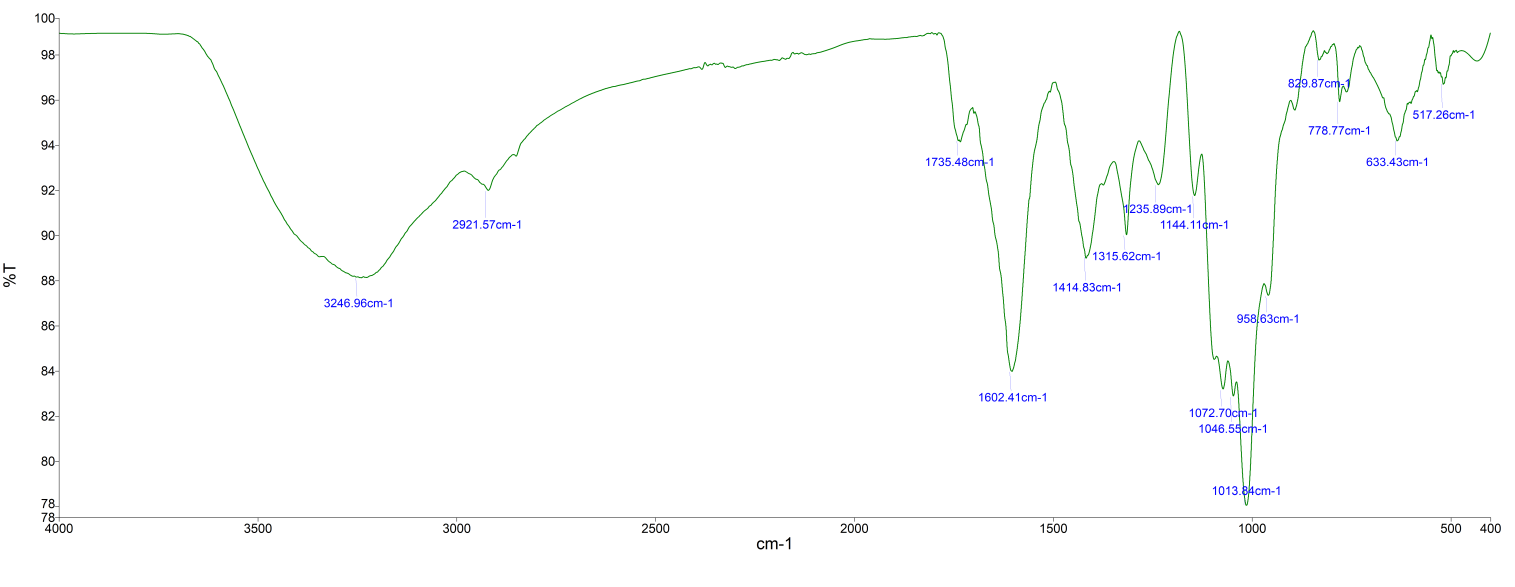
The graph above shows the effect of temperature on the alkaline extraction of lignin from Cannabis sativa L. As the temperature increases, a significant decrease in the lignin content is observed. For example, at 60°C, the lignin content is approximately 6.5%, while at 140°C it drops to 1.2%. This indicates that lignin is degraded at high temperatures by hydrolytic and oxidative reactions involving hydroxide ions.

High temperature in NaOH solution accelerates the hydrolysis of β-O-4 ether bonds of lignin macromolecules. The cleavage of these bonds leads to the decomposition of lignin into water-soluble, low molecular weight fragments. It is this decomposition that ensures the separation of lignin from the fiber.

However, with increasing temperature, the degree of fiber polymerization also decreases. The initial value of the degree of polymerization, which was around 1600–1700, approached 900 at 140°C. This is due to the partial hydrolysis of cellulose in an alkaline environment, especially at high temperatures, i.e., a reduction in chain length through the cleavage of glycosidic bonds. Under conditions of NaOH and high temperatures, cellulose is degraded and its molecular weight decreases, which reduces the degree of polymerization.

Thus, although high temperatures are effective in ensuring complete lignin separation, this leads to a decrease in the degree of polymerization, which is important for the crystallinity and continuity of the fibers. As a result, the physical and mechanical properties of the fibers, in particular strength and elasticity, can be negatively affected.

When choosing the optimal process temperature, it is necessary to find a balance between minimizing the amount of lignin and maintaining the quality of the fibers. In practice, temperatures around 100°C can be considered to provide an optimal balance between lignin degradation and cellulose preservation.



**FIGURE 10.** Infrared spectrum of fiber obtained from the stem of Cannabis Sativa L after processing

In the studies, the infrared spectrum of Cannabis sativa L fiber obtained after the extraction process carried out in an alkaline medium showed that a large part of the lignin was separated. This can be understood by the significant weakening of the absorption peaks characteristic of aromatic rings observed in the absorption range of 1515–1600 cm⁻¹. However, the absorption peak recorded at 1602.41 cm⁻¹ corresponds to the vibration of the aromatic C=C carbon-carbon bonds, indicating that traces of the aromatic structure of lignin are preserved.

Also, the broad absorption at 3246.96 cm⁻¹ belongs to the –OH groups, which indicates that some phenolic groups or hydroxyl chains are preserved. This indicates that the lignin is not completely separated, but partially.

The absorptions at 1414.83 cm⁻¹ and 1315.62 cm⁻¹, which are characteristic of the aromatic structure, as well as the absorption peaks around 1144.11 and 1235.89 cm⁻¹, which are related to the C–O carbon-oxygen bonds, are attributed to the ether or phenolic oxygen bonds of lignin. This confirms that a small amount of structural fragments are preserved even after extraction. The decrease in the intensity of the peaks observed in the FTIR spectrum, especially in the –OH, C–O–C and aromatic group regions, confirms the overall quantitative reduction of lignin. This indicates that the dissolved part of lignin in the alkaline solution (water-soluble lignin) has gone into solution and only the structurally stable fractions remain in the solid phase.

**CONCLUSION**

**In this study, the efficiency of chemical-alkaline extraction of lignin from the stem of Cannabis Sativa L was studied. During the study, the main factors such as the concentration of sodium hydroxide (NaOH) solution, the duration of the process and the temperature were experimentally determined to determine how they affect lignin separation and the structural integrity of cellulose. These parameters were evaluated as important factors in optimizing the efficiency of lignin extraction.**

**According to the results of the analysis, during the alkaline extraction process, the water-soluble fraction of lignin goes into solution, and only structurally stable components remain in the solid phase. The lignin content was 9.6% in a 3% NaOH solution, but decreased to 7.3% in 7% NaOH, and with increasing temperature and duration of the process, i.e., when the temperature was 140°C and the duration was 60 minutes, the lignin content decreased to 1.2%. However, due to the decrease in the degree of cellulose polymerization at high alkali and temperature, 3% NaOH concentration, 100°C temperature and 60 min duration were recommended as the optimal conditions.**

**The lignin composition before and after extraction was analyzed by FTIR spectroscopy. The main wavenumbers such as 3418, 1605, 1516, 1267, 1033 cm⁻¹ confirmed the presence of phenolic –OH, aromatic C=C and guaiacyl/syringyl units. The decrease in the intensity of these peaks after extraction indicated a decrease in the lignin content from a chemical point of view.**

**The practical significance of the study is that it offers an environmentally friendly, energy-efficient and economically viable technological approach to extract lignin from Cannabis Sativa L biomass. The results will expand the possibilities of producing high-value-added bioactive materials, biofuels, composites and “green” chemical products based on lignin in the future.**

**The approach and results of this study can be applied to other lignocellulosic biomasses, providing a scientific basis for future comparative experiments and large-scale processing strategies.**

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